160622 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-06-22

WEDNESDAY, 6/22

Today we received sequencing results back from Macrogen (first batch of Induction parts on low-copy backbones). For the most part we have one confirmed version of every part on every tier of backbone, except for WM16_015 on medium-copy backbone (3K3).

Today Likhitha, Callan, and Christine set up Colony PCRs of last night's Transformants, which were:

Table1

	A	B	C	D	E
1	**Transformed Plasmid 1**	**Transformed Plasmid 2**	**Strain**	**Grew?**	
2	WM16_025 3K3		5 alpha		
3	WM16_025 3C5		5 alpha		
4	WM16_025 3T5		5 alpha	No	
5	WM16_014 1A3	WM16_016 3K3	5 alpha		
6	WM16_014 1C3	WM16_016 3K3	5 alpha		
7	WM16_014 3K3	WM16_016 3T5	5 alpha		
8	WM16_014 3K3	WM16_016 1A3	5 alpha		
9	WM16_014 3C5	WM16_016 3T5	5 alpha	No	
10	WM16_014 3T5	WM16_016 3K3	5 alpha		
11	WM16_014 1A3	WM16_016 3K3	10 beta		
12	WM16_014 1C3	WM16_016 3K3	10 beta		
13	WM16_014 3K3	WM16_016 3T5	10 beta		
14	WM16_014 3K3	WM16_016 1A3	10 beta		
15	WM16_014 3C5	WM16_016 3T5	10 beta	No	
16	WM16_014 3T5	WM16_016 3K3	10 beta		
17	WM16_014 1A3	WM16_016 3K3	BL21		
18	WM16_014 1C3	WM16_016 3K3	BL21		
19	WM16_014 3K3	WM16_016 3T5	BL21		
20	WM16_014 3K3	WM16_016 1A3	BL21		
21	WM16_014 3C5	WM16_016 3T5	BL21	No	
22	WM16_014 3T5	WM16_016 3K3	BL21	No	

160621 Transformation Plate Growth Results

Likhitha and Callan also inoculated streaked-out glycerol stocks of Minipreps that had run low

Andy and I also diluted overnight inoculates from earlier permutations of WM16_014 + WM16_016 cotransformations, which were:

Table2

	A	B	C	D	E
1	**Plasmid 1**	**Plasmid 2**	**Strain**	**Grew?**	**Dilution Grew?**
2	WM16_014 3T5	WM16_016 1C3	5 alpha	No	
3	WM16_014 1C3	WM16_016 3T5	5 alpha	No	
4	WM16_014 1A3	WM16_016 3T5	5 alpha	No	
5	WM16_014 3T5	WM16_016 1C3	10 beta	No	
6	WM16_014 1C3	WM16_016 3T5	10 beta	No	
7	WM16_014 1A3	WM16_016 3T5	10 beta	No	
8	WM16_014 3T5	WM16_016 1C3	BL21	Yes	No
9	WM16_014 1C3	WM16_016 3T5	BL21	No	
10	WM16_014 1A3	WM16_016 3T5	BL21	Yes	Yes

160621 Inoculation Overnight Growth results + Dilution Results

After today's failures at the plate-growth and liquid-growth level, our progress on the permutations of WM16_014 and WM16_016 on different backbones is:

Table3

	A	B	C	D	E
1	**WM16_014**	**WM16_016**	**5 alpha status**	**10 beta status**	**BL21 Status**
2	High	High	Measured 160620		
3	High	Medium			
4	High	Low	Failed at Liquid Growth	Failed at Liquid Growth	Failed at Liquid Growth
5	Medium	High			
6	Medium	Medium			
7	Medium	Low			
8	Low	High	Failed at Liquid Growth	Failed at Liquid Growth	
9	Low	Medium	Failed at Plate Growth		
10	Low	Low	Failed at Plate Growth	Failed at Plate Growth	Failed at Plate Growth

Progress Report on Backbone Permutations for WM16_014 + WM16_016

160623 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-06-23

THURSDAY, 6/23

In @ 9:00 AM

Analyzed the Sequence Results from Macrogen. A lot of disconfirmed parts overall-- however most of the IPTG-inducible parts were not disconfirmed on an entire class of backbone (ie. if 3T5 was disconfirmed, a 3C5 was confirmed). Things are still looking okay for IPTG. aTc and Arabinose inductions are not looking as good, but we are going to hold off on characterizing those until we find the optimal backbone-copy combination through the IPTG assay on WM16_014 + WM16_016.

Part_Progress.xlsx

Today we received the Plate Reader from BioTek and their representative came and trained us on it. For the most part it seemed quite intuitive to use. The big thing is that we shouldn't use it as a plate vortexer / shaker exclusively, and that during Kinetics measurements the shaking feature shouldn't be set above the Medium level. It also won't hurt to use a smaller volume in the wells to prevent splashing of liquid during shaking, although BioTek claims 300 uL will be fine.

Likhitha is Minprepping the glycerol stock streaks that grew in liquid culture. She will also Miniprep the WM16_025 onto low-copy backbone assemblies, but none of those had growth in liquid media as of 10:00 AM.

Callan is setting up PCRs to create:

- WM16_015 on pSB3K3 (by moving 15 1C3 onto 3K3)
- WM16_031 on pSB1C3 (by creating it from 15 1A3 (insert) and 15 1C3 (backbone))

Callan is going to set up dilutions of overnight co-transformation cultures after the PCR-- as of 10:00 AM, only the BL21s were growing.

> *Consistently, only BL21 cultures grow well in the M9 with diluted antibiotic concentration (2000x Amp, 3000x Chlor, 1000x Kan, 1000x Tet). What's going on here?*

I'm setting up a FACS Measurement of Overnight Inductions of WM16_014 1A3 + WM16_016 1C3 in BL21 in M9.

- Startup went fine (pinhole alignment was not an issue) but I got "Event Rate
- Too Low" errors in three consecutive tries of QC despite shaking the beads in the tube and in the dropper.
- Ran a Low Pressure Wash with DI H2O. Doesn't look like it took up any of the water.
- Ran a High Pressure Wash with DI H2O. It definitely took up water.
- Ran QC again. Event Rate remains at 0 throughout the procedure.
- Called Matt and left a message. Perhaps the broken laser he mentioned during his last visit is causing this issue.
- Shut Down with DI H2O

Callan and I set up dilutions of colonies that had growth after overnight inoculation. Into 1 mL M9 media with the appropriate antibiotic we added 50 uL overnight growth (turbidity was low in most of the samples). Each tube was diluted into six tubes corresponding to the six molarities of IPTG induction. Once they reach midlog they will be induced with IPTG and put into the Plate Reader for bulk measurement. The diluted colonies were:

Table1

	A	B	C	D
1	**WM16_014 Backbone**	**WM16_016 Backbone**	**Grew?**	**Diluted?**
2				
3				
4				
5				

Out @ 2:30 PM
In @ 3:00 PM

Smith Meeting went great!

Tried to set up FACS measurements from earlier today... still having trouble getting the FACS machine to work. Sample gets drawn up but none of it gets detected as a fluorescent cell, let alone a cell at all. After 1.8 million events, only 3 end up being within the R1 gate for "correct size".

Some of Callan's PCRs disappeared when run out on a gel. Re-run of gel proved the same thing. It seems like sample may have not been loaded into the PCR reaction, as the primers were double-checked for correctness and annealing temp and extension time etc. Will have to re-run tomorrow.

Out @ 7:00 PM
In @ 9:30 PM

Callan Induced the dilutions with IPTG (0, 1 uM, 10 uM, 100 uM, 1 mM, 10 mM per sample) and loaded them into the Plate Reader. It took a while to figure out how to use it but we started it on a 16-hour kinetic run collecting fluorescence data. We wanted it to simultaneously collect OD600 measurements with the fluorescence readings, but the manual seems to suggest ths isn't possible. Will have to call BioTek tomorrow to ask about this.

Out @ 11:30 PM
In @ 1:55 AM

Finally went back and did a successful FACS Measurement of WM16_014 1A3 + WM16_016 1C3 in BL21 in M9. At this point they have been sitting in 37C 250 rpm with IPTG induction for over 48 hours. Results:

Table2

	A	B	C	D	E
1	**Sample**	**Number of Peaks**	**Mean 1**	**Mean 2**	**Proportion of fluorescent cells in each peak**
2	#1 Uninduced	1	1198		
3	#1 1 uM	1	1299		
4	#1 10 uM	1 with low tail	1158		
5	#1 100 uM	2	363	1421	did not record
6	#1 1 mM	1	1215		
7	#1 10 mM	1	1197		
8	#2 Uninduced	2	1	1231	20-80
9	#2 1 uM	2	2	1372	20-80
10	#2 10 uM	2	19	1330	20-80
11	#2 100 uM	1	1126		
12	#2 1 mM	1	1164		
13	#2 10 mM	1	1295		
14	#3 Uninduced	2	1	1525	10-90
15	#3 1 uM	2	1	1451	15-85
16	#3 10 uM	2	8	1419	15-85
17	#3 100 uM	1 with low tail	1072		
18	#3 1 mM	1	1231		
19	#3 10 mM	1	1297		
20					

- Shut Down with DI H2O.

It seems that everything is pretty much maximally expressing after such a long induction time.

Out @ 2:25 AM

160624 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-06-24

FRIDAY, 6/24

In @ 10:00 AM

Likhitha found out that one reason the 5 alpha strain might not be growing in M9 media is that 5 alpha is unable to produce its own Thiamine. LB contains Thiamine, but M9 does not.. apparently one has to add it in manually.

Christine found out that 10-beta does not produce its own Leucine, and so it cannot grow in M9 media without Leucine added in.

Likhitha and Christine also found out that:

- You can't use Tet as a selector in M9, because the Magnesium inhibits Tet activity.
  - But why do 3T5 plasmids seem to be lost at the plate level, according to Colony PCRs? Our tetracycline concentration seems to be fine.
- You can't grow up 5 alpha in M9 using Chlor as a selector (from a questionable paper?)

Here is the 14 + 16 backbone permutation progress so far, with Green representing FACS measurements and Purple being the currently in-progress Plate Reader measurements:

Table3

	A	B	C	D	E
1	**WM16_014**	**WM16_016**	**5 alpha status**	**10 beta status**	**BL21 Status**
2	High	High	Measured 160620	Measured 160624	
3	High	Medium			Measured 160624
4	High	Low	Failed at Liquid Growth	Failed at Liquid Growth	Measured 160624
5	Medium	High			Measured 160624
6	Medium	Medium			
7	Medium	Low			
8	Low	High	Failed at Liquid Growth	Failed at Liquid Growth	Measured 160624
9	Low	Medium	Failed at Plate Growth		
10	Low	Low	Failed at Plate Growth	Failed at Plate Growth	Failed at Plate Growth

Callan is PCRing to create

- WM16_015 on pSB3K3 (by moving 15 1C3 onto 3K3)
- WM16_031 on pSB1C3 (by creating it from 15 1A3 (insert) and 15 1C3 (backbone))
- K577882 (pBAD RFP) onto pSB3K3
- WM16_014 on pSB3C5 (by using WM16_014 1A3 MP2 160603 and the only confirmed 3C5 backbone we have (MP 1))

        - Looking back at old PCRs, every 3C5 + 3T5 combination failed.. however the component parts involved in these cotransformation seemed to all be good (JPM p.57 for Induction Insert MPs used, JPM p. 60 for backbone MPs used to create the low-copy parts; JPM p. 74 for specific MPs in the cotransformation). Given that WM16_014 3C5 is not sequence confirmed, we are trying the MP2 of source WM16_014 1A3 instead of the MP1 originally used.
    - WM16_016 on pSB3C5
        - If this and the above are successful then we will be able to have a Med-Low combination of 14 and 16 that works in M9 (no Tet in M9).
    - WM16_014 1C3 with P019, P013 (to make a UNS 1C3 backbone on which we can place WM16_029)

Successful completion of these assemblies should take care of all induction-related parts on all backbone copy number levels (High, Medium, Low), provided that a re-inoculation of the WM16_025 transformations in LB (instead of M9) works.

Ethan and Joe successfully achieved complete BsmBI cutting of 1 ug monomer DNA!

IDT shipment arrived! We received:

- P038 and P039, which are used to move the 85x tetO array from addGene onto a standard UNS backbone.
- WM16_028-029A geneBlock, which can be combined with WM16_029B geneBlock (already arrived earlier) to assemble WM16_029 in a UNS backbone!
    - WM16_028C is scheduled to arrive on Monday, June 27

Adam is resuspending gBlocks and Primers

Adam is setting up a PCR to amplify the 85x tetO array insert with P038 and P039.

Out @ 12:40 PM
In @ 8:00 PM

We are setting up inoculations to FACS the co-transformations which were recorded on the Plate Reader today:

Table1

	A	B	C	D	E	F
1	**Plate Date**	**14 Backbone**	**16 Backbone**	**Strain**	**Media**	**Grew on 160625?**
2	160621	1C3	3K3	BL21	LB	
3	160621	3K3	3T5	BL21	LB	
4	160621	3K3	1A3	BL21	LB	
5	160620	3T5	1C3	BL21	LB	
6	160621	1C3	3K3	BL21	M9	
7	160621	3K3	1A3	BL21	M9	

Callan and Adam transformed the following Gibsons:

- WM16_015 3K3
- K577882 (pBAD RFP) 3K3
- WM16_014 3C5
- WM16_016 3C5
- WM16_029 1C3
- ICA tetO 16bp 3-mer (probably didn't work) 1C3

Adam PCR'd the 85x tetO array from addGene with P038 and P039 and got a band of the correct size! (MP 1). This will be Gibson'd tomorrow into 1C3.

Out @ 10:20 PM

160625 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-06-25

SATURDAY, 6/25

In @ 1:00 PM

Ethan found out that Glucose can inhibit the arabinose induction of a pBad promoter.

Ethan is making new M9 media with Glycerol instead of Glucose for better induction in general.

Callan and Andy set up PCRs to assemble the following:

- WM16_024 onto 3C5
- WM16_030 onto 1A3
- WM16_031 onto 1C3
- WM16_031 onto 3K3
- K577882 (pBad RFP) onto 1A3
- K577882 (pBad RFP) onto 3C5
- K1493504 (pTet GFP) onto 1A3

Here are the specs from Andy's 160625 (they also used P036 and P037 to transfer K577882 (pBad RFP) and K1493504 (pTet GFP) onto the UNS backbones:

Insert PCRs

Table1

	A	B	C	D	E	
1	part name	location	Date	Forward Primer	Reverse Primer	Anr Ten
2	WM16_024-Amp-mp2	Box 3, slot 29	160609	WM16_P008	WM16_P009	
3	WM16_030 Chlor Mp1	Box 3 slot 58	160614	WM16_P008	WM16_P009	
4	WM16_031 on pSB3T5 MP 2	Box 4 slot 56	160620	WM16_P008	WM16_P009	
5	K577882 pBAD RFP Chlor MP1	Box 3 slot 43	160611	WM16_P030	WM16_P031	
6	K1493504 (pTet GFP Chlor MP1)	Box 4 slot 13	160615	WM16_P030	WM16_P031	

Backbone PCRs:

Table2

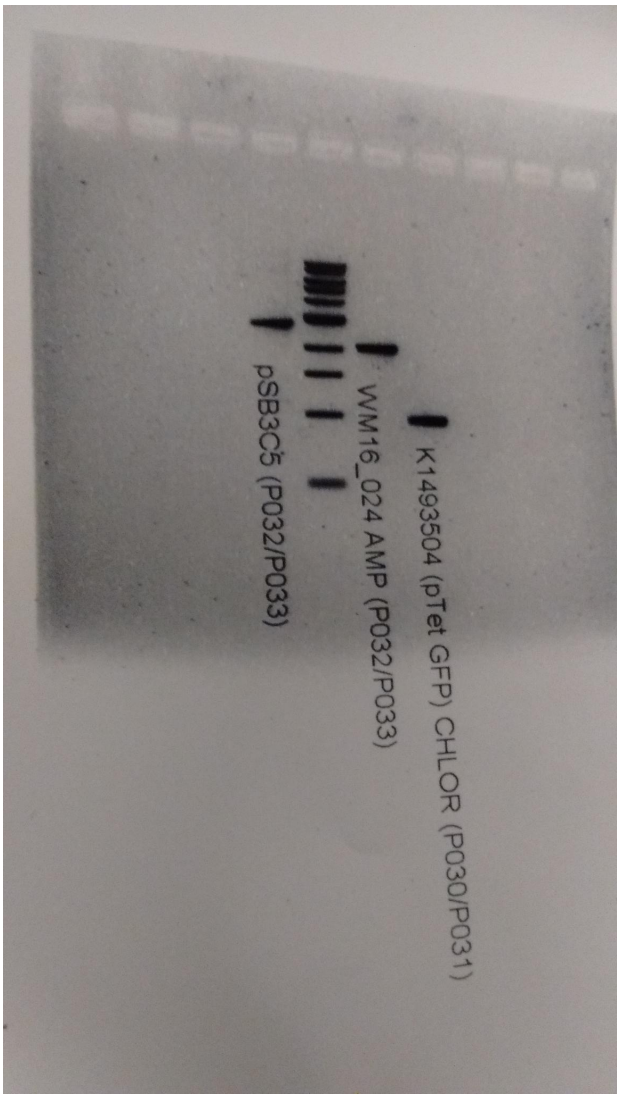
	A	B	C	D	E	F
1	Template	Location	Date	Forward Primer	Reverse Primer	Annealing temperature
2	WM16_024-Amp-mp2	Box 3, slot 29	160609	P13	P19	67
3	WM16_030 Chlor Mp1	Box 3 slot 58	160614	P13	P19	67
4	WM16_024-Amp-mp2	Box 3, slot 29	160609	P32	P33	70
5	pSB3C5 MP1	Box 4, slot 19		P36	P37	72
6	WM16_026 on pSB3K3 MP1	Box 4, slot 42	160620	P36	P37	72
7	pSB3C5 MP1	Box 4, slot 19		P32	P33	70

Table3

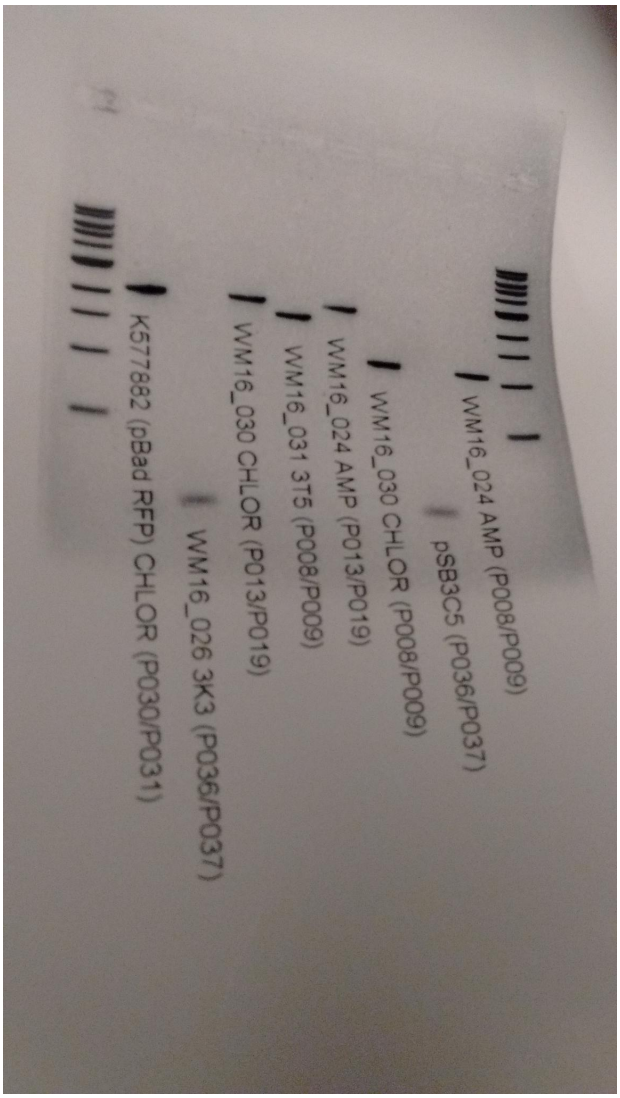
	A	B	C	D
1	Thermal cycler	annealing temperature	extension time	Samples
2	1	64	30s	WM16_024-Amp-mp2 P8/P9; WM16_030 Chlor Mp1 P8/P9
3	2	64	1m	WM16_031 on pSB3T5 MP 2 P8/P9
4	3	70	1m	K577882 pBAD RFP Chlor MP1 P30/31; WM16_024-Amp-mp2 P32/33
5	4	70	30s	K1493504 (pTet GFP Chlor MP1) P30/P31
6	Bradley lab	67	1m	WM16_024-Amp-mp2 P13/P19; WM16_030 Chlor Mp1 P13/P19
7	Saha lab	72	1m30s	pSB3C5 MP1 P36/P37; WM16_026 on pSB3K3 MP1 P36/P37
8	Core lab	70	1m30s	pSB3C5 MP1 P32/P33

Gels from these PCRs:

IMG_20160625_182305696.jpg



IMG_20160625_182311461.jpg



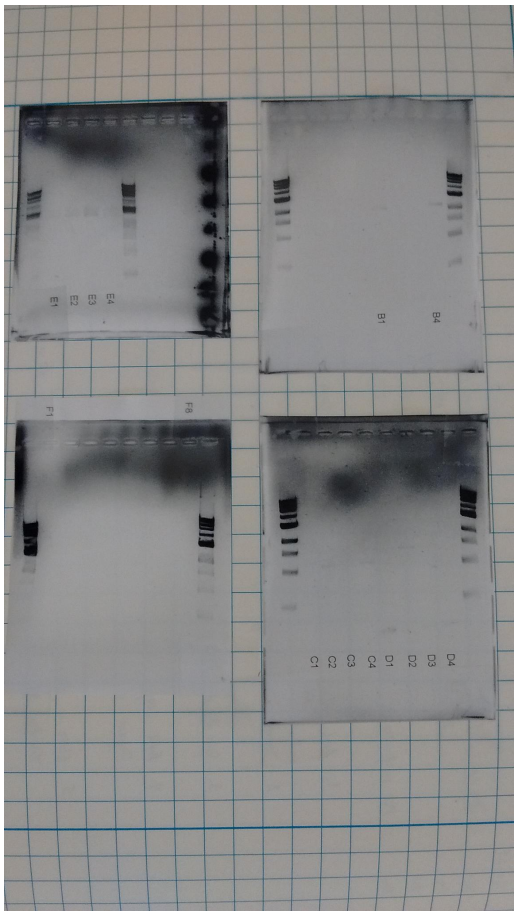
...it seems like the P038 P039 combo isn't working :(

All the inoculations from last night grew. Andy is diluting them for IPTG induction upon reaching midlog.

Incubator @ 1:15 PM

Callan and Christine are setting up Colony PCRs to assess the transformations from last night. Good ones will be inoculated for minipreps tomorrow.

IMG_20160625_183506783.jpg



They don't look so good.....

Callan set up the PCRs for DpnI reaction.

Callan set up the PCR Purification of the PCRs for Gibson.

Callan set up the Gibson Assemblies:

- (1): WM16_030 1A3
- (2): WM16_031 1C3
- (3): K577882 (pBad RFP) 1A3
- (4): K577882 (pBad RFP) 3C5
- (5): K1493504 (pTet GFP) 1A3
  (We'll need to re-do the ones from earlier today's list that failed at the PCR stage)

Andy and I set up IPTG inductions of the dilutions. 500 uL per dilution condition per sample. We are just adding IPTG solution into the culture, no spin-downs or resuspensions.

Incubator @6:20 PM

Ethan is setting up inoculations of the 160624 transformation colonies that looked good on Colony PCR (in LB).

Out @ 6:40 PM. See ADH 160625 for night details.

PCR for gibsons

Introduction

Dr. Kary Mulliis's gift to the biochemical world! This allows you to amplify a selected region of template DNA in an exponential fashion. The starting point for our cloning pipeline. Can be done off of any kind of dsDNA (plasmid or fragment). Do not PCR gBlocks!

Materials

- NEB 2x Q5 HiFi MM
- NFW
- Forward/Reverse Primer
- Template DNA
- 1% Agarose Gel (optional)

Procedure

PCR w/ MasterMixx

1. Set up MasterMix according to below table

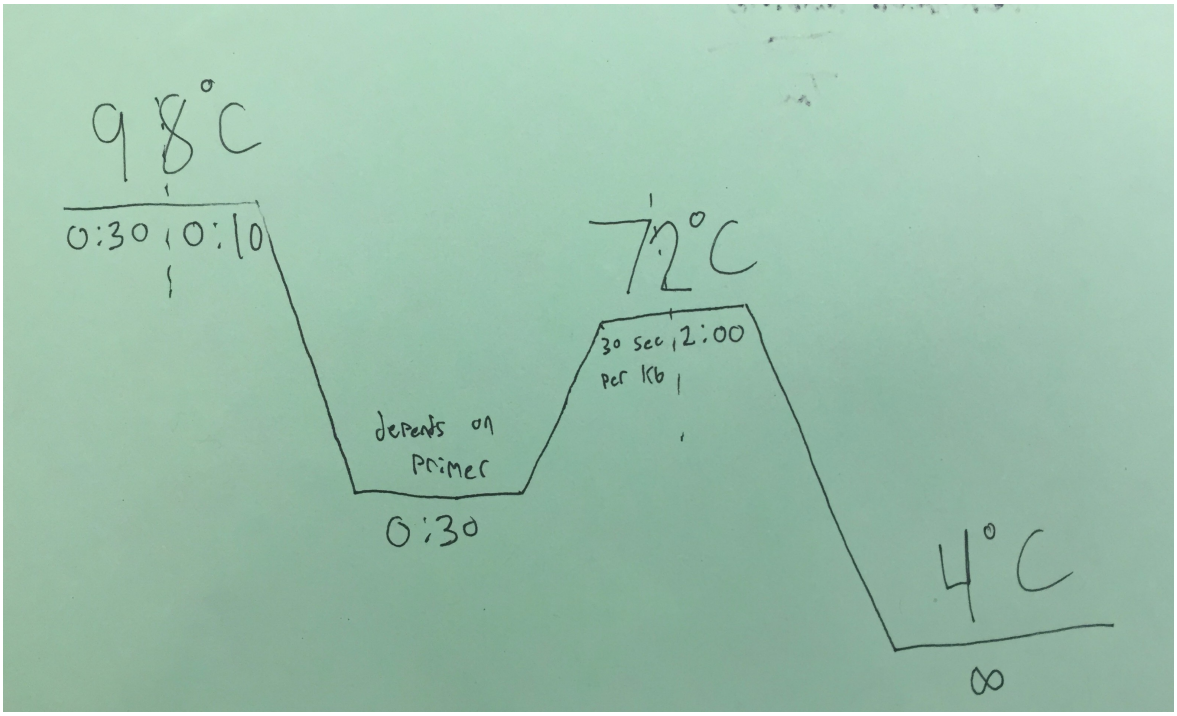
   Set up MasterMixx using the table below. Use a Upscale factor of at least 1.1. **Write into the yellow boxes.**

Table2

	A	B	C
1	Number of Reactions		
2	Percent Upscale		
3		uL / Tube	uL - > MM
4	10 uM Forward		0
5	10 uM Reverse		0
6	Nuclease Free Water		0
7	Q5 2x-HiFI MM		0

2. Thermal Cycle.

   Look up appropriate temperature for your primers if you don't know.
   Extension Time should be 30 seconds for each 1000 bp you are trying to amplify.



- [x] 3. Run a Gel of your Thermal Cycled PCR!

PCR for gibson redo

Introduction

Dr. Kary Mulliis's gift to the biochemical world! This allows you to amplify a selected region of template DNA in an exponential fashion. The starting point for our cloning pipeline. Can be done off of any kind of dsDNA (plasmid or fragment). Do not PCR gBlocks!

Materials

- NEB 2x Q5 HiFi MM
- NFW
- Forward/Reverse Primer
- Template DNA
- 1% Agarose Gel (optional)

Procedure

PCR w/ MasterMixx

1. Set up MasterMix according to below table

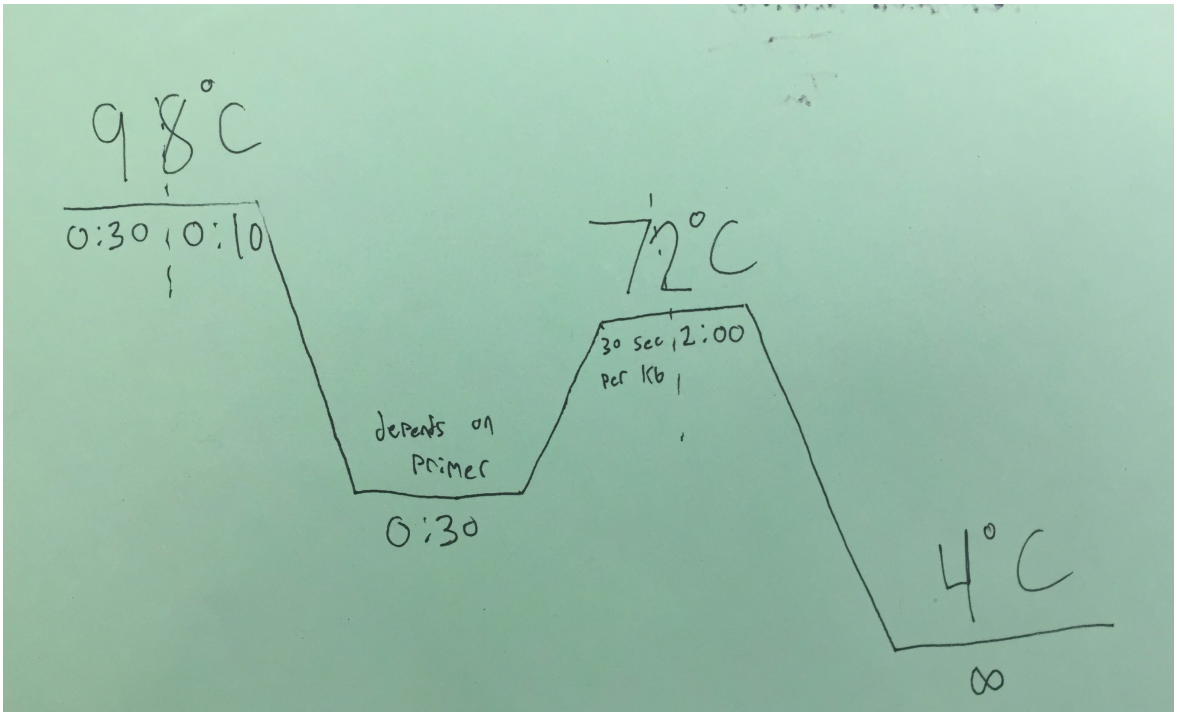
   Set up MasterMixx using the table below. Use a Upscale factor of at least 1.1. **Write into the yellow boxes.**

Table2

	A	B	C
1	Number of Reactions		
2	Percent Upscale		
3		uL / Tube	uL - > MM
4	10 uM Forward		0
5	10 uM Reverse		0
6	Nuclease Free Water		0
7	Q5 2x-HiFI MM		0

2. Thermal Cycle.

   Look up appropriate temperature for your primers if you don't know.
   Extension Time should be 30 seconds for each 1000 bp you are trying to amplify.



- [x] 3. Run a Gel of your Thermal Cycled PCR!

160626 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-06-26

SUNDAY, 6/26

In @ 1:40 PM. See ADH 160626 for morning details.

Joe Miniprepped the assembly inoculations from last night.

Adam is setting up Gibson Assemblies of the following parts:

- WM16_024 3C5
- WM16_026 3T5
- 85x tetO array 1C3

This will complete the assemblies that were missed yesterday. Setup:

Table2

	A	B	C	D	E	F	G
1	**backbone**	**1C3 with UNS**	**0.05**	**2150**	**50.6**	**1.402173913**	
2	insert	85x tetO with UNS	0.15	3188	107	2.94964486	0.64818
3	**backbone**	**pSB3T5 from WM16_031**	**0.06**	**3252**	**133.1**	**0.9675371901**	
4	insert	WM16_026 from 3K3	0.18	1025	72.9	1.67037037	2.362
5	**backbone**	**pSB3C5**	**0.06**	**2738**	**55.6**	**1.950086331**	
6	insert	WM16_024 from 1A3	0.18	1083	133.1	0.9666446281	2.0832

Joe is Colony PCRing the transformations from last night. Recall that they were

- (1): WM16_030 1A3
- (2): WM16_031 1C3
- (3): K577882 (pBad RFP) 1A3
- (4): K577882 (pBad RFP) 3C5
- (5): K1493504 (pTet GFP) 1A3

Good colonies will be inoculated.

Andy and I are FACSing the parts that were measured at 3-4 hour induction last night by ADH and EMJ, to test efficacy of an 18-hour IPTG induction relative to the shorter time. Recall that the samples are (MP sources from Callan p.28 (160621 Transformations):

- Sample 1 - WM16_014 on pSB1C3 (MP1 160605), WM16_016 on pSB3K3 (MP1 160620) in LB
- Sample 2 - WM16_014 on pSB3K3 (MP1 160620), WM16_016 on pSB3T5 (MP1 160620) in LB
- Sample 3 - WM16_014 on pSB3K3 (MP1 160620), WM16_016 on pSB1A3 (MP1 160614) in LB
- Sample 4 - WM16_014 on pSB3T5 (MP1 160620), WM16_016 on pSB1C3 (?) in LB
- Sample 5 - WM16_014 on pSB1C3 (MP1 160605), WM16_016 on pSB3K3 (MP1 160620) in M9 (Glucose)
- Sample 6 - WM16_014 on pSB3K3 (MP1 160620), WM16_016 on pSB1A3 (MP1 160614) in M9 (Glucose)

And the ID convention is "Sample.Replicate.Induction" where the inductions are

- 1 = 0

- 2 = 1 uM
- 3 = 10 uM
- 4 = 100 uM
- 5 = 1 mM
- 6 = 10 mM

We're having issues with getting the FACS machine to work properly... the same issue that occured on 160623 is happening, where we run samples through and they don't get detected as the correct size (R1 gate) despite picking up millions of events. We restarted the machine and ran QC twice and it became okay.

Table1

	A	B	C	D	E	F
1	Sample	Peak 1 Mean	Peak 2 Mean	% of cells in plot in peak	Notes	Fl1 Gain
2	1.1.1	1.26				460
3	1.1.2	1.64				460
4	1.1.3	11.6				460
5	1.1.4	68.5				460
6	1.1.5	89.5				460
7	1.1.6	83				460
8	1.2.1	1.2		95		460
9	1.2.2	1.4		94		460
10	1.2.3	18.6		100		460
11	1.2.4	73		99		460
12	1.2.5	92		99		460
13	1.2.6	86.1		99		460
14	1.3.1	1.85		93		460
15	1.3.2	3.1		97		460
16	1.3.3	8.2		99		460
17	1.3.4	78.6		99		460
18	1.3.5	86		99		460
19	1.3.6	83.8		99		460
20	2.1.1	3.4		100		700
21	2.1.2	2.9		100		700
22	2.2.3	3.6		100		700
23	2.1.4	6.6		99		700
24	2.1.5	8.6		99		700
25	2.1.6	9.9		95		700
26	2.2.1	3		100		700
27	2.2.2	2.9		100		700
28	2.2.3	3.4		100		700

29	2.2.4	7.3		100		700
30	2.2.5	8.1		99		700
31	2.2.6	8.3		100		700
32	2.3.1	2.9		100		700
33	2.3.2	3.1		100		700
34	2.3.3	3.3		100		700
35	2.3.4	6.4		100		700
36	2.3.5	7.2		100		700
37	2.3.6	7.7		100		700
38	3.1.1	6.5		100		700
39	3.1.2	6.4		100		700
40	3.1.3	31.8		100		700
41	3.1.4	127.9		100		700
42	3.1.5	146.7		100		700
43	3.1.6	150.4		100		700
44	3.2.1	6.7		100		700
45	3.2.2	8.5		100		700
46	3.2.3	40.7		100		700
47	3.2.4	143.3		100		700
48	3.2.5	146.3		100		700
49	3.2.6	172.3		100		700
50	3.3.1	5.8		100		700
51	3.3.2	6.5		100		700
52	3.3.3	36		100		700
53	3.3.4	148		100		700
54	3.3.5	160.2		100		700
55	3.3.6	180.7		100		700
56	4.1.1	7		100		550
57	4.1.2	7.9		100		550
58	4.1.3	47.6		99		550
59	4.1.4	105.8		100		550
60	4.1.5	140		100		550
61	4.1.6	132.5		99		550
62	4.2.1	5.2		100		550
63	4.2.2	6		100		550
64	4.2.3	36.1		100		550

65	4.2.4	84.4		100		550
66	4.2.5	100.3		100		550
67	4.2.6	106.9		100		550
68	4.3.1	4.2		100		550
69	4.3.2	4.6		100		550
70	4.3.3	16		99		550
71	4.3.4	45		99		550
72	4.3.5	80		99		550
73	4.3.6	83.8		99		550
74	5.1.1	1.1		99		460
75	5.1.2	1.1		99		460
76	5.1.3	6.7		97		460
77	5.1.4	67		99		460
78	5.1.5	163.7		100		460
79	5.1.6	199.4		100		460
80	5.2.1	1		99		460
81	5.2.2	1		99		460
82	5.2.3	1.6		99		460
83	5.2.4	26.3		99		460
84	5.2.5	112.9		99		460
85	5.2.6	179.9		99		460
86	5.3.1	1		100		460
87	5.3.2	1.1		99		460
88	5.3.3	1.9		100		460
89	5.3.4	32.1		98		460
90	5.3.5	149		99		460
91	5.3.6	178		99		460
92	6.1.1	5.1		100		700
93	6.1.2	4.9		100		700
94	6.1.3	12.4		99		700
95	6.1.4	48		100		700
96	6.1.5	236.5		100		700
97	6.1.6	256.8		100		700
98	6.2.1	4.3		100		700
99	6.2.2	4.9		100		700
100	6.2.3	9		100		700

101	6.2.4	44.4		100		700
102	6.2.5	191.4		100		700
103	6.2.6	247.6		100		700
104	6.3.1	4.7		100		700
105	6.3.2	5.1		100		700
106	6.3.3	8.8		100		700
107	6.3.4	49.1		100		700
108	6.3.5	218.2		99		700
109	6.3.6	259.8		99		700

It seems that the sample which represents the best induction is 5.1, and 5 in general (WM16_014 1C3 + WM16_016 3K3 in M9 Glucose BL21). Other good choices were 6 (WM16_014 3K3 WM16_016 1A3 in M9 Glucose BL21).

> Ethan read that most people put the repressor on a low-copy plasmid and the reporter on a medium-copy. Given that we know we can't use Tet in M9, we don't have that combination currently available... we're content rolling forward with the high reporter / medium repressor combo.

Adam and Joe set up Transformations of the three Gibson Assemblies from earlier today.

160627 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-06-27

MONDAY, 6/27

In @ 9:50 AM

Transformants from last night grew (2/3 of them):

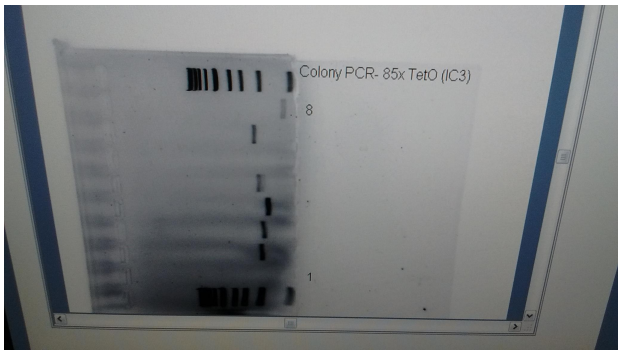
- 85x tetO 1C3 grew
- WM16_024 3C5 grew
- WM16_026 3T5 didn't grow.

Adam is setting up Colony PCRs of the ones that grew. Here are gels:

IMG_20160627_181253263.jpg

WM16_024 3C5....???

IMG_20160627_181139707.jpg



85x tetO looks like differing numbers of repeats were assembled. Inoculated all of them.

Adam is setting up dilutions of the inoculations from last night. He also inoculated one colony of WM16_025 3K3 from a 160621 plate (it previously failed at the liquid growth stage, but it was in M9 and it is a 5-alpha strain). They are:

Table2

	A	B	C	D
1	**Tube**	**4 mL of Media**	**Antibiotics**	**Inoculant**
2	1	M9 Glucose	Chlor	WM16_031 1C3 #3
3	2	LB	Amp	pTet GFP 1A3 #1
4	3	LB	Amp	pTet GFP 1A3 #2
5	4	M9 Glycerol	Amp	pBad RFP 1A3 #1
6	5	M9 Glycerol	Amp	pBad RFP 1A3 #2
7	6	M9 Glycerol	Amp	pBad RFP 1A3 #3
8	7	M9 Glycerol	Chlor	pBad RFP 3C5 #2
9	8	LB	Kan	WM16_025 3K3

(In @ 11:00 AM)

Callan is setting up Macrogen Sequencing of the following MPs (all from 160626):

Table4

	A
1	ICA 3mer 1c3 MP1
2	ICA 3mer 1c3 MP2
3	ICA 3mer 1c3 MP3
4	ICA 3mer 1c3 MP4
5	ICA 3mer 1c3 MP5
6	ICA 3mer 1c3 MP6
7	ICA 3mer 1c3 MP7
8	ICA 3mer 1c3 MP8
9	WM16_029 1C3 DH5alpha MP1
10	WM16_029 1C3 dh5alpha MP2
11	WM16_029 1C3 dh5alpha MP3
12	K577882 3K3 pBAD RFP dh5alpha MP1
13	K577882 3K3 pBAD RFP dh5alpha MP2
14	Wm16_014 3C5 dh5alpha MP1
15	Wm16_014 3C5 dh5alpha MP2
16	WM16_016 3C5 Dh5alpha MP1

Ethan is annealing Initiator, Terminator, and Caps for ICA.
Then Ethan and Joe did an ICA to make a 3-mer and a 9-mer.
Adam is resuspending the gBlock of 28B, which arrived today. He is resuspending to a concentration of 0.1 pmol/uL
I set up PCRs in standard conditions to assemble the following parts:

- WM16_015 3K3
- WM16_031 1C3
- WM16_031 3K3

Using:

Table1

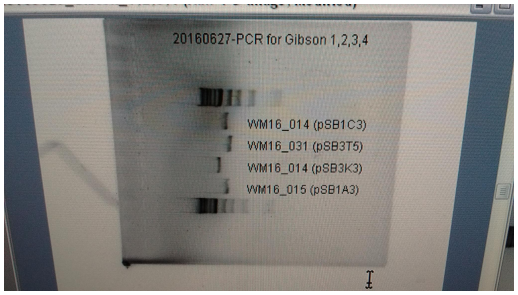
	A	B	C	D	E	
1	**Insert**	**Primer 1**	**Primer 2**	**Annealing Temp**	**Extension Time**	**Key**
2	WM16_015 1A3 160608 MP1	P008	P009	64C	1:00	1
3	WM16_014 3K3 160620 MP3	P019	P013	67C	1:30	2
4	WM16_031 3T5 160620 MP2	P008	P009	64C	1:00	3
5	WM16_014 1C3 160605 MP1	P019	P013	67C	1:00	4

Backbones are in Grey

Gel:

Gels look good!

IMG_20160627_135712393.jpg



The Sigma 54 parts arrived from Orna Atar! Andy will resuspend the dried plasmid parts later today.

Adam and Callan set up Gibsons:

Table3

	A	B	C	D	E	F	G
1	20160627 Gibsons		*pmol*	*length (bp)*	*concentration (ng/uL)*	***You need this many uL:***	***you need much H20***
2	**backbone**	**3K3 (from WM16_014 3K3)**	**0.06**	**2830**	**148**	**0.7572162162**	
3	insert	WM16_015	0.16	1820	105.9	1.814844193	2.4279
4	**backbone**	**1C3 (from WM16_014 1C3)**	**0.06**	**2150**	**117.5**	**0.7245957447**	
5	insert	WM16_031	0.18	1745	142.8	1.451722689	2.8236
6	**backbone**	**3K3 (from WM16_014 3K3)**	**0.06**	**2830**	**148**	**0.7572162162**	
7	insert	WM16_031	0.18	1745	142.8	1.451722689	2.7910
8	**backbone**	**1C3 (from WM16_014)**	**0.1**	**2150**	**117.5**	**1.207659574**	
9	insert	WM16_028A	0.1	1262	83	1.003518072	
10	insert	WM16_028B	0.1	1249	82	1.005292683	
11	insert	WM16_028C	0.1	791	52	1.003961538	
12	insert	Example Additional Insert	0	0	1	0	***You need much H20***
13		***TOTAL MOLES (want .01 - .25 pmol for 1-2 Fragments, and .1 - .5 pmol for 3+ fragments) :***	0.4		***TOTAL FRAGMENT VOLUME (cannot exceed 5 uL) :***	4.220431868	0.7795681

Callan and Christine are setting up aTc and Arabinose inductions of the pTet GFP and pBad RFP inoculants.

- Arabinose is being diluted to 0, 1 uM, 10 uM, 100 uM, 1 mM, 10 mM
- aTc is being diluted to 0, 31.25 ng/mL, 250 ng/mL, 500 ng/mL, 1000 ng/mL, 2000 ng/mL

Incubator @ 7:40 PM

(It seems that overnight growth is recommended for Arabinose, and that overnight growth is allowable for aTc)

Likthia and I transformed the following parts:

Table5

	A	B	C	D
1	**Plasmid 1**	**Plasmid 2**	**Strain**	**Key ID**
2	WM16_015 3K3 (from Gibson)		5 alpha	1
3	WM16_031 1C3 (From Gibson)		5 alpha	2
4	WM16_031 3K3 (from Gibson)		5 alpha	3
5	WM16_028 1C3 (from Gibson)		5 alpha	4
6	WM16_029 1C3 MP 1 160626		BL21	5
7	WM16_029 1C3 MP 1 160626	pTet GFP 3K3 MP 1 160620	BL21	6

We used 1 uL of 1:100 dilution for the MPs and 2 uL of undiluted Gibson assembly.

Out @7:40PM
In @ 8:30 PM

Adam plated out the transformations at 10:00 PM (2.5 hrs outgrowth).

160628 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-06-28

TUESDAY, 6/28

In @ 9:50 AM

Responded to Adriel from NOVA iGEM re. their modeling. It looks like their project (introducing yeast which can degrade starches into a production plant water filtration system) is perfect for a compartmental flux model using NetLogo.

Joe called BioTek and found out that you actually can take OD600 measurements concurrently with Kinetic measurements within a Kinetic Loop. All you have to increase the interval time to accomodate the extra read step. Joe added OD600 step into our current Kinetic Loop program for sfGFP and set the interval time to 2 minutes, and the sequence was validated by the software.

Adam Callan and Likhitha set up Colony PCRs of last night's transformants. All transformants grew!

All of the 5 alpha transformation will be inoculated in LB for miniprep. All of the BL21 strains will be inoculated in M9 Glycerol (for solo 29) or LB (for 29 + pTet GFP) for measurement tomorrow.

(See AJR and LK 160628 for the gel pics and decisions of colonies to inoculate)

All overnight inductions grew. Adam is adding 10 uL of overnight induction to 500 uL PBS. Then I FACS'd them (15 hour induction):

- Ran Startup (no problems)
- Ran QC (no problems)
- Measured (no problems)
- Shut Down with DI H2O (no problems)

Table1

	A	B	C	D	E	F	
1	**Strain**	**Media**	**Plasmid 1**	**Plasmid 2**	**Induction Level**	**Peak 1 Mean**	**Peak 2**
2	BL21	M9 Glycerol	pBad RFP 3C5		0 uM	4.5	
3	BL21	M9 Glycerol			1 uM	4.8	
4	BL21	M9 Glycerol			10 uM	6.7	
5	BL21	M9 Glycerol			100 uM	14	
6	BL21	M9 Glycerol			1 mM	22.1	
7	BL21	M9 Glycerol			10 mM	24.5	
8	BL21	M9 Glycerol	pBad RFP 1A3 #3		0 uM	4.3	
9	BL21	M9 Glycerol			1 uM	4.2	
10	BL21	M9 Glycerol			10 uM	4.2	
11	BL21	M9 Glycerol			100 uM	4.2	
12	BL21	M9 Glycerol			1 mM	4.2	
13	BL21	M9 Glycerol			10 mM	4.2	
14	BL21	M9 Glycerol	pBad RFP 1A3 #2		0 uM	4.3	

15	BL21	M9 Glycerol			1 uM	4.2	
16	BL21	M9 Glycerol			10 uM	4.2	
17	BL21	M9 Glycerol			100 uM	4.2	
18	BL21	M9 Glycerol			1 mM	4.2	
19	BL21	M9 Glycerol			10 mM	4.2	
20	BL21	M9 Glycerol	pBad RFP 1A3 #1		0 uM	4.2	
21	BL21	M9 Glycerol			1 uM	4.3	
22	BL21	M9 Glycerol			10 uM	4.6	
23	BL21	M9 Glycerol			100 uM	4.2	
24	BL21	M9 Glycerol			1 mM	4.1	
25	BL21	M9 Glycerol			10 mM	4.2	
26	BL21	M9 Glucose	WM16_031 1C3		0 uM	1.3	
27	BL21	M9 Glucose			1 uM	1.3	
28	BL21	M9 Glucose			10 uM	1.3	
29	BL21	M9 Glucose			100 uM	1.3	
30	BL21	M9 Glucose			1 mM	1.4	
31	BL21	M9 Glucose			10 mM	1.3	
32	BL21	LB	pTet GFP 1A3 #2		0 ng/mL	559.2	
33	BL21	LB			31.25 ng/mL	545.7	
34	BL21	LB			250 ng/mL	575.1	
35	BL21	LB			500 ng/mL	612.23	
36	BL21	LB			1000 ng/mL	587.7	
37	BL21	LB			2000 ng/mL	629.4	
38	BL21	LB	pTet GFP 1A3 #1		0 ng/mL	5.5	
39	BL21	LB			31.25 ng/mL	11	
40	BL21	LB			250 ng/mL	10.1	
41	BL21	LB			500 ng/mL	9.5	
42	BL21	LB			1000 ng/mL	7.9	
43	BL21	LB			2000 ng/mL	6.7	

A question: Are the pTet GFPs co-transformed with a constitutive tetR plasmid? Looking back at the past few days' records I am unable to find out whether this is the case or not. Regardless, the pBad RFP does not seem to be inducing well, especially the 1A3 construct. It could be that this particular part (result of transformation from 160625) has failed, but the 3C5 is still having quite weak induction in M9.

Likhitha Miniprepped three WM16_024 3C5 constructs and yesterday's re-inoculation of WM16_25 3K3 (which formerly failed at the liquid growth stage after a transformation).

I set up a PCR for Gibson Assembly to make constitutive tetR (I739001) on pSB3K3.

- (I) I739001 1C3 MP3 160608; P30 P31; 70C; 0:30

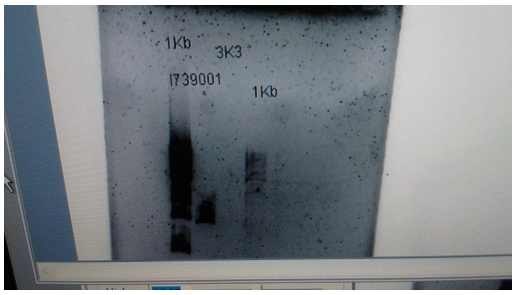
- (II) WM16_014 3K3 MP3 160620; P32 P33; 70C; 1:30

Out @ 1:40 PM
In @ 3:00 PM

I set up DpnI of the PCRs.

I also loaded the gel for the two PCRs: Ladder, I, II, Ladder.

IMG_20160628_162731614.jpg



Had to flip over the gel tray in the TAE prior to activating the gel, so I think sample flowed out of the wells. I'm going to re-run the gel after the DpnI to see if this is the case.

Adam re-imaged the gel after DpnI and the bands looked correct this time.

Adam PCR Purified the two PCRs

Adam set up a Gibson Assembly of the PCRs to create constitutive tetR on pSB3K3.

Adam Transformed the following parts:
- pBad RFP 1C3 onto BL21
- pBad RFP 1C3 onto 10 Beta
- tetR 3K3 into 5 alpha
- tetR 3K3 + pTet GFP 1_3 into BL21

Callan struck out a glycerol stock of pBad RFP 1C3 5 alpha

> *The pBad RFPs will be inoculated tomorrow in order to be induced on 160630. Before then we need to figure out how arabinose induction works! We also need to obtain Leucine and Thymine to allow the non-BL21 strains to grow in M9.*

Joe plated out the transformations of the sigma 54 parts that arrived from Orna Atar. These transformations were done last night but not plated out for lack of Kan plates. Some of the constructs were plated out last night, which had grown by now, so Joe inoculated these to make sure they can grow.

Had a big-picture meeting about the Circuit Control Toolbox. Big take-aways:
- Need to make a list of what parts are still needed, especially if we want to reverse the order of the tet component and the lac component within the modified portion of the circuit.
- Need to really determine what experiments and measurements will be needed in order to obtain sufficient empirical evidence to back up the calculator.
- Need to really think about how we are going to empirically assess the impact of IPTG / aTc concentrations on shifting the overall transfer function
- Need to think about how fluorescent reporters are bumping into each other
- A user who created a genetic circuit will need to obtain their empirical transfer function by swapping out the coding region of the last protein in their circuit with an *insulated* fluorescent protein (with RiboJ), so that we can rest assured that their empirical transfer function will carry over to our beginning to code with LacI-mCherry or tetR-mCherry.

Out @ 8:50 PM
In @ 10:30 PM

Macrogen results arrived! I assessed the sequences of everything except the ICA 3-mer attempts.

WM16_029 looks good, thankfully. We accidentally sequenced pSB3K3 with VF2 and VR (insert primers), so we gained no information on that once again. Honestly we should just assume it works since it's straight from the kit, multiple parts have been assembled onto it and grown in Kan media, and it presents a unique level of induction in concert with reporters on high-copy plasmid backbone.

Out @ 11:25 PM

iGEM DpnI Digestion

Introduction

DpnI Digestion allows you to specifically eliminate methylated DNA. This is useful becuase it allows you to eliminate template plasmid from your PCR, so you can be sure that your transformants did not take up old plasmid.

Materials

- DpnI Enzyme
- Cutsmart
- PCR Product
  - 3K3 backbone (no UNS; from WM16_014 3K3 MP3 160620)
  - constitutive tetR insert from I739001 1C3 MP3 160608

Procedure

1. 24 uL of PCR product (this is assuming you did a 25 uL PCR and ran 1 uL on a gel).

   We recommend adding other reagents directly into your PCR tube to save time and money.
2. 2.7 uL 10X cutsmart buffer
3. 0.5 uL DpnI enzyme
4. Thermal Cycler on DpnI program

   37 for 60 min, 80 for 20 min, hold at 4.

160629 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-06-30

THURSDAY, 6/30

See AJR 160629 and LK 160629 for lab notes. The gist is that we colony PCR'd and set up inoculations to test out pBad RFP inductions tomorrow (160630).

I spent the whole day prepping for / in meetings discussing the project and getting funding for the project.

I also made Leucine-supplemented M9 media with Glycerol with Likhitha using 4 mg into 400 mL.

160630 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-06-30

THURSDAY, 6/30

In @ 10:00 AM

Ethan found out that the Orna Atar strain is recA positive, which means that repetitive arrays like the decoy binding plasmids will work with (potentially severely) reduced efficiency in this strain. Need to keep this in mind when we test circuits in the strain.

Inoculations all grew from last night! These include:

- Orna Atar parts for miniprepping
- Constitutive tetR on 3K3 for minprepping
- 85x and 240x tetO arrays from addGene glycerol re-streaks for miniprep for restriction digest
- pBad RFP 1C3s on all three strains in LB for induction
- pBad RFP 1C3 on 10beta in M9 glycerol leucine

Callan and Likhitha are setting up dilutions of the pBad RFPs for measurement. 100 uL overnight culture going into 3 mL of fresh antibiotic appropriate media (1 tube per tube; each dilution tube will become 6 tubes with 500 uL each).

Incubator @ 11:15 AM

Ethan is miniprepping the Orna Atar parts and the constitutive tetR and the tetO arrays. The tetO arrays need to have sufficiently high yield to restriction digest to make smaller repeat-number arrays.

Callan is setting up glycerol stocks of the minpreps that didn't come from glycerol stocks

Joe is starting the electrocomp protocol which we received from Orna Atar for the Orna Atar strain

We received sequence data about the plasmids from Orna Atar. We can now design primers to move the parts into BioBrick backbones.

Smith Meeting:

- Good suggestions for error functions that would be appropriate to compare Hills
- Covered fast-slow dynamics
- Talked about pushing plasmid fluctuation distributions through the lacI competitive binding model

Callan inoculated the pBad RFP constructs in M9 and LB.

Likhitha is making more M9 Glycerol.

Likhitha borrowed some thiamine from Dr. Young's lab. She's making an aqueous solution that will be added into M9 glycerol to make M9 glycerol thiamine.

Out @ 7:30 PM
In @ 1:30 AM

Joe cotransformed Sigma 54 parts using the new electrocompetent LG.300:

- Helper Plasmid (pACT-Tet) + 57S (XXO (2/3 tetR binding sites in the cassette))
    - In two conditions: Undiluted miniprep and 1 ng miniprep in the electroporation

I inoculated pBad RFP 1C3 5 alpha from GS2 (160628 plate) into M9 glycerol Thiamine for Induction tomorrow

- Incubator @ 2:00 AM

Andy and I are FACSing the arabinose-inducible constructs that Callan induced 10 hours ago. The nomenclature scheme is X.Y.Z, where

- X = Sample (refer to CEM 160630):
  - 1 = Orange = WM16_029 1C3 5 alpha from GS in LB
  - 2 = Yellow = pBad RFP 1C3 5 alpha from GS2 in LB
  - 3 = Green = pBad RFP 1C3 10 beta in LB
  - 4 = Blue = pBad RFP 1C3 BL21 in LB
  - 5 = Red = pBad RFP 1C3 10beta in M9 w/ glycerol + leucine
- Y = Replicate (1-3)
- Z = Concentration:
  - 1 = 0 uM
  - 2 = 1 uM
  - 3 = 10 uM
  - 4 = 100 uM
  - 5 = 1 mM
  - 6 = 10 mM

We added 10 uL of cell culture into 500 uL of PBS for measurement.
Refer to LK 160629 for drylab findings about starin compatibility of pBad.

Table1

	A	B	C	D	E	F	G
1	Sample	Replicate	Arabinose	Mean of Peak 1	Mean of Peak 2	Mean of Peak 3	% of Plot ir Peak 1
2	WM16_029 1C3 5alpha LB	1	0 uM	2.7			
3			1 uM	7			
4			10 uM	13.2			
5			100 uM	40.5			
6			1 mM	37.4			
7			10 mM	4.5	96		
8		2	0 uM	2.7			
9			1 uM	7.6			
10			10 uM	15.5			
11			100 uM	42.9			
12			1 mM	41.2			
13			10 mM	4.7	96.6		
14		3	0 uM	2.9			
15			1 uM	7			
16			10 uM	11			
17			100 uM	37.8			
18			1 mM	42.3			
19			10 mM	90.3			
20	pBad RFP 1C3 5	1	0 uM	2.2			

20	alpha LB						
21			1 uM	2.4			
22			10 uM	2.9			
23			100 uM	3.2			
24			1 mM	5.5			
25			10 mM	9			
26	pBad RFP 1C3 10 beta in LB	1	0 uM	2.1			
27			1 uM	2.3			
28			10 uM	3.2			
29			100 uM	3.7			
30			1 mM	4.2			
31			10 mM	5.4			
32		2	0 uM	2.1			
33			1 uM	2.4			
34			10 uM	3.4			
35			100 uM	4.4			
36			1 mM	4.5			
37			10 mM	5.8			
38		3	0 uM	2.8			
39			1 uM				
40			10 uM				
41			100 uM	4.2			
42			1 mM	4.8			
43			10 mM	5.5			
44	pBad RFP 1C3 BL21 in LB	1	0 uM	2.1			
45			1 uM	2.8			
46			10 uM	6.2			
47			100 uM	9.7			
48			1 mM	9.2			
49			10 mM	8.6			
50		2	0 uM	2			
51			1 uM	2.5			
52			10 uM	6			
53			100 uM	8.5			
54			1 mM	7.6			
55			10 mM	7.4			

55			10 mM	7.4			
56		3	0 uM	2.1			
57			1 uM	2.6			
58			10 uM	6.5			
59			100 uM	11.5			
60			1 mM	12.5			
61			10 mM	10.1			
62	pBad RFP 1C3 10beta in M9 w/ glycerol + leucine	1	0 uM	2.4			
63			1 uM	2.3			
64			10 uM	2.8			
65			100 uM	8.2			
66			1 mM	25.1			
67			10 mM	24.4			
68		2	0 uM	2.2			
69			1 uM	2.4			
70			10 uM	2.9			
71			100 uM	10.4			
72			1 mM	35			
73			10 mM	26.8			
74		3	0 uM	2.2			
75			1 uM	2.2			
76			10 uM	2.8			
77			100 uM	6.8			
78			1 mM	23.8			
79			10 mM	23			

We then put the cultures back into the incubator to finish out their overnight growth to be re-measured to assess the effect of longer induction time on arabinose induction.

Given that it looks like the WM16_029 pBad tetR-RFP construct induces better than the pBad-RFP construct for some reason, Andy and I set up inoculations of the same WM16_029 1C3 5 alpha into M9 Glycerol + Thiamine.

This was done by taking 10 uL of the uninduced #1, #2, #3 culture sof WM16_029 1C3 5alpha LB culture and adding it to 4 mL M9 Glycerol + Thiamine.

Incubator @ 3:35 AM

Out @ 3:40 AM

160701 JPM

Made with Benchling

Project: iGEM 2016

Authors: Andy Halleran

Date: 2016-07-01

FRIDAY, 7/1

In @ 10:00 AM

The M9 glcyerol + thiamine inoculations I did last night (for pBad RFP 1C3 and WM16_029 1C3, both 5 alpha) are growing but not at midlog turbulence yet. Onc they get to midlog we'll go straight to arabinose induction.

Everyone is working on Dry Lab to figure out usage instances and protocols for Spinach / Broccoli Aptamers, designing primers to move Sigma 54 constructs onto BioBrick backbones, modeling, ...

Out @ 1:50 PM

In @ 3:30 PM

Likhitha induced the pBad RFP / WM16_029 5 alpha M9 glycerol thiamine cultuse that I inoculated last night, with the same arbinose molarities as usual.

Induced @ 3:00 PM-- these will be ready to FACS at 9:00 PM (ADH will do it)

Andy and I are FACSing the same pBad parts from last night, except this time after 24 hours of arabinose induction.

Table1

	A	B	C	D	E	F	G
1	Sample	Replicate	Arabinose	Mean of Peak 1	Mean of Peak 2	Mean of Peak 3	% of Plot ir Peak 1
2	WM16_029 1C3 5alpha LB	1	0 uM	10.1	10.1		
3			1 uM	13.6	13.6		
4				10 uM	20	20	
5							
6			100 uM	10.5	105.6		
7			1 mM	106	106		
8			10 mM	17.5	264.7		
9		2	0 uM	10.2	10.2		
10			1 uM	12.9	12.9		
11			10 uM	21	21		
12			100 uM	9.6	104.3		
13			1 mM	10.8	97.6		
14			10 mM	17.4	260		
15		3	0 uM				
16			1 uM	13.7	13.7		
17			10 uM	19	19		
18			100 uM	10.9	68.5		

19			1 mM	103.5	103.5		
20			10 mM	18.2	238.5		
21	pBad RFP 1C3 5 alpha LB	1	0 uM	9.4			
22				1 uM	9.6		
23							
24			10 uM	10.1			
25			100 uM	10			
26			1 mM	17.8			
27			10 mM	52.1			
28	pBad RFP 1C3 10 beta in LB	1	0 uM	9.9			
29			1 uM	10.7			
30				10 uM	12.5		
31							
32			100 uM	13.7			
33			1 mM	13.8			
34			10 mM	21			
35		2	0 uM	9.5			
36			1 uM	10.3			
37			10 uM	12.9			
38			100 uM	15.1			
39			1 mM	15.7			
40			10 mM	24.4			
41		3	0 uM				
42			1 uM	10			
43			10 uM	10.7			
44			100 uM	10.8			
45			1 mM	17			
46			10 mM	20.8			
47	pBad RFP 1C3 BL21 in LB	1	0 uM	9.4			
48			1 uM	12.5			
49				10 uM	22.4		
50							
51			100 uM	31.2			
52			1 mM	32.4			
			10 mM	32.5			

53			10 mM	32.5			
54		2	0 uM	9.9			
55			1 uM	10.9			
56			10 uM	20.2			
57			100 uM	26.5			
58			1 mM	24.9			
59			10 mM	28.9			
60		3	0 uM	9.5	9.5		
61			1 uM	9.6	9.6		
62			10 uM	10.5	10.5		
63			100 uM	9.6	38.2		
64			1 mM	9.6	40.3		
65			10 mM	10.1	34.2		
66	pBad RFP 1C3 10beta in M9 w/ glycerol + leucine	1	0 uM	10.8	10.8		
67			1 uM	11.1	11.1		
68				10 uM	19	19	
69							
70			100 uM	32.1	32.1		
71			1 mM	77.6	77.6		
72			10 mM	52	180		
73		2	0 uM	10.6	10.6		
74			1 uM	11.2	11.2		
75			10 uM	15.7	15.7		
76			100 uM	35.1	35.1		
77			1 mM	87.3	87.3		
78			10 mM	46.3	183.5		
79		3	0 uM	10	10		
80			1 uM	10.3	10.3		
81			10 uM	12.5	12.5		
82			100 uM	24.7	24.7		
83			1 mM	69.5	69.5		
84			10 mM	48.9	176.8		

Given that the Arabinose curves don't look like they're leveling off by 10 mM, we should probably try a 100 mM arabinose induction condition. We are probably currently underestimating the possible fold change in our pBad constructs.

Adam inoculated the 57S + pACT-Tet cotransformation.

Likhitha made Amp Chlor Kan Plates.

Joe and Christine are cotransforming the following parts to test (1) Decoy Binding Array, and (2) aTc Induction. Both of these things have not been tested rigorously so far.

- Decoy Binding Array (We have obtained a good IPTG induction curve on this 14/16 combination in BL21 before (160626), so we are going to apply the lacI plasmid from addgene (which presumably has 256 lacO sites) in order to determine if the induction curve will shift):
  - WM16_014 1C3 MP1 160605 + WM16_016 3K3 MP1 160620 + lacI-sce1-tetO (amp) MP3 160619 into BL21
  - WM16-014 1C3 MP1 160605 + WM16_016 3K3 MP1 160620 into BL21
- aTc Induction (into each of 5 alpha, BL21, and 10 beta, do:)
  - pTet GFP 1C3 160615 MP 1 + tetR 3K3 160630 MP 1
  - pTet GFP 1C3 160615 MP 1 + tetR 3K3 160630 MP 2
  - pTet GFP 1C3 160615 MP 1 + tetR 3K3 160630 MP 3

  (for a total of 9 cotransformations. These tetR constructs have not been sequence confirmed so we are trying all three minipreps for tetR 3K3, since the triplicate can be fit in one tube of chem. comp. cells and so we are not wasting much in terms of expensive consumables by doing so).

Joe and Christine added 25 uL cells for the Decoy Binding Array transformations, and 15 uL of cells each for the aTc cotransformations. They also used 1 uL of undiluted minprep for DNA amounts.
See JLM 160701 for the Transformation Key.

Adam and Callan are streaking from Glycerol Stock all stocks of sequence-confirmed reporter constructs from the Ribozyme project (WM16_014, 015, 024, 025, 027, 028, 030, 031) on 1A3 so that they can be inoculated tomorrow for solo FACS. This will lead to a final and conclusive determination of which stock is the representative reporter construct on 1A3.

We are doing this because (1) there has not yet been a conclusive solo FACS test of the fluorescence of all 8 ribozyme reporters, and (2) sequence-confirmed parts have been exhibiting behavior that suggests part invalidity, such as not fluorescing or not growing under correct antibiotic pressure. This suggests mix-ups etc. have happened in between the confirmed minprep / glycerol stock and these assays.

As such we are going to run a conclusive test of part validity, and follow these up with cotransformations with WM16_016 3K3 to get IPTG induction curves in arbitrary units.

Callan and Ethan designed primers to move the Sigma 54 constructs onto a BioBrick backbone. These need to be reviewed.

Out @ 8:30 PM

160703 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-07-03

SUNDAY, 7/3

In @ 10:30 PM

Talked with Andy about progress over the weekend.

Me and Andy are FACSing the aTc inductions of the pTet GFP + tetR cotransformations (#1 - 23 on the 160703 JLM key).

Table1

	A	B	C	D	E	F	G
1	Sample	Strain	[aTc] (ng/mL)	Peak 1 Mean	Peak 2 Mean	Peak 3 Mean	Peak 1 %
2	1	10B	0	18.65			
3		10B	1	22.3			
4		10B	5	37.2			
5		10B	10	54.2			
6		10B	50	172.3			
7		10B	100	364.6			
8	2	10B	0	9.6			
9		10B	1	12.1			
10		10B	5	20.1			
11		10B	10	28.1			
12		10B	50	108.6			
13		10B	100	245.8			
14	3	10B	0	6.6	330.8		
15		10B	1	7.6	315.9		
16		10B	5	6.7	340		
17		10B	10	6.9	319.8		
18		10B	50	7.1	314.2		
19		10B	100	6.9	306.2		
20	4	10B	0	3.1	250.2	1781.6	
21		10B	1	3.1	265.4	1960.8	
22		10B	5	3	260.6	1940	
23		10B	10	same as above			
24		10B	50	same as above			
25		10B	100	same as above			

26	5	10B	0	same as above			
27		10B	1	same as above			
28		10B	5				
29		10B	10				
30		10B	50				
31		10B	100				
32	6	10B	0	3.9			
33		10B	1	4.1			
34		10B	5	4.1			
35		10B	10	4.8			
36		10B	50	4.8			
37		10B	100	4.5			
38	7	10B	0	5			
39		10B	1	5.3			
40		10B	5	4.8			
41		10B	10	4.8			
42		10B	50	5.4			
43		10B	100	5.3			
44	8	10B	0	7.3	390.2		
45		10B	1	7.2	384.2		
46		10B	5	same as above			
47		10B	10	same as above			
48		10B	50	same as above			
49		10B	100				
50	9	5 alpha	0	8.6			
51		5 alpha	1	9.1			
52		5 alpha	5	13.1			
53		5 alpha	10	14.5			
54		5 alpha	50	28.6			
55		5 alpha	100	35			
56	10	5 alpha	0	7.7			
57		5 alpha	1	11.6			
58		5 alpha	5	14.2			
59		5 alpha	10	16.9			
60		5 alpha	50	32.2			
61		5 alpha	100	45.8			
	11	5 alpha	0	7			

62	11	5 alpha	0	7			
63		5 alpha	1	9.8			
64		5 alpha	5	11			
65		5 alpha	10	12.6			
66		5 alpha	50	20.7			
67		5 alpha	100	33.7			
68	12	5 alpha	0	low peak and lots of noise above			
69		5 alpha	1	same as above			
70		5 alpha	5	same as above			
71		5 alpha	10	same as above			
72		5 alpha	50	same as above			
73		5 alpha	100	same as above			
74	13	5 alpha	0	two low peaks and a short spike very high			
75		5 alpha	1	same as above			
76		5 alpha	5	same as above			
77		5 alpha	10	same as above			
78		5 alpha	50	same as above			
79		5 alpha	100	same as above			
80	14	5 alpha	0	low peak and a high spike at 10^4			
81		5 alpha	1	same as above			
82		5 alpha	5	same as above			
83		5 alpha	10	same as above			
84		5 alpha	50	same as above			
85		5 alpha	100	same as above			
86	15	5 alpha	0	low peak and a high spike at 10^4			
87		5 alpha	1	same as above			
88		5 alpha	5	same as above			
89		5 alpha	10	same as above			
90		5 alpha	50	same as above			
91		5 alpha	100	same as above			
92	16	5 alpha	0	low peak and lots of noise and a high spike at			

				10^4			
93		5 alpha	1	same as above			
94		5 alpha	5	same as above			
95		5 alpha	10	same as above			
96		5 alpha	50	same as above			
97		5 alpha	100	same as above			
98	17	BL21	0	4.5			
99		BL21	1	4.9			
100		BL21	5	5.4			
101		BL21	10	5.2			
102		BL21	50	5.4			
103		BL21	100	6.6			
104	18	BL21	0	3.7			
105		BL21	1	4.5			
106		BL21	5	4.9			
107		BL21	10	5.5			
108		BL21	50	5.8			
109		BL21	100	7.3			
110	19	BL21	0	4.5			
111		BL21	1	4.2			
112		BL21	5	5.5			
113		BL21	10	5.7			
114		BL21	50	5.5			
115		BL21	100	7.7			
116	20	BL21	0	low peak and slight noise higher throughout			
117		BL21	1	same as above			
118		BL21	5	same as above			
119		BL21	10	same as above			
120		BL21	50	same as above			
121		BL21	100	same as above			
122	21	BL21	0	12.9			
123		BL21	1	same as above			
124		BL21	5	same as above			
125		BL21	10	same as above			
126		BL21	50	same as above			

126		BL21	50	same as above			
127		BL21	100	same as above			
128	22	BL21	0	7.5			
129		BL21	1	same as above			
130		BL21	5	same as above			
131		BL21	10	same as above			
132		BL21	50	same as above			
133		BL21	100	same as above			
134	23	BL21	0	low peak and slight noise higher throughout			
135		BL21	1	same as above			
136		BL21	5	same as above			
137		BL21	10	same as above			
138		BL21	50	same as above			
139		BL21	100	same as above			

It seems that the only minprep of tetR 3K3 from 160630 that functionally works is the MP1. Furthermore, best induction happens in strain 10 beta (~30 fold), lackluster induction happens in strain 5 alpha (~3 fold), and hardly any induction happens in strain BL21 with this functional construct set.

Andy and I are checking the primers that Likhitha designed to move the Sigma-54 parts (the actual enhancer plasmid and the helper plasmid) onto BioBrick backbones. Some optimization needed to be done but it was mostly conceptually sound.

Andy designed WM16_034, which is the basic part for characterization of the promoters with and without RiboJ. Note that the Spinach sequence contains a SpeI cut site, but we will eventually replace this with Broccoli so it will be Okay.

Andy and I ordered WM16_034 and WM16_P040 - P045.

Tomorrow we should transform working IPTG and Tet induction constructs into the 3.300 strain to assess induction potential there.

We should also transform known-to-work constructs into Joe's new electrocomp cells to assess their efficacy.

Out @ 2:45 AM

160704 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-07-04

MONDAY, 7/4

In @ 10:00 AM

Joe, Ethan, Andy and I talked through the gels of the Restriction Digest cut (JLM 160702). EcorI doesn't cut on the 240x plasmid, but we don't know yet which of EcorI or Cla1 is cutting on the 85x.
Joe and Ethan will re-run individual digests for all enzymes, on all minipreps of both 240x and 85x, to diagnose cuttability on each plasmid.

Callan, Likhitha, and Christine are Minprepping the resuspended promoter constructs that were transformed on 160702.

Andy and I went through the results of the 160703 solo FACS characterization of the Ribozyme parts. The strong-RBS versions seem okay, except for the fact that 26 is too low to seem believable. Also, we went ahead and accepted constructs for 15, 25, 27, and 31 despite the fact that the fluorescence values are awfully low.. however the decreasing trend (with RiboJ removal and/or switching to pTac) seems to be preserved (although it's hard to tell what with the different gains and all). Table below reproduced from ADH 160703 (except for the first column; with Green indicating "best" construct):

Table2

	A	B	C	D	E	F
1	160704 Sequence Analysis	Sample	Code	FL1 Gain	Peak 1 Flx	Peak 2 Fl
2	Sequence doesn't begin until bp 140, which is a little later than most P008 sequencings. Forward cuts out around bp 800, and the reverse hardly matches anything.	W16_027 AMP GS3 160614	1-1	660	3.4	
3	*Need sequence file (not on Dropbox)*	WM16_027 AMP GS1 160618	1-2	660	40	
4	*Need sequence file (not on Dropbox)*	WM16_027 AMP GS2 160618	1-3	660	41.2	
5	*Need sequence file (not on Dropbox)*	WM16_027 AMP GS3 160618	1-4	660	35	
6	Completely confirmed	WM16_015 AMP GS1 160608	2-1	620	13.5	
7	Completely confirmed	WM16_015 AMP GS3 160608	2-2	620	14.2	
8	Completely confirmed	WM16_026 AMP GS1 160609	3-1	620	25.3	
9	Completely confirmed	WM16_026 AMP GS2 160609	3-2	620	22.9	
10	Completely confirmed	WM16_026 AMP GS3 160609	3-3	620	20.7	
11	bps 649-873 in the construct (end of cI, beginning of sfGFP) were not reached by the sequence; otherwise confirmed.	WM16_031 AMP GS1 160614	4-1	660	2.3	

12	bps 491-627 in the construct (middle of cI) were not reached by the sequence; otherwise confirmed	WM16_031 AMP GS2 160614	4-2	660	2.6	
13	completely confirmed	WM16_030 CHLOR GS1 160614	5-1	460	1.04	
14	completely confirmed	WM16_030 CHLOR GS3 160614	5-2	460	1.8	
15	Completely confirmed	WM16_024 AMP GS2 160609	6-1	460	42.9	
16	completely confirmed	WM16_024 AMP GS3 160609	6-2	460	1	
17	completely confirmed	WM16_025 AMP GS2 160614	7-1	560	2.4	
18	completely confirmed	WM16_025 AMP GS3 160614	7-2	560	1.4	
19	completely confirmed	WM16_014 AMP GS1 160603	8-1	420	1	
20	completely confirmed	WM16_014 AMP GS2 160603	8-2	420	1	

The minipreps themselves are, as they were initially, very well-confirmed. Andy thinks some of the issues (especially with the trimodal distributions on some of the parts) couuld be arising from multiple regrowths leading to selective pressure towards lessening the fluorescence / tossing the plasmid etc.

I then went through some of the "non-functional" stocks' minpreps' sequences to see if the sequence is *actually* confirmed or what. This info is in the first column of the above table.

Joe set up transformations of the following:

- IPTG induction parts (WM16_014, WM16_016) into strain 3.300 to make sure induction can happen
- aTc induction parts (pTet GFP, tetR) into strain 3.300 to make sure induction can happen
- 57S + pACT-Tet into 3.300 retry to get more colonies to inoculate
- WM16_014 + WM16_016 + addGene lacI+tetO array (all three MPs) into BL21 to get a transfer function shift to happen

Table reproduced from JLM 160704:

Table1

	A	B	C	D	E
1	KEy	Strain	PLas 1	Plas 2	Plas 3
2	1	LG3.300	14 1C3 MP1 160605	16 3K3 MP1 160620	
3	2	LG3.300	pTet GFP 1C3 MP1 160615	TetR 3K3 MP1 160630	
4	3	BL21	14 1C3 MP1 160605	16 3K3 MP1 160620	LacI Spe TetO AMP 160619 MP1
5	4	BL21	14 1C3 MP1 160605	16 3K3 MP1 160620	LacI Spe TetO AMP 160619 MP2
6	5	BL21	14 1C3 MP1 160605	16 3K3 MP1 160620	LacI Spe TetO AMP 160619 MP3
7	6	LG3.300	57S OA Kan MP1 160630	pACT TET AMP MP1 160630	

Ethan got restriction cuts on the 85x tetO array to work (the sites which flank the entire array). The cuts on the interior sites did not work... this means we won't be able to make the smaller subset arrays, but the 85x should be movable into a BioBrick backbone! He'll do the process tomorrow.

Out @ 6:15 PM
In @ 9:45 PM

Andy and I designed primers for swapping promoters into WM16_034. Thee primers need to be made for each promoter we want to characterize with and without RiboJ. Also inclued were primers to try and PCR up an Anderson promoter which we don't have, by coding for the promoter sequence on single-stranded ultramers with overhangs to the appropriate regions of WM16_034. We also designed RBS primers with this same strategy, but swapping into WM16_014.

We ordered them. The RBS primers that work with WM16_014 were ordered Same Day, but the others waiting on the WM16_034 geneBlock were ordered with standard shipping to save money.

Out @ 10:30 PM

160705 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-07-05

TUESDAY, 7/5

In @ 10:30 AM

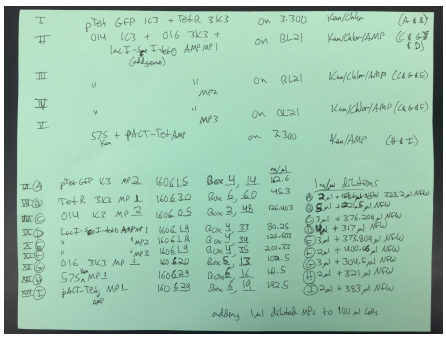
Only the 14+16 transformation into 3.300 grew on plate, unfortunately. People are colony PCRing this one transformation to inoculate. Recap:

Table1

	A	B	C	D	E	F
1	KEy	Strain	PLas 1	Plas 2	Plas 3	**Grew 160705?**
2	1	LG3.300	14 1C3 MP1 160605	16 3K3 MP1 160620		**Yes**
3	2	LG3.300	pTet GFP 1C3 MP1 160615	TetR 3K3 MP1 160630		**No**
4	3	BL21	14 1C3 MP1 160605	16 3K3 MP1 160620	LacI Spe TetO AMP 160619 MP1	**No**
5	4	BL21	14 1C3 MP1 160605	16 3K3 MP1 160620	LacI Spe TetO AMP 160619 MP2	**No**
6	5	BL21	14 1C3 MP1 160605	16 3K3 MP1 160620	LacI Spe TetO AMP 160619 MP3	**No**
7	6	LG3.300	57S OA Kan MP1 160630	pACT TET AMP MP1 160630		**No**

Joe redid the trasnformations, including control conditions with single-transformations. 160705 JLM (https://benchling.com/wmigem/f/uDbWaeJV-jlm/etr-yXzpd9GE-160705-jlm/edit)

IMG_2686.JPG



All single-transformation went into electrocompetent BL21 made by us.

Jmitch successfully numerically solved the ODE system for the lacI+lacO+IPTG system for various concentrations of lacI_total and IPTG_total. The transfer function shifts to the right with increasing IPTG_total, as expected. This is all at steady state.

I built up a SSA implementation of the lacI+lacO+IPTG system.

Ethan and Joe worked on cloning the 85x tetO array into the 1C3 backbone using restriction digest. They got up to the ligation step.

160706 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-07-06

WEDNESDAY, 7/6

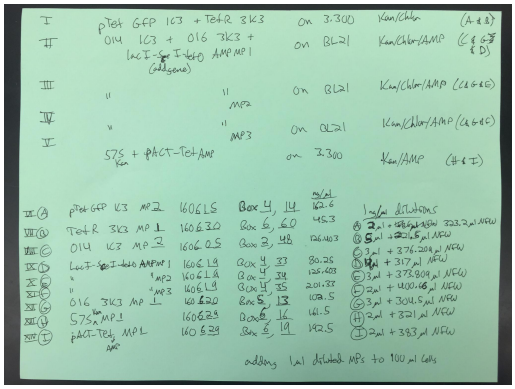
In @ 10:00 AM

Only three plates from last night's transformations grew. They were:

- VII: tetR 3K3 MP1 160630 into BL21
- XII: WM16_016 3K3 MP1 160620 into BL21
- XIII: 57S (kan) MP1 160629 into BL21

Recall that the transformations were

IMG_2686.JPG



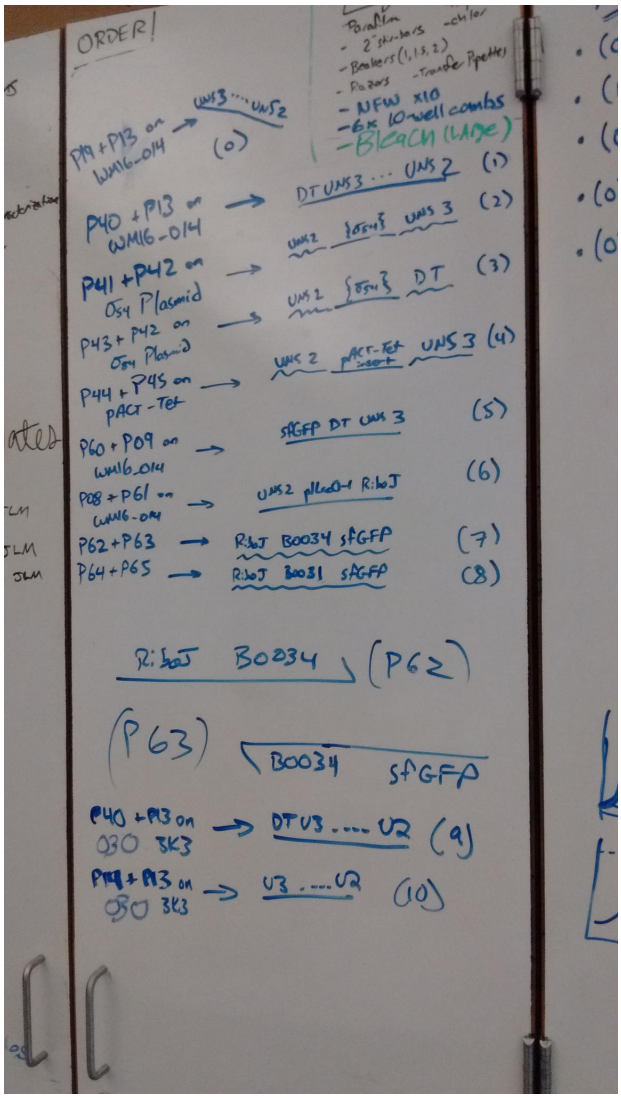
All single-transformations went into electrocompetent BL21 made by us.

Many of these controls have been successfully transformed into BL21 before (14, 16, pTet, tetR, etc.). This sugWMgests that the electro-transformation procedure using the BL21 cells is much more inefficient, either due to the quality of the electrocomp. cells or the procedure itself.
While we could try and remedy this by using chemical procedure for triple-transformation, this would likely also be pretty low efficiency... but might still be worth a shot. Chemical transformation is not an option for 3.300 because they came incompetent and we only received a protocol for making them electrocompetent.
One thing to note is that the recommended voltage for electrically transforming DNA into the 3.300 cells is higher than the voltage allowed on Dr. Young's electroporator (by decree, not design), which we are currently using.

Primers arrived! WM16_P045-P065. P066 was never ordered, it seems (P060 was ordered twice instead), so we need to do that.

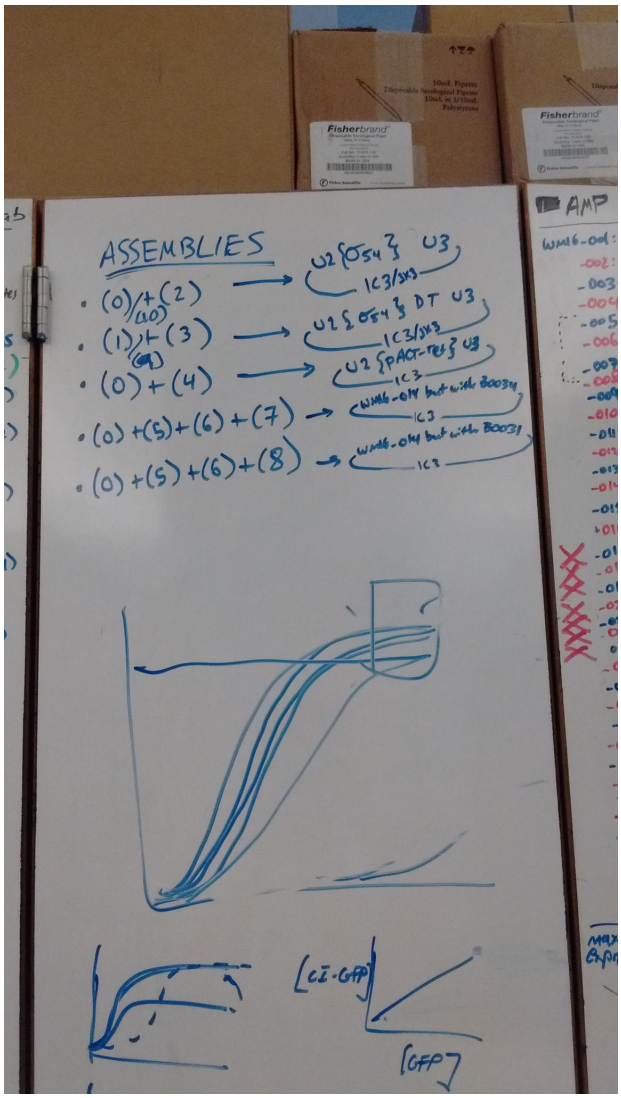
Callan and Adam are setting up PCRs to move sigma 54 parts into the BioBrick backbone, as well as to swap out the RBS in WM16_014 with either B0034 or B0031. The assembly concepts are:

160706_assembly_pcrs.jpg



For actual templates and PCR parameters see 160706 CEM (https://benchling.com/wmigem/f/mnsihkXQ-cem/etr-Ba6DvJhz-160706-cem/edit)

160706_assemblies.jpg



Out @ 1:05 PM
In @ 1:40 PM

One of the thermal cyclers (containing PCRs 5 and 6 (57S amplifications with/without DT)) was left paused at 98C for the past hour. Adam is re-doing those two PCRs.

It looks like Thermal Cycler #5 will tend to pause on its own.

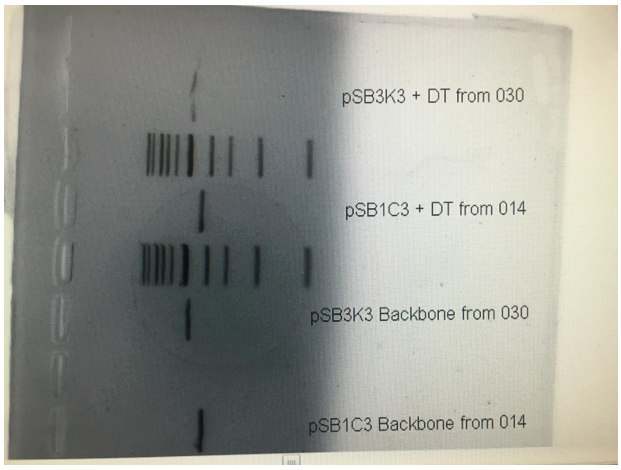
Adam set up DpnI of the nine PCRs which don't include #5 and 6.

Likhitha is making plates (AmpChlorKan)

Callan is making 50x TAE.

Here are the gels from the PCRs (except for #5,6):

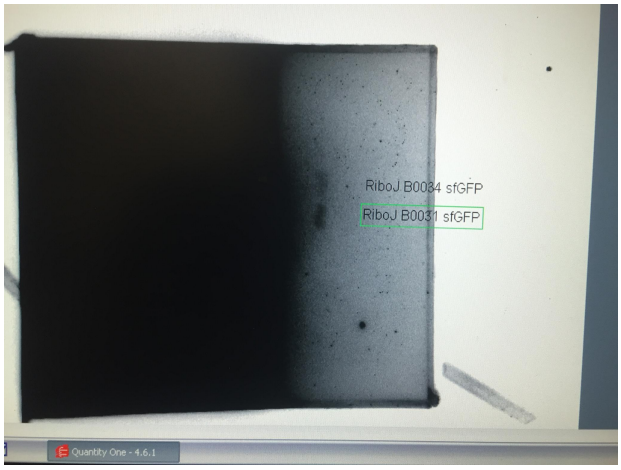
IMG_7961.JPG



IMG_7962.JPG

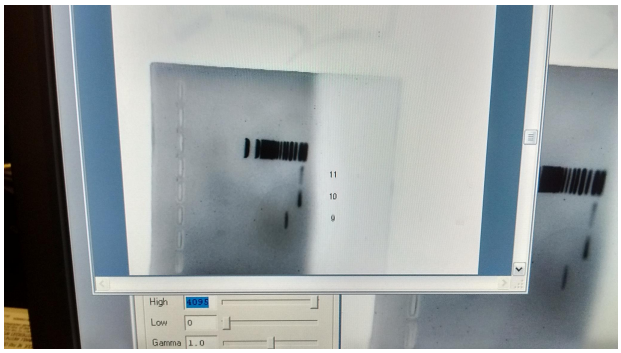
The B0031 and B0034 bands are present (see next picture)

IMG_7963.JPG



But I neglected to run them with 50bp ladder. So we'll have to re-run it after PCR Purification to make sure it's ok.

20160706_91011.jpg



11 = RiboJ B0031 sfGFP (64bp)
10 = RiboJ B0034 sfGFP (64bp)
9 = UNS2 plLacO-1 (199bp)

=> it all looks good, assuming a gel shift is occurring between the ladder and the #9.

Adam is setting up PCR Purifications of the DpnI'd constructs. He also ran the gel of PCRs #5,6 and started DpnI of those. He then PCR Purified 5 and 6.

I told Adam to elute the RBS primer-primer PCRs in 6 uL volume and the smaller 14 constructs in 10 uL volume (everything is within the recommended 5-20 uL elution range of the Monarch PCR Purification Kit) because the small size of these parts will require a high concentration for the Gibson.

Ethan found out that the restriction digest cloning of the 85x tetO array into pSB1C3 was done using the EcorI and XbaI cut sites, which are inside the prefix... hence if the ligation works, then we will have an 85x tetO array followed by UNS2 followed by WM16_014 insert, UNS3, Suffix.

However, he also found that a SpeI cut site exists directly outside of the XbaI cut site in the 85x tetO array, and SpeI is a cut site in the Suffix. This means that we should be able to do the same digestion process using EcorI and SpeI to move 85x tetO array into pSB1C3.

This makes today's restriction digest just a 'test-run' of restriction digesting. We can still colony PCR it using VF2 and UNS2 Reverse tomorrow to check if it worked.

John Mitchell is working on saving a vector of ODE system solution evaluated at specific values of "model time" rather than "vector index", so that we can create composite plots of [free pLac] w.r.t. [lacI total] and [IPTG total] at steady state *and* at any time point (of model time) that we want to specify (in increments of 0.01 seconds).

Adam and Callan are setting up Gibson reactions from the earlier PCRs. I am having them proceed with the WM16_014 RBS swap Gibsons despite the fact that the UNS2-plLacO1 construct did not appear on the gel, because its size (~300bp) puts it right where the EtBr front would be (hence would be obscured in either image).

We are going to re-run the PCR purfications of the B0034, B0031, and UNS2-plLacO1 PCRs on gels using leftover volume after the Gibson reactions, using 50bp ladder this time.

Gibson reactions are

Table1

	A	B	C	D	E	F
1	20160706 Gibsons		*pmol*	*length (bp)*	*concentration (ng/uL)*	***You need this many uL:***
2	**backbone**	**WM16_014 1C3 P19 P13**	**0.06**	**2150**	**85.4**	**0.9969555035**
3	insert	57S P41 P42	0.16	2669	115.4	2.442343154
4	**backbone**	**WM16_030 3K3 P19 P13**	**0.06**	**2830**	**72.2**	**1.552188366**
5	insert	57S P41 P42	0.18	2669	115.4	2.747636049
6	**backbone**	**WM16_014 1C3 P40 P13**	**0.05**	**2279**	**109.5**	**0.6868219178**
7	insert	57S P43 P42	0.15	2798	76.6	3.616214099
8	**backbone**	**WM16_030 3K3 P40 P13**	**0.045**	**2959**	**55.4**	**1.586323105**
9	insert	57S P43 P42	0.135	2798	76.6	3.254592689
10	**backbone**	**WM16_014 1C3 P19 P13**	**0.06**	**2150**	**85.4**	**0.9969555035**
11	insert	pACT-Tet P44 P45	0.18	3005	220.1	1.621962744
12	**backbone**	**WM16_014 1C3 P19 P13**	**0.12**	**2150**	**85.4**	**1.993911007**
13	insert	WM16_014 P60 P09	0.12	760	195.1	0.3085187084
14	insert	WM16_014 P08 P61	0.12	309	37.2	0.6578709677
15	insert	P62 P63	0.12	64	41.8	0.1212631579
16	insert	Example Additional Insert	0	0	1	0
17		***TOTAL MOLES (want .01 - .25 pmol for 1-2 Fragments, and .1 - .5 pmol for 3+ fragments) :***	0.48		***TOTAL FRAGMENT VOLUME (cannot exceed 5 uL) :***	3.081563841
18	**backbone**	**WM16_014 1C3 P19 P13**	**0.12**	**2150**	**85.4**	**1.993911007**
19	insert	WM16_014 P60 P09	0.12	760	195.1	0.3085187084
20	insert	WM16_014 P08 P61	0.12	309	37.2	0.6578709677
21	insert	P64 P65	0.12	64	34.6	0.1464971098
22	insert	Example Additional Insert	0	0	1	0
23		***TOTAL MOLES (want .01 - .25 pmol for 1-2 Fragments, and .1 - .5 pmol for 3+ fragments) :***	0.48		***TOTAL FRAGMENT VOLUME (cannot exceed 5 uL) :***	3.106797793

It turns out that WM16_014 P08 P61 is supposed to be 199bp.

Adam and Callan are setting up Transformations of the Gibsons into 5 alpha. For specs and key, see 160706 AJR (https://benchling.com/wmigem/f/jZYrfUIA-ajr/etr-EDpgvkSs-160706-ajr/edit)

160707 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-07

THURSDAY, 7/7

In @ 10:00 AM

All of last night's transformations grew! Colony PCRs looked interesting, but at least 3 colonies from each transformation worked. See 20160707 LK (https://benchling.com/wmigem/f/aY4Q6dee-lk/etr-HRS4ijIw-20160707/edit)

The big news is that **the restriction digest of the 85x tetO array into the Prefix of WM16_014 on pSB1C3 successfully worked!** We had previously tried this via Gibson Assembly into a UNS backbone with P038 P039, but the initial PCR was smeary and the Gibsons products were variable in length (none of which exceeded 1kb (remember that the 85x tetO array is ~3kb)).

Ethan is saying that, however, even the modified Restriction Digest (using SpeI) that will put the array between the Prefix and Suffix will introduce a scar that makes the part non-compatible with BioBrick standards... This would be a problem since the whole point is to make the thing submittable. If this is the case, we might want to try going back to the Gibson method...

These colony PCRs were all inoculated, see 20160707 LK (https://benchling.com/wmigem/f/aY4Q6dee-lk/etr-HRS4ijIw-20160707/edit)

IDT primers arrived; we now have everything up to WM16_P065. I ordered WM16_P066.

We sent out WM16_031 and WM16_028 (both on 1C3) to Lidia to sequence confirm.

We transformed WM16_014 + WM16_016 + addgene lacI array, once again, into BL21 (chemically competent) as a re-try. See 160707 AJR (https://benchling.com/wmigem/f/jZYrfUIA-ajr/etr-V74STHzY-160707-ajr/edit)

We also assembled and transformed the pACT-Tet onto 3K3 and the sigma 54 plasmid onto 3C5, for testing purposes in case the higher-copy version doesn't properly display the staircase induction curve. See 160707 AJR (https://benchling.com/wmigem/f/jZYrfUIA-ajr/etr-V74STHzY-160707-ajr/edit)

We also did the proper restriction digest of 85x tetO array into pSB1C3 today. This has been transformed; we also did an additional overnight ligation (started today) just in case the colony PCRs for the non-overnight ligation look bad tomorrow. See 160707-EMJ (https://benchling.com/wmigem/f/yMfgp3Ra-emj/etr-I9PPZq4t-160707-emj/edit)

We also re-struck out Glycerol stocks of MPs that were running low (WM16_014, WM16_016, and addgene lacI that were used in the triple transformation). See 20160707 LK (https://benchling.com/wmigem/f/aY4Q6dee-lk/etr-HRS4ijIw-20160707/edit)

It turns out that LG3.300 cells can be made chemically competent. Joe will do this tomorrow.

Andy and Ethan worked out that we will be able to fit all of our Toolbox constructs (not including the Binding Arrays) onto a single plasmid with a total insert length of only ~5kb. We should hopefully be able to account for copy number difference by using variable RBS strengths.

Had a good meeting with Dr. Smith.

- *Created an Overleaf document to globally document all important modeling results (nobody knows LaTeX so I will have to teach them tomorrow)*
- *Learned the proper way to deal with k+ and k- when all you know is a K_D or a K_A. (assume some physicially realistic value for k+ and calculate k- using the known K value). Had JMitch re-scale the parameter values in the ODE system accordingly.*

- *JMitch's assignment: apply a protein production model on top of [free pLac] and plot this protein out ont eh z axis of a [lacI total] and [IPTG total] 3D plot.*
- *Kalen's assignment: stop working on the Ribozyme modeling question for now, and focus on finding analytical solutions to the 3-reaction competitive binding model for lacI for [free pLac]_{ss}, and also [protein produced by pLac]_{ss}. Use these solutions to gain insights about what terms/parameter clusters are driving desirable properties of the steady-state solution*
- *My assignment: stop work on the SSA for now and focus on making sure the transition to the protein production model is smooth for everyone. This bit will involve the rapid buffering approximation that Dr. Smith showed us.*

Out @ 7:30 PM
In @ 8:40 PM

Worked on setting up a crowdfunding page at Experiment. Definitely this is up Joe's alley-- will delegate to him tomorrow.

Out @ 10:30 PM

160708 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-08

FRIDAY, 7/8

In @ 10:00 AM

Broccoli arrived from addGene! We're going to try and do a Colony PCR on it to see if we can assemble that into UNS backbone from the get-go. We're also streaking it out onto a plate for real colony PCR tomorrow.

Joe is making chemically competent LG3.300 cells. See 160708 JLM (https://benchling.com/wmigem/f/uDbWaeJV-jlm/etr-6bsAquB4-160708-jlm/edit)

Likhitha is writing up a profile for our project on the scientific crowdfunding platform Experiment.

Adam is minprepping inoculations of the 160706 assemblies (sigma 54 parts onto BioBrick backbones, and two RBS swaps (B0034 and B0031) into WM16_014 1C3). We are not carrying through the modified WM16_014 parts into measurement steps because the FACS machine computer is still broken. See 160708 AJR (https://benchling.com/wmigem/f/jZYrfUIA-ajr/etr-jBbWVMjo-160708-ajr/edit)

All the transformations except for the triple transformation worked! Callan is Colony PCRing them. See 160608 CEM (https://benchling.com/wmigem/f/mnsihkXQ-cem/etr-3W2O3rZ5-160608-cem/edit)

> *Something needs to be done about the triple transformation not working. We have tried chem comp and electrocomp (BL21) twice each, and this last chem comp run used 5 uL of undiluted MP solution for each plasmid. 14+16 cotransformations routinely work chemically in this strain, and the addGene plasmid was transfomred successfully into 5alpha (chemically) so the antibiotic resistance should be intact and correct.*
> *Perhaps there's some interaction between someting on the addGene plasmid and the genotype of BL21? Seems unlikely, but we haven't tried a 5 alpha chemical triple transformation. We might just end up having to wait until we get our own electroporator.*

Spherotech's absolute fluorescence beads arrived from addGene!

Andy and I talked through the transition between the empirical transfer function that a Calculator User will measure (using RiboJ-insulated GFP as their reporter to measure the circuit's current transfer function) and the user-built circuit driving expression of RiboJ-insulated lacI that we will include as our modification.

- Since transfer functions' measurements occur at steady state, their expression values are given by PRODUCTION/DEGRADATION, where the former PRODUCTION term is a function of both transcription rates and translation rates. The insulation by RiboJ supposedly eliminates any promoter influence in the translation term so we can assume that the steady-state value is given by (1) the mRNA concentration, (2) the translation efficiency of the transcript, and (3) the degradation rate of the protein. Since we are not changing the n-1 th promoter in their circuit, (1) should remain the same between their empirical function and our lacI-driving modification. If we assume that cell division is the dominant term in setting the degradation rates of these proteins (as they are not degradation-tagged), then (3) is taken care of. This leaves (2) as the only variable element between the two functions.
- If we experimentally measure pBad driving GFP vs pBad driving lacI-RFP (with the same RBSs!), then we will be able to determine the ratio of translational efficiency between lacI-RFP and GFP. Fixing the RBS should help support the assumption that this ratio is the protein-intrinsic ratio of translational efficiency between these two proteins.
  - This experiment should probably be done with different choices of fixed RBS to evaluate the validity of the intrinsic-ness assumption
- Once we know this ratio, we will be able to convert the empirically-measured [input] vs. [insulated GFP] transfer function to an [input] vs. [insulated lacI-RFP] transfer function by scaling the empirical function accordingly. We will then be able to feed this scaled empirical function through our Toolbox elements to predict the reality of what is happening inside the cell.

- The final issue is that the actual implementation of our toolbox will use lacI rather than lacI-RFP, and we don't have a good way to evaluate the validity of assuming that the lacI-RFP:GFP ratio is approximately equal to the lacI:GFP ratio. This part still needs some thought... the inelegant solution is to use lacI-(disabled XFP) in our real Toolbox rather than lacI, but that has problems of its own.

Out @ 12:40 PM
In @ 1:50 PM

I talked to my high school Bio teacher, Dr. Walck, about what sort of things she would like to see in a learnsynbio.org. Highlights include:

- Preferable length for a video / module would be 20-30 mins. if it is to be used in-class.
- She wouldn't really care whether the site advertised itself as complying with curricular standards, because she just looks for educational materials based on quality and supplements / reinforces her teaching around said materials to meet curricular requirements anyway.
- She loves the idea of the worksheets following the Khan Academy style videos-- got to integrate the learned information into critical thinking scenarios and problems!
- Some suggestions for advertising the site once it launches would be to contact local cities' Science Coordinators and to advertise with listservs etc. for organizations like VAST (VA Association of Science Teachers)

Working on the crowdfunding page on the platform Experiment.

Adam re-did the Colony PCRs on the 85x tetO array using P30 and P31, but no bands appeared on the gel. See 160708 AJR (https://benchling.com/wmigem/f/jZYrfUIA-ajr/etr-jBbWVMjo-160708-ajr/edit)

This is in agreement with Ethan's assessment of the scarring in the Prefix

Likhitha inoculated the colony PCRs. 20160708 LK (https://benchling.com/wmigem/f/aY4Q6dee-lk/etr-FVa4yAQ8-20160708-lk/edit)

Out @ 7:30 PM
In @ 8:45 PM

Joe and Ethan are transforming sigma 54 parts, IPTG inducible construct, and aTc inducible construct into chemically competent 3.300. See 160708-EMJ (https://benchling.com/wmigem/f/yMfgp3Ra-emj/etr-wEDfSICL-160708-emj/edit) 160708 JLM (https://benchling.com/wmigem/f/uDbWaeJV-jlm/etr-6bsAquB4-160708-jlm/edit)

Joe is setting up a Big Dye reaction to in-house sequence the WM16_014 with modified RBS's.

We got sequencing results for WM16_028 back from the Core Lab; we'll have to analyze these tomorrow.

Working on writing up the Project Description for Experiment.

Out @ 1:30 AM
In @ 3:00 AM

Finished up the Experiment profile

Out @ 4:00 AM

160709 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-09

SATURDAY, 7/9

In @ 3:00 PM

At least one colony grew on all of the plates from last night's transformations!

We are Colony PCRing from the plates that grew. See 160609 CEM (https://benchling.com/wmigem/f/mnsihkXQ-cem/etr-ottzlzXJ-160609-cem/edit)

Everything had at least 3 colonies that worked, other than the pTet GFP + tetR double transformation (but these look like some sort of technical error in the pipeline process is likely). These will be inoculated tonight, so that we can make glycerol stocks of these cells tomorrow. Once the FACS machine gets repaired we will be able to measure IPTG and aTC induction in LG3.300 cells.

We decided not to minprep the inoculations from last night as they were overgrown when we came in. See 160709 AJR (https://benchling.com/wmigem/f/jZYrfUIA-ajr/etr-mAsPm1Vh-160709-ajr/edit)

Joe is sequencing the WM16_014 with modified RBSs (B0034 and B0031).

The seal on the big dye plate was not properly applied, so a lot of sample solution evaporated overnight. Joe is re-doing the big dye reaction.

I asked Joe to add 160615 tetR Amp MP1 (using P30 and P31) to the sequencing set, as this is the only non-disconfirmed tetR 1A3 MP that we have and we do not have a confirmed construct of this. This will allow for a high/high backbone combination of constiutive tetR and pTet GFP for atc induction.

I checked the sequences of the 160629 MPs of WM16_031 1C3 and WM16_028 1C3 that we got back from Lidia yesterday. All confirmed!

I worked on storyboarding and scripting and writing out the video for the Experiment platform.

Out @ 8:00 PM
In @ 9:00 PM

Callan diluted the overgrown inoculations from last night and put them back into the shaker. She also inoculated Colony PCR colonies and streaked-ou glycerol stocks which were low. The colony PCR colonies will become glycerol stocks and the glycerol stock streaks will become minipreps. See 160609 CEM (https://benchling.com/wmigem/f/mnsihkXQ-cem/etr-ottzlzXJ-160609-cem/edit)

I created part pages and new WM16 IDs for variants of WM16_014 and the Sigma 54 parts on UNS backbone. The new IDs are as follows:

Table1

	A	B
1	**Name**	**Decription**
2	WM16_035	WM16_014 with B0034
3	WM16_036	WM16_014 with B0031
4	WM16_037	pACT-Tet on UNS
5	WM16_038	57S on UNS (no DT)
6	WM16_039	57S on UNS (with DT)
7	WM16_040	WM16_034 with J23100 RiboJ
8	WM16_041	WM16_034 with J23100 no RiboJ
9	WM16_042	WM16_034 with J23115 RiboJ
10	WM16_043	WM16_034 with J23115 no RiboJ

160710 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-10

SUNDAY, 7/10

In @ 1:00 PM

Likhitha and Adam are setting up Minpreps and Glycerol Stocks of the inoculants from last night. All single-trasnformations are getting minprepped, whereas all double-transformations are only getting glycerol stock'd. These stocks will be re-streaked for FACS once the machine comes back online.

Andy and I checked through the corelab sequences that Joe ran yesterday. Everything was confirmed with at least one MP (honestly the sequences were extremely good), however Big Dye was not added to the tetR Amp miniprep.

85x tetO array in 1C3 sequence was questionable on BLAST-- Ethan is checking this one.

It turns out that this is the 85x tetO array which was inserted into the prefix region of WM16_014 1C3. Furthermore, it was sequenced with P008 and P009 so none of the array is covered in the sequence data.

At this point, the only important parts that we have no sequence-checked are the 57S on 3C5 and pACT-Tet on 3K3 and the one tetR Amp minprep from 160615.

Likhitha is setting up a diagnostic test PCRs of P054 - P059, just to make sure they work. These will be thrown out after the gel because we don't have the geneBlock WM16_034 yet. It should hopefully arrive later in the week.

They seem to have worked out! See 20160710 LK (https://benchling.com/wmigem/f/aY4Q6dee-lk/etr-hstWw4Gg-20160710-lk/edit)

Likhitha and Adam are setting up PCRs of the addGene Broccoli plasmid to put into UNS backbone; this Gibson Pipeline will be completed tomorrow.

Likhitha and Adam are setting up transformations of the following: 20160710 LK

- 85x tetO 1C3 (not the WM16_014 version) + pTet GFP 3K3 + tetR 1A3 into 10Beta
- pTet GFP 3K3 + tetR 1A3 into 10Beta
- WM16_035 1C3 + WM16_016 3K3 into BL21
- WM16_036 1C3 + WM16_016 3K3 into BL21
- [all 8 Ribozyme parts] 1C3 + WM16_016 3K3 into BL21

Likhitha and Adam set up glycerol streaks of:

- WM16_014 1C3 + WM16_016 3K3 in LG3.300
- pTet GFP 1C3 + tetR 3K3 in LG3.300
- 57S + pACT-Tet (as they were from Orna Atar) in LG3.300
- WM16_035 1C3 in 5 alpha
- WM16_036 1C3 in 5 alpha

These will be inoculated tomorrow for induction FACS on Tuesday, to confirm the ability to induce with IPTG and aTc in the 3.300 strain, as well as the replication of an initial Phillips paper result for the Synthetic Enhancer.

We should also put pBad RFP in LG3.300 to test arabinose induction there.

Likhitha and Adam also inoculated:

- All of the Interlab Measurement parts
- Broccoli (bacterial) from addGene

These will be diluted tomorrow for FACS. The Interlab parts will be measured with the absolute fluorescence calibration beads, so this will complete the interlab measurement project.

Adam found out that some of our plates in storage have been contaminated with fungal growth. He threw them out-- see 160710 AJR (https://benchling.com/wmigem/f/jZYrfUIA-ajr/etr-l58NTEBe-160710-ajr/edit) for what they were.

Out @ 7:30 PM
In @ 9:00 PM

Andy and I designed primers to swap out the RBS in WM16_014 with the rest of the commonly used iGEM RBS Community Collection
(http://parts.igem.org/Ribosome_Binding_Sites/Prokaryotic/Constitutive/Community_Collection)
These make up WM16_P067 through WM16_P078

Andy and I set up the Big Dye reaction for the sequencing of the following parts:

Table1

	A	B	C	D	E	F
1	**Key**	**Sample**	**Primer 1**	**Primer 2**	**Concentration**	**Location**
2	**A, A2**	tetR 1A3 160615 MP1	VF2	VR	52.1	Box 4 Slot 16
3	**B, B2**	85x tetO 1C3 160709 MP1	VF2	VR	623.1	Box 7 Slot 55
4	**C, C3**	85x tetO 1C3 160709 MP2	VF2	VR	464.5	Box 7 Slot 56
5	**D, D3**	85x tetO 1C3 160709 MP3	VF2	VR	473.2	Box 7 Slot 57
6	**E, E3**	WM16_037 160709 3K3 MP2	P008	P009	41	Box 7 Slot 54
7	**F, F3**	WM16_039 160709 3C5 MP 2	P008	P009	432.3	Box 7 Slot 52
8	**G, G3**	WM16_039 160709 3C5 MP 3	P008	P009	219.8	Box 7 Slot 53

200-400 ng of plasmid + enough water to bring up to 7 uL + 1 uL Primer + 4 uL Big Dye
(12 uL total)

Out @ 1:00 AM

160711 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-11

MONDAY, 7/11

In @ 10:00 AM

All of the transformations from last night grew except for pTet GFP + tetR. However, this cotransformation was plated on the wrong antibiotic selection plate. Likhitha is setting up a re-plate of this from the cells that we saved in 4C from last night. 20160711 LK (https://benchling.com/wmigem/f/aY4Q6dee-lk/etr-7XlzmWHZ-20160711-lk/edit)

All of the glycerol stock streaks grew except for WM16_035. Likhitha noticed that this glycerol stock had separation between the cell solution and the glycerol, so the storage may have been ineffective.

Adam is setting up PCRs to assemble pTet GFP into 1A3. See 160711 AJR (https://benchling.com/wmigem/f/jZYrfUIA-ajr/etr-7SI4Rmin-160711-ajr/edit)

Callan and Christine are setting up Colony PCRs of the transformations (11/12 successful). See 160711 CEM (https://benchling.com/wmigem/f/mnsihkXQ-cem/etr-arpwnjFC-160711-cem/edit)

The BioRad technician is scheduled to arrive today to fix the FACS machine. One this arrives we will dilute the inoculations of the Interlab parts and the addGene Broccoli from last night for non-induction FACS at midlog.

Andy is setting up sequencing for which we set up the Big Dye last night.

Worked on the modeling-- derived out the reduced steady-state system with [free pLac], [free IPTG], and [free lacO] as the LHS quantities. Asked Jmitch to check my formulas against his numerical ODE solver... still waiting to hear about that.

> If my equation are right, it turns out that bistability in steady-state concentrations is possible provided that the [free molecule of interest, say pLac] at steady state is sufficiently greater than [free lacI] at steady state. The required extent of differnce is governed by the conserved [total molecule of interest] as well as the dissociation constant K_D of molecule of interest's binding to lacI. This basically means that any [pLac] or [lacO] steady states will definitely not be sufficiently above [lacI], and [IPTG]'s required difference is made very large by its high K_D... so even though [IPTG] > [lacI] routinely at steady state, the system will not exhibit bistability.

Out @ 1:40 PM
In @

FACS'd the interlab parts . Used absolute bead calibration so these should be converted to absolute units tomorrow.

Forgot that the Broccoli parts from addGene need to be induced with IPTG to produce the aptamer, whic then needs to be activted wtih DFHBI. Andy set up inoculations of addGene broccoli to induce tomorrow.

Andy diluted out DFHBI.

Out @ 12:00 AM

160712 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-07-12

TUESDAY, 7/12

In @ 10:00 AM

Joe and I pulled off the sequencing from the 160711 corelab set. Everything was NNNNN :(

Recall that these sequences were

Table1

	A	B	C	D	E	F
1	**Key**	**Sample**	**Primer 1**	**Primer 2**	**Concentration**	**Location**
2	**A, A2**	tetR 1A3 160615 MP1	VF2	VR	52.1	Box 4 Slot 16
3	**B, B2**	85x tetO 1C3 160709 MP1	VF2	VR	623.1	Box 7 Slot 55
4	**C, C3**	85x tetO 1C3 160709 MP2	VF2	VR	464.5	Box 7 Slot 56
5	**D, D3**	85x tetO 1C3 160709 MP3	VF2	VR	473.2	Box 7 Slot 57
6	**E, E3**	WM16_037 160709 3K3 MP2	P008	P009	41	Box 7 Slot 54
7	**F, F3**	WM16_039 160709 3C5 MP 2	P008	P009	432.3	Box 7 Slot 52
8	**G, G3**	WM16_039 160709 3C5 MP 3	P008	P009	219.8	Box 7 Slot 53

We'll have to set these up again... send them to Macrogen

Likhitha is setting up Colony PCRs of the three transformations from last night. These all grew, but the Broccoli-on-UNS 1C3 gibson assembly grew extremely well (almost a lawn!). See 20160712 LK (https://benchling.com/wmigem/f/aY4Q6dee-lk/etr-flssDyA6-20160712-lk/edit)

Adam and Callan are setting up dilutions of last night's inoculations (70 uL overnight culture into 3.5 mL fresh media)-- these will be induced at midlog to FACS later today. For the key and table see 160712 JLM (https://benchling.com/wmigem/f/uDbWaeJV-jlm/etr-WopFRksc-160712-jlm/edit)

Note that the WM16_014 1C3 + WM16_016 3T5 in LG3.300 cotransformation is correct in its backbones... I don't know why it was not done with 16 3K3 in the 160708 JLM transformation. (https://benchling.com/wmigem/f/uDbWaeJV-jlm/etr-6bsAquB4-160708-jlm/edit)

In @ 11:50 AM

Callan induced these dilutions with IPTG (one aTc induction for the 57S + pACT-Tet in LG3.300) 160708 CEM (https://benchling.com/wmigem/f/mnsihkXQ-cem/etr-3W2O3rZ5-160708-cem/edit)

Joe started making part pages.

Upon reaching midlog, Andy and I FACS'd the solo inoculations of WM16_035 1C3 and WM16_036 1C3 in 5alpha. We obtained:

- WM16_035 1C3 5alpha: Two peaks at FL1 380.
  - 239 au (12%)
  - 1984 au (88%)
- WM16_036 1C3 5alpha: One peak at FL1 380.
  - 591 au (99%)

After 2 hours of IPTG induction, I induced expression of Broccoli in both replicates' 0 uM IPTG and 10 mM IPTG tubes. For each sample I spun down 250 uL induced cell culture for 3 min at 8,000 rpm. I then resuspended the cells in 200 uM DFHBI-5T in PBS (500 uL for the 10 mM IPTG solution, 100 uL for the 0 uM IPTG) and incubated them at 37C 250 rpm for 1 hour. I then FACS'd them.

> *NOTE that DFHBI-5T is extremely light sensitive!! This means that I did all of the procedure with the lights off, and that I wrapped the incubation tubes and FACS tubes in aluminum foil.*

The results were:

Table2

	A	B	C	D
1	**mean FL1 (at gain 540)**	**IPTG concentration**	**DFHBI-5T concentration**	**Replicate**
2	3.1	0 mM	0 uM	1
3	2.6	0 mM	0 uM	2
4	3.1	10 mM	0 uM	1
5	2.7	10 mM	0 uM	2
6	3.4	0 mM	200 uM	1
7	3.1	0 mM	200 uM	2
8	5.3	10 mM	200 uM	1
9	5	10 mM	200 uM	2

It turns out that we needed to express the addGene Broccoli in BL21 since the Broccoli is under the control of the T7 promoter, and the BL21 strain has a T7 RNAP expressed under a pLac induced by IPTG. The above Broccoli was trasnformed in 5 alpha, which should explain the low fluorescence values. That being said, one could convince oneself that basal transcription rates of the broccoli have shifted.

REGARDLESS, we'll have to re-transform this into BL21.

After 6 hours of induction, Andy and I FACS'd the Ribozyme parts 1C3 (cotransformed with WM16_016 3K3), as well as the 14+16 cotransformation in LG3.300 and the 57S+pACT-Tet cotransformation in LG3.300. We decided to not use the size gates because the old gates set up by Matt were lost in the hard drive wipe that happened a while ago. However, clearing the size gates does not introduce much noise in the histogram and the samples seem quite pure.

> *We realized that we forgot to induce the 57S + pACT-Tet combination with IPTG prior to aTc induction :(Therefore it's no wonder that no induction occurred.*

But it's really cool that we can get IPTG induction to work in LG3.300!!

Table3

	A	B	C	D	E
1	Sample	ID	Mean	% of cells	Gain

2	14 1C3 + 16 3K3 in BL21	1.1.1	3	97	560
3					
4					
5					
6		1.1.2	3.3	97	
7		1.1.3	8.3		
8			118	96	
9			1015	95	
10			892.7	99	
11		1.2.1	2.6	83	
12			3.7	81	
13			7.3	82	
14			90.9	84	
15			821.6	84	
16			766	86	
17		1.3.1	2.8	95	
18			3.3	95	
19			5.1	97	
20			94.8	93	
21			988	93	
22					
23					
24					
25		1.3.6	857	96	
26	15 1C3 + 16 3K3 in BL21	2.1.1	3.3	100	600
27			3.3	100	
28			4.3	100	
29			26.6	100	
30			255.5	100	
31			322.2	99	
32		2.2.1	2.7	99	
33			2.7	100	
34			3.5	100	
35			19.7	97	
36			228	98	

37			258.3	99	
38		2.3.1	2.7	99	
39			2.9	99	
40			3.5	100	
41			24	98	
42					
43					
44					
45			225	99	
46			257	98	
47	24 1C3 + 16 3K3 in BL21	3.1.1	2.7	98	380
48			3.9	97	
49			14.1	96	
50			128	98	
51			217.2	100	
52			228.4	100	
53		3.2.1	2.8	98	
54			3.3	98	
55			12.3	96	
56			135.4	99	
57			224	100	
58			219	100	
59		3.3.1	1.9	99	
60			4.2	97	
61			6.5	100	
62			141	99	
63			223	100	
64					
65					
66					
67			223	100	
68	25 1C3 + 16 3K3 in BL21	4.1.1	2.8	100	600
69			2.8	100	
70			3.6	100	
71			7.3	100	

72			9.1	100	
73			11	100	
74		4.2.1	2.6	100	
75			2.8	100	
76			3.4	100	
77			8	100	
78			9.3	100	
79			10.1	100	
80		4.3.1	2.8	100	
81			3.2	100	
82			3.5	100	
83			10.1	100	
84			10.3	100	
85					
86					
87					
88			11.8	100	
89	26 1C3 + 16 3K3 in BL21	5.1.1	4.4	100	600
90			5.3	100	
91			8.7	100	
92			43.6	100	
93			295	99	
94			390	100	
95		5.2.1	7.1	99	
96			7.9	99	
97			15.4	98	
98			85.2	96	
99			595	98	
100			642	98	
101		5.3.1	4.1	100	
102			4.8	100	
103			8.1	100	
104			39.9	98	
105					
106					
107					

108			298	99	
109			322	99	
110	27 1C3 + 16 3K3 in BL21	6.1.1	3.9	100	660
111			3.8	100	
112			4.2	100	
113			11.1	100	
114			26.6	99	
115			37.4	100	
116		6.2.1	2.8	100	
117			3	99	
118			3.8	100	
119			8.9	100	
120			21.8	100	
121			26.3	100	
122		6.3.1	2.9	100	
123			2.8	100	
124			3.7	100	
125			7.2	87	
126					
127					
128					
129			25.5	100	
130			26.6	99	
131	30 1C3 + 16 3K3 in BL21	7.1.1	3.9	99	460
132			5	98	
133			18.2	99	
134			311	99	
135			833.4	100	
136			830.2	100	
137		7.2.1	4	98	
138			4.8	98	
139			18.8	95	
140			351.4	97	
141			835.6	100	
142			836.4	100	

143		7.3.1	4.1	98	
144			6	99	
145					
146					
147					
148			20.8	95	
149					
150					
151					
152			4.3	99	
153			831.5	100	
154			906	100	
155	31 1C3 + 16 3K3 in BL21	8.1.1	4.7	99	660
156			3.5	100	
157			6	100	
158			63.3	99	
159			249.4	98	
160			256.3	98	
161		8.2.1	23.2	100	
162			22.3	100	
163			65.9	97	
164			1496	99	
165			4492	100	
166			4498	100	
167		8.3.1	3.5	99	
168			3.2	99	
169			4.8	99	
170			63.8	99	
171					
172					
173					
174			260.1	97	
175			270	98	
176	35 1C3 + 16 3K3 in BL21	9.1.1	4.1	99	580
177			3.3	99	

178			7.7	98	
179			76.2	98	
180			773	98	
181			957.7	99	
182		9.2.1	2.7	98	
183			3.3	98	
184			6.5	98	
185			63.1	98	
186			855	100	
187			1022	99	
188		9.3.1	3.5	98	
189			4.1	99	
190					
191					
192					
193			8.2	99	
194			81	98	
195			1025	98	
196			998	99	
197	36 1C3 + 16 3K3 in BL21	10.1.1	3.6	95	660
198			4	95	
199			4.8	95	
200			24.9	97	
201			468	96	
202			489	97	
203		10.2.1	3.3	99	
204			3.6	99	
205			4.5	99	
206			29.2	99	
207			450.1	98	
208			467.8	99	
209	14 1C3 + 16 3K3 in LG3.300	11.1.1	4	100	560
210					
211					
212					
213			6.2	100	

213			6.2	100	
214			11.3	100	
215			268.4	99	
216			601.2	99	
217			702	100	
218		11.2.1	2.7	100	
219			4.4	100	
220			9.9	100	
221			177.8	100	
222			421	100	
223			450	100	
224		11.3.1	2.5	100	
225			4.1	100	
226			8.4	100	
227			144.1	100	
228			417.6	100	
229			468	100	
230	57S + pACT-Tet in LG3.300	12.1.1	5.1	100	780 (FL3)
231			4.8	100	
232			6.1	100	
233			5.9	100	
234			6	100	
235			6.1	100	
236		12.2.1	6.5	100	
237			6	100	
238			7.4	100	
239			7.8	100	
240			7.7	100	
241			8	100	

Note that the last sample is measured on FL3 because it is an mCherry reporter.
Also, I don't know why there are empty rows in the table here. For more concise presentation (with graphs), see the Excel file in Dropbox/iGEM 2016/workspace/john/20160712 FACS.xlsx

During the 12.3 samples the FACS machine gave up on us and started not reading events :(We ran samples through it for a while, tried unclog + debubble, did a low-pressure wash with water, re-ran QC (got a Event Rate Too Low error)... no solution :(

We'll have to re-run the absolute fluorescence beads at the gains used in the above table tomorrow in order to do the FlowCal conversion :(

Out @ 5:00 AM

160715 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-15

FRIDAY, 7/15

In @ 10:00 AM

All the RBS swaps of WM16_014, as well as the WM16_034 assembly onto 1C3, grew!

Likhitha is Colony PCRing the WM16_034 inserts in order to:
a) check the insert length
b) assemble into a J23100/J3115 promoter swap with/without RiboJ
c) swap the spinach with Broccoli
Also she is colony PCRing the RBS swaps. See 20160715 LK

Adam and Callan are diluting the following:

- 57s + pACT-Tet cotrasnformation in LG3.300
  - for IPTG induction followed by aTC induction
- triple / double transformations of pTet GFP + tetR + 85x tetO
  - for aTC induction

see 160715 CEM

Ethan checked sequences from Macrogen.

- Broccoli UNS 1C3 worked
- 85x tetO 1C3 worked (!!)
- 57S + DT 3C4 disconfirmed (hold off on redoing these until we can get functional 57S+pACT-Tet induction)
- pACT Tet 3K3 disconfirmed (hold off on redoing these until we can get functional 57S+pACT-Tet induction)
- pTet GFP 1A3 disconfirmed (again! but hold off on redoing this because we are going to try and move pTet GFP and tetR together onto one plasmid because triple cotransformations are just not working.)

Ethan and Joe are miniprepping 85x tetO 3C5.

Out @ 1:30 PM
In @ 2:30 PM

Out @ 7:00 PM
In @ 8:00 PM

Andy and I FACSd the innoculations that Callan induced (5 samples 3 replicates). They were taken out of the incubator around 12:15 AM.
We chose not to FACS the cotransformations which included pTet GFP 1A3 (Samples #4, 5 from Callan's key) because that part was found to be disconfirmed-- indeed, the cultures were visibly red as they were on the plate.

...But the FACS machine is unable to read a sufficiently high event rate :(
We tried following the manual and going into the admin setting to calibrate the sample pressure offset, which got past the event rate problem but led to every CV being out of range, or the event rate being too high...
at this point we need to just talk to tech support :(

160716 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-16

SATURDAY, 7/16

In @ 12:00 PM

Ethan and Callan are miniprepping the inoculations from last night (RBS swaps on WM16_014 and WM16_034). 160716-EMJ

However Ethan found that the nanodrop stopped reading concentrations :(
He turned it off and on and did a bunch of attempts but to no avail :(
Logged it in without nanodrop values... however the few that were successfully measured were quite good on both yield and purity 160716-EMJ

Ethan and Callan are Colony PCRing the transformations from last night for inoculation for miniprep tomorrow (Broccoli into WM16_034 / J23100 or J23115 into the Spinach version of WM16_034). 160716 CEM

Ethan and Callan are setting up sequences of today's minipreps to send to Macrogen on Monday.

I finished up the script for the Experiment video and edited the project page to be more layperson-friendly.

Ethan and Callan set up inoculations of their colony PCR products. I put these into the shaking incubator (37C 250 rpm) at 9:00 PM. For parts see 160716 CEM

There is also one glycerol streak for a replenishment miniprep.

Out @ 7:00 PM
In @ 8:30 PM

The inoculations that went in will need to be Minprepped tomorrow, after which they will need to go into a Gibson Assembly pipeline. We want to eventually create

- J23100 - RiboJ- RBS - mCherry - Broccoli - DT
- J23100 - RBS - mCherry - Broccoli - DT
- J23115 - RiboJ - RBS - mCherry - Broccoli - DT
- J23115 - RBS - mCherry - Broccoli - DT
- J23107 - RiboJ - RBS - mCherry - Broccoli - DT (we have this one inoculated)
- J23107 - RBS - mCherry - Broccoli - DT

And we have inoculated

- J23100 - RiboJ - RBS - mCherry - Spinach - DT
- J23100 - RBS - mCherry - Spinach - DT
- J23115 - RiboJ - RBS - mCherry - Spinach - DT
- J23115 - RBS - mCherry - Spinach - DT
- J23107 - RiboJ - RBS - mCherry - Broccoli - DT

If the J23107 - RiboJ - RBS - mCherry - Broccoli - DT part grows, then the best way to do this is to ignore the four J23100 / J23115 inoculations and do the promoter swaps onto the J23107 part just like we did onto the WM16_034 construct:

- Insert PCRs from:

Table1

	A	B	C	D
1	J23100 Miniprep	p054, p055	35 bp	65 deg C
2	J23100 Miniprep	p054, p056	35 bp	65 deg C
3	J23115 (no template)	p057, p058	35 bp	72 deg C
4	J23115 (no template)	p057, p059	35 bp	72 deg C

Promoter Insert PCRs. Reproduced from 20160710 LK

- Along with using P066 + P013 (no RiboJ) // P015 + P013 (keep RiboJ) on the J23107 - RiboJ - RBS - mCherry - Broccoli - DT part for the backbones PCRs.

  If the J23107-RiboJ-RBS-mCherry-Broccoli-DT part doesn't grow, but the other four do, then we can use the Spinach -> Broccoli swap PCRs on those four parts to make the required constructs.

These assemblies will be transformed and eventually miniprepped and sequenced... as soon as these are sequence confirmed we will order the big batch of primers for all of the Anderson promoter swaps.

Out @ 9:40 PM

160718 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-18

MONDAY, 7/18

In @ 10:00 AM

Likhitha set up Colony PCRs of the four transformations Andy and I did last night (J23100 and J23115 w/ and w/out RiboJ on the WM16_034 backbone on 1C3 in 5 alpha). 20160718 LK20160718 LK

These were inoculated for miniprepping tomorrow.

We forgot to send out sequences to Macrogen. We'll prepare the additional minipreps (the J23100 and J23115 34 variants from Sunday 0717) as well as Saturady 0716's minpreps to send to sequence tomorrow.

We're making Chlor plates.

Calling BioRad about the FACS machine struggling at QC with the Event Rate Too Low error.
Finally got it to work! See cell sorter log

Out @ 8:00 PM
In @ 9:00 PM

Math meeting was good. Assigned Kalen to start thinking about the synthetic enhancer binding model. Assigned John Mitchell to conclusively determine if affecting transcription vs translation rate in a protein production model makes a difference.

Andy and I struck out glycerol stocks of WM16_034 and its existing variants (J23107 w/ Broccoli; J23100 w/ RiboJ w/ Spinach; J23100 w/out RiboJ w/ Spincah; J23115 w/ RiboJ w/ Spinach; J23115 w/out RiboJ w/ Spinach)
These are going to be inoculated tomorrow morning to FACS and determine if Spinach / Broccoli work. Additionally, when we inoculate these plates we also need to dilute out into new tubes some cultures of WM16_040, 041, 042, and 043 which are currently in the incubator for minprepping tomorrow. This way they will all grow up for FACS sometime in the evening.

Out @ 12:00 AM

160719 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-19

TUESDAY, 7/19

In @ 10:00 AM

Inoculants of 40, 41, 42, 43 didn't grow :(

Ethan and Adam are sending out sequences to Macrogen

None of the inoculations of 40, 41, 42, 43 grew from last night so we cannot miniprep them. However, yesterday's Colony PCRs looked good and none of the Gibson operations weren't performed before supposedly successfully... see 20160718 LK
20160718 LK

I'm going to guess technical error. Adam is re-inoculating colonies from plate now (in the morning) so we can assess growth by this evening.

Callan is setting up inoculations of glycerol streaks of cotransformations which haven't been measured yet (57S + pACT-Tet in LG3.300; pTet GFP + tetR; pTet GFP + tetR + 85x tetO both in BL21) as well as the 34 variants that exist in MP form currently. See 160719 CEM

These will be induced (with IPTG or aTc or DFHBI) at 3:00.

Most of these didn't grow!! Only the 57S + pACT-Tet culture grew, which Callan is pushing through the IPTG + aTC induction. We are concerned because the 40, 41, 42, 43 inoculants were in LB + Chlor (new LB, new Chlor dilution), and the inoculation we started this morning also didn't grow (and all but one sample of these were in new LB + new Chlor). The new Chlor was diluted yesterday by Callan and Adam, who followed the standard iGEM 2015 dilution protocol. It may be that the scale they used to weigh out Chlor malfunctioned, causing them to dilute out an over-concentrated amount.

To test this theory I had Likhitha inoculate glycerol stock streaks of J23100-with RiboJ-Spinach and J23100-without RiboJ-Spinach into LB with no antibiotic. These went in around 4:00 PM.

Today we should co-transform the RBS swaps with 16 and get those characterizations from the FACS on Thursday!

Tips and Culture Tubes arrived.

Out @ 12:10 PM
In @ 12:45 PM

Adam finished checking the thermodynamics of the Anderson promoter characterization primers.

I ordered these at Standard Shipping rate.

Likhitha and Ethan are setting up cotransformations of the remaining RBS variants of 14 on 1C3 with 16 on 3K3 into BL21. See 20160719 LK

Out @ 5:30 PM
In @ 7:30 PM

Inoculations have started to grow!

Overnight inoculations of 40, 41, 42, 43 have that:

- Two replicates of 41 (J23100 w/out RiboJ) and two replicates of 42 (J23115 w/ RiboJ) grew
- Nothing else grew

Some of the 34 variants grew, so we can induce them with DHFBI. These were:

- (1) WM16_034 #2
- (2) WM16_34 w/ J23100 w/out RiboJ #1
- (3) WM16_34 w/ J23100 w/out RiboJ #2
- (4) WM16_34 w/ J23100 w/out RiboJ #3
- (5) WM16_34 w/ J23115 w/ RiboJ #3

At both the spinach level (34 variants) and the broccoli level (40-43), the J23100 w/out RiboJ and J23115 w/ RiboJ constructs managed to grow whereas the J23100 w/ RiboJ and J23115 w/out RiboJ constructs did not.

I set up DFHBI activation of Spinach of the 34 variants (#1-5 above) which grew. I followed the methods in "The Spinach Aptamer as a Tool for Characterization in Synthetic Biology":

- I created 500 uL of 100 uM DFHBI *(note that the paper used 200 uM)* in LB solution by adding 1.25 uL stock (40mM) DFHBI into 498.75 uL LB (*All done in a dark room since DFHBI is light sensitive!!)*
- I added 10 uL cell culture into 100 uL LB solution in a black 1.5 mL tube, and put them in the shaking incubator 37C 250 rpm for 10 minutes
- These were placed on ice for 10 minutes, then FACS'd

We also included a set of no-DFHBI controls with otherwise the same procedure.

Andy and I FACS'd the DFHBI-activated 34 variants. Discouraging results:

- None of the J23100 parts had fluorescence in the FL3 (mCherry) channel. The J23107 and J23115 parts had a little bit, and the J23115 part had less than the J23107 part as expected, though.
- None of the parts had fluorescence in the FL1 (spinach) channel.

However these parts have also been questionable at the liquid-growth stage. Furthermore, minpreps that were conducted on 0716 and 0717 have shown visibly red pellets, so I still think the parts are fine.
We should re-do 34 -> 40, 41, 42, 43 assemblies.

We also collected measurements for the Spherotech absolute beads at the gain levels used in the previous Ribozyme part etc. measurements.

However, after the FACS I went back and looked at the cultures in the incubator and found that more of them had grown. At least one replicate from each sample of the 34 variants now had cloudy growth. I spun down 500 uL of each culture at 8,000 rpm for 3 minutes to look at the color of the pellet, and did the same to the two replicates of 41 and 42 that grew. Interestingly, 41 (J23100 w/out RiboJ on Broccoli) had a distinctly red pellet whereas the 34 w/ J23100 cultures did not. This might suggest that the promoter-swap assembly did not work on the 34 template, but did work on the 34 w/ Broccoli template. Perhaps it's a low-efficiency assembly? Regardless, it suggests that we still have some hope to hold on to. None of the 34 variants had particularly red-looking pellets except for maybe the lone replicate of 34 (J23107 w/ Brocoli).

I set up DFHBI activation of Broccoli of the two replicates each of 41 and 42. I followed the supplemental methods in "Broccoli- Rapid Selection of an RNA Mimic of Green Fluorescent Protein":

- I created 400 uL of 200 uM DFHBI by adding 4 uL stock (40 mM) DFHBI into 796 uL PBS. (*All done in a dark room since DFHBI is light sensitive!!)*
- I spun down 500 uL of cell culture for 3 min. at 8,000 rpm and removed supernatant
- I resuspended the pellets in 100 uL of the DFHBI + PBS solution
- I transfred the new solution to a culture tube wrapped in aluminum foil and incuated for 45 min. at 37C 250 rpm.
- I transfered 30 uL of this solution to 300 uL PBS for FACS.
- I also included uninduced "controls" which transferred 30 uL of the original LB solution (sitting at room temp. for the past 45 min) into 300 uL of PBS for FACS.

I also used
Andy and I then FACS'd the 41 and 42 samples.

They are very strange... overall the fluorescence levels are dim, if at all, on FL3 (mCherry) and nonexistent / noncorrelated with induction condition on FL1 (Broccoli). :(But the "controls" were also poorly executed as well so I'm not sure about the results here.

However, I then realized that the inoculation which Adam put in this morning of 40, 41, 42, 43 (from the same plate that was inoculated yesterday) had now grown to turbidity-- furthermore, every single sample and replicate grew!! (these colonies were chosen at random without colony PCR).

I spun down these pellets and found that 40 and 42 had distinctly red pellets (particularly 40!! 42 had fainter but still definitely red pellets for replicates #2,3. #1 was plain color.) However, 41 and 43 had definitely not-red pellets. This correlates with the not-RiboJ promoter swap assemblies, so that's unfortunate-- will really have to look at the sequencing that comes back.

I prepared DFHBI activation of Broccoli for the three replicates each of 40 and 42, using the above protocol (but resuspending in 200 uL PBS solution), however I also included *actual* uninduced controls which were resuspended in non-DFHBI PBS solution and put into the shaking incubator with the others:

- I created 1200 uL of 200 uM DFHBI by adding 6 uL stock (40 mM) DFHBI into 1194 uL PBS. (*All done in a dark room since DFHBI is light sensitive!!)*
- I spun down ~500 uL of cell culture for 3 min. at 8,000 rpm and removed supernatant
- I resuspended the pellets in 200 uL of the DFHBI + PBS solution
- I transfered the new solution to a culture tube wrapped in aluminum foil and incubated for 45 min. at 37C 250 rpm.
- For the control, I spun down ~500 uL of more cell culture for 3 min. at 8,000 rpm and removed supernatant
- I resuspended the pellets in 200 uL of PBS solution
- I transfered the new solution to a culture tube and incubated for 45 min. at 37C 250 rpm.
- I transfered 30 uL of theses solution to 300 uL PBS for FACS.

Andy and I FACS'd the pACT-Tet + 57S in LG3.300 cotransformations and the pTet GFP (3K3) + tetR (1A3) in BL21 cotransformations, which had finished a 4 hour aTc induction by this point.

The pACT-Tet + 57S samples looked strange. Lots of events were being detected along the edges of the size and shape channels ("garbage" in the solution), and it was difficult to get a high density within the size gates. We had to settle for obtaining 250 events before recording mean of fluorescence distribution. We ended up not seeing any increase with induction :(

The pTet GFP + tetR samples had amazing induction!!

All FACS Data is in Dropbox/iGEM 2016/FACS Data/160719 FACS

Andy and I FACS'd the more recent batch of 40 and 42.

The WM16_040 (J23100 w/ RiboJ w/ Broccoli) is definitely expressing mCherry. That's good news. Broccoli induction seems to be happening at a slow but consistent level but this might be a protocol issue.
Similar case for WM16_042 (J23115 w/ RiboJ w/ Broccoli), but to a lower mCherry magnitude (as expected).

Both samples had a lot of "garbage" on the size gates, but given their turbidity that is probably expected.

We need to make sure the presence of DFHBI doesn't increase fluorescence on the FL1 channel... should run RFP constructs without aptamers and run some with and some without DFHBI.

Out @ 1:45 AM

160720 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-21

THURSDAY, 7/21

In @ 10:00 AM

Met with Scott Hale about an alumni/donor-based crowdfunding platform promoted by the development office which would feature iGEM

Recorded some takes of the Experiment video. Still need to do vocal narration and video editing.

Outreach all day today-- had middle school students come in for Biology Bistro and Strawberry DNA Extraction.

Meanwhile, the cotransformations of the remaining RBS swaps onto 14 all grew, and looked good on the colony PCR. See 20160720 LK

Likhitha inoculated these for FACS tomorrow.

Out @ 8:00 PM
In @ 1:30 AM

Macrogen results arrived! Checked the sequences for 34 and its variants.

Most parts look phenomenally sequence confirmed! One 34 replicate was disconfirmed.

This result is funky because notes from LK when she did the minipreps, as well as me and Andy's FACSing yesterday, suggest that a lot of these cells aren't growing red at all (others are). What's up with that? Is there a compatibility issue with mCherry expression and our strain or something?

The Broccoli swaps seem to be questionable... all three replicates were perfect in all regions of the construct EXCEPT for a 20bp region in the middle of the 2x broccoli sequence (bp 1020-1040 in the part).

Turns out it's not just the 20bp region, but that more might be missing (the sequence strand realigns with the post-20bp region of the query at a different location from where it left off)

I didn't check the other sequences in the batch. Here's a table of the sequences of 34 variants I used:

Table1

	A	
1	**Name**	**Sequence**
2	34 with Broccoli	AACAAACGCAGAATCCAAGCtttacggctagctcagccctaggtattatgctagcagctgtcaccggatgtgctttccggtctgatgagtcc ctccaaggcctacgtgaagcaccccgccgacatccccgactacttgaagctgtccttccccgagggcttcaagtgggagcgcgtgatgaacttcga gcgcctacaacgtcaacatcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtacgaacgcgccgagggccgccact ATGATCCAGACGGTCGGGTCCATCTGAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTC
3	34 with J23100 with RiboJ	AACAAACGCAGAATCCAAGCttgacggctagctcagtcctaggtacagtgctagcagctgtcaccggatgtgctttccggtctgatgagtc gctccaaggcctacgtgaagcaccccgccgacatccccgactacttgaagctgtccttccccgagggcttcaagtgggagcgcgtgatgaacttcg ggcgcctacaacgtcaacatcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtacgaacgcgccgagggccgccac ACTAGTCGCGTCCGGCCGCGGGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCAAATACTCTACGGTC
4	34 with J34100 without RiboJ	AACAAACGCAGAATCCAAGCttgacggctagctcagtcctaggtacagtgctagcaaagaggagaaaTACTAGatggtgagcaa agtgggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgacccaggactcctccttgcaggacggcgagttcatctacaaggtgaag acgaacgcgccgagggccgccactccaccggcggcatggacgagctgtacaagtaataaCTCTACGACAACCTCTTCACAGCC CCAAATACTCTACGGTCACATACccaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtc
5	34 with J23115 with RiboJ	AACAAACGCAGAATCCAAGCtttatagctagctcagcccttggtacaatgctagcagctgtcaccggatgtgctttccggtctgatgagtcc ctccaaggcctacgtgaagcaccccgccgacatccccgactacttgaagctgtccttccccgagggcttcaagtgggagcgcgtgatgaacttcga gcgcctacaacgtcaacatcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtacgaacgcgccgagggccgccact CTAGTCGCGTCCGGCCGCGGGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCAAATACTCTACGGTCA
6	34 with J23115 without RiboJ	AACAAACGCAGAATCCAAGCtttatagctagctcagcccttggtacaatgctagcaaagaggagaaaTACTAGatggtgagcaag gtgggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgacccaggactcctccttgcaggacggcgagttcatctacaaggtgaagc cgaacgcgccgagggccgccactccaccggcggcatggacgagctgtacaagtaataaCTCTACGACAACCTCTTCACAGCCA CAAATACTCTACGGTCACATACccaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtcg

Final impressions: It's great that the promoter swaps seem to work for both from-template and not-from-template AND for including RiboJ and excluding RiboJ! Seems like something is up with the Broccoli assembly that we need to look into.

Out @ 2:25 AM

160723 JPM

Made with Benchling

Project: iGEM 2016

Authors: Adam Reiss

Date: 2016-07-23

SATURDAY, 7/23

In @ 3:00 PM

Macrogen results came in for 52S and 55AS. Ethan checked sequences. At least one confirmed part for everything.

Ethan is making the broth that was used in the Phillips paper to grow up the LG3.300 strains constaining the synthetic enhancer constructs following overnight LB growth. However, it turns out we are out of Magnesium Sulfate so we can't proceed... we'll make this up first thing in the morning tomorrow.

Ethan is setting up PCRs for re-doing the assembly of Broccoli into 34. This time we are going from the sequence-confirmed UNS Broccoli construct instead of the addGene plasmid to get the Broccoli insert. See 160723-EMJ

I fixed up primers and redesigned them, in order to

(i) Move pTet GFP and tetR into UNS backbones

(ii) Combine pTet GFP and tetR into one plasmid, both with and without UNS flanking.

These are P140, 141, 142, 143, 144, 154, 155

I ordered these, as well as P097 and P099 to remove RiboJ from 34 to test the J23107 promoter.

Ethan set up transformations of the following RBS swaps of WM16_014 (solo) into BL21:

Table1

	A	B
1	WM16_035	160708 MP1
2	WM16_036	160708 MP1
3	WM16_044	160716 MP2
4	WM16_045	160716 MP1
5	WM16_046	160716 MP2
6	WM16_047	160716 MP3
7	WM16_048	160716 MP3
8	WM16_049	160716 MP2

160723-EMJ

He also re-did two RBS swap cotransformations into BL21:

Table2

	A	B	C
1	**Co**	**Reporter MP**	**Repressor MP**
2	46 1C3 + 16 3K3	160716 MP2	160709 MP2
3	48 1C3 + 16 3K3	160716 MP3	160709 MP2

160723-EMJ

He also did cotransformations of the new sequence-confirmed Orna Atar parts 52S, 55AS with pACT-Tet into LG3.300:

Table3

	A	B	C
1	**Co**	**Enhancer MP**	**Helper MP**
2	52S Kan + pACT-Tet Amp	160716 MP2	160630 MP2
3	55AS Kan + pACT-Tet Amp	160716 MP1	160630 MP2

160723-EMJ

Out @ 8:00 PM
In @ 10:00 PM

I inoculated the solo glycerol streaks of the eight RBS swaps in 5 alpha into Chlor LB.
In @ 10:30 PM

Ethan plated the cells @ 10:30 PM

I labeled the glycerol streaks of the six sequence confirmed cotransformations of RBS swap of 14 1C3 + 16 3K3 as having been measured on 160721, then stored them in the 4C fridge. (all six had good growth)

Tomorrow we'll need to MP the eight RBS swaps in order to send them to collaborators-- we'll also have to dilute out some of the culture to FACS when at midlog.

We'll also need to colony PCR the cotransformations and inoculate them for FACS. If the two RBS swap cotransformations look good then we'll have to inoculate the other six from plates I put in storage so we can measure all eight at once in the same conditions.

We also need to make the broth and also make up more LB-- I think we're running low on some plates as well.

We really also should make a spreadsheet of the primers and what they are.

Ethan ran the gel on the PCRs and the broccoli looks double-banded... will have to re-run on 50bp ladder tomorrow.

Out @ 10:50 PM

160722 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-07-23

SATURDAY, 7/23

In @ 12:00 AM

Today we went to the mid-Atlantic meetup. We launched a collaboration with Pitt where they will characterize our parts in their cell-free extract.

Andy and I are streaking out glycerol stocks for re-FACS (and Minprep for the solo transformations) of the eight RBS swaps of 14 (cotransformed with 16 3K3).

The minpreps will go to our collaborators to characterize in cell-free system

The re-FACS is to re-measure all eight simultaneously in the same conditions and to get another replicate for measurements.

The glycerol stocks we used for solo transformations are (all sequence confirmed and in 5 alpha):

Table1

	A	B
1	WM16_035	160708 GS1
2	WM16_036	160708 GS1
3	WM16_044	160716 GS2
4	WM16_045	160716 GS1
5	*WM16_046*	*160716 GS2*
6	WM16_047	160716 GS3
7	*WM16_048*	*160716 GS3*
8	WM16_049	160716 GS2

Note that these correspond to the plasmids which were used in the cotransformations which LK and EMJ performed, which were FACS'd on 160721, with the exception of 46 which used the disconfirmed MP1 and 48 which used the disconfirmed MP2. 20160719 LK

The glycerol stocks we used for cotransformation restreaks are (all using sequence confirmed minipreps in BL21 with reporter on 1C3 and repressor on 3K3, with functional FACS characterization taken from 160721 measurements):

Table2

	A	B
1	**Reporter**	**GS**
2	WM16_035	160713 GS3
3	WM16_036	160713 GS1
4	WM16_044	160721 GS1
5	WM16_045	160721 GS3
6	WM16_046	seq. confirmed cotransformation doesn't exist
7	WM16_047	160721 GS3
8	WM16_048	seq. confirmed cotransformation doesn't exist
9	WM16_049	160721 GS3

Plates went in at 1:00 AM

The re-FACS of the eight simulatenous cotransformations will have to hold off because we discovered that two of the cotransformations (46, 48) were done using disconfirmed minipreps.

Tomorrow we will have to re-do these cotransformations into BL21. We also need to transform the same single-part RBS swaps of 14 into BL21 to match up with our induction results from the cotransformations in BL21.

Tomorrow we also want to make the broth from the Phillips paper and start growing up an overnight culture of 57S + pACT-Tet in LB for dilution into the Phillips broth (and also M9) for FACS on Sunday

We also need to make the experiment video and finalize it by July 25!

Out @ 1:10 AM

160724 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-07-24

SUNDAY, 7/24

In @ 12:00 PM

Likhitha is setting up Colony PCRs of the solo and cotransformations of the RBS swaps that Ethan set up last night.

The 46/48 + 16 cotransformations didn't look good :(See 20160724 LK

The double transformations of 52S and 55AS with pACT-Tet didn't grow :(

Adam is setting up minipreps of the eight solo RBS swaps in 5 alpha

WM16_035 didn't grow... only one colony was on the plate from the glycerol streak.

Will re-streak the other sequence-confirmed glycerol stock (160708 MP2) tonight.

WM16_046 had one extremely green replicate and two not-very-green replicates

Adam is re-running the gel of the UNS broccoli insert PCR that Ethan did yesterday, using 50 bp ladder.

Multiple banding is occuring but the strongest band is at the correct size (~270 bp). Will proceed with Gibson and select with both insert primers and broccoli-region primers at the colony PCR stage tomorrow. See 160724 AJR

Adam set up Dpn I and PCR Purification of the Broccoli insert and the 34 + backbone w/out spinach.

Out @ 5:30 PM

In @ 8:00 PM

Adam assembled and transformed the new 34 w/ Broccoli into 5 alpha, as well as the WM16_035 160716 MP1 which had bad growth off the glycerol streak.

Likhitha inoculated all eight RBS swaps of 14 in BL21, as well as the cotransformations of 46 and 48 with 16 (all into M9 glucose), and glycerol streaks of 57S + pACT-Tet (these into both LB and into Phillips broth), and also some colonies off the original 160714 WM16_034 1C3 plate (into LB) for dilution and induction (IPTG; IPTG+aTC; DFHBI, respectively) FACS tomorrow.

In @ 9:00 PM

Adam also did glycerol streaks of WM16_035 (this time with 160716 MP3, the other sequence confirmed version of this part) and WM16_034 (sequence confirmed).

Likhitha re-did Colony PCRs on the 46 and 48 cotransformations with 16, and this time they looked fine (there was an extension time error the first time) 20160724 LK

So today we'll need to either re-inoculate the two 46, 48 cotransformations along with the six other RBS swaps in storage, or keep the current inoculations alive via rediluting so we can FACS everything tomorrow.

Out @ 10:40 PM

160725 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-25

MONDAY, 7/25

In @ 10:00 AM

Today was mostly occupied by making the Experiment video and updating the page's description for the launch of the campaign on Wednesday.

Likhitha did Colony PCRs of the 34 w/ Broccoli that we transformed lastnight. She did insert checks with P8 P9 and Broccoli-size checks with P48 P49. We had prominent triple-banding on most of the colonies-- she went ahead and inoculated the best-looking ones where the correct-size band was the brightest (in M9 Glucose, mistakenly, and later in LB). We'll miniprep these (LB) tomorrow as well as FACS them (LB and M9). 20160725 LK

inoculations of 34 didn't grow-- Likhitha and Adam set up new inoculations of 34 (from sequence-confirmed glycerol stock) in M9 Glucose and LB. If they grow, they should be ready to FACS tomorrow.

Andy and Joe FACS'd the eight RBS swaps' solo transformations in BL21, but found that the quality of the measurements was once again bad... lots of 'trash' in the solution which appeared at the edges of the size scatter plots. Andy thinks this may be caused by the cells sitting out too long on the bench.

> We set up re-inoculations of the eight RBS swaps (35, 36, 44-49) solo in BL21 in M9 Glucose to FACS again tomorrow.

The 46, 48 cotransformations with 16 in BL21 grew well last night. These were diluted again in the evening along with inoculations of the other six RBS swaps cotrasnformed with 16 in BL21 in M9 Glucose. People also made glycerol stocks of them to put into the cotransformation glycerols box (now we have all eight sequence-confirmed RBS swaps cotrasnformed with 16 in BL21 in glycerol stock!).

We set up dilutions -> inductions of pACT-Tet + 57S cotrasnformations in LG3.300 (from glycerol streak). The protocol was LB overnight -> LB to midlog -> IPTG (1mM) with Phillips Broth resuspension for 4 hours -> aTC for 4 hours -> FACS.

> The wording was not the clearest on the Phillips paper, so we decided that the proper protocol was actually to add aTC at the same time as IPTG in the induction step. We inoculated more colonies from this cotransformation glycerol streak in LB in order to dilute and follow the proper protocol tomorrow if the FACS doesn't work tonight.

People did a lot of reading about Broccoli and Spinach activation protocols (especially for following up with Flow Cytometry or FACS). The general consensus seems to be to grow things up in normal broth (LB for example), induce them with DFHBI, and then to transfer some culture into PBS for measurement but making sure that there is still the same molarity of DFHBI in the new PBS solution. Apparently people use 40 uM DFHBI for FACS. We'll check these out tomorrow when we activate them for FACS.

Andy and I FACS'd the 57s + pACT-Tet LG3.300 cotransformation which underwent the sequential iPTG -> aTC induction but otherwise followed the phillips protocol. The induction curves aren't monotonic but there is a general slight increase... definitely not a staircase function though.

> Remember you can find data in Dropbox / iGEM 2016 / FACS Data / 160725 FACS

It's quite unfortunate. Also the samples looked like they had a lot of 'trash' like they did before, with many events being detected at the low extremes of the size gates and causing the functional sample rate to be pretty low (we went for 500 events before we recorded the mean of the distribution).

> Remember these cells were induced 4 hours each on IPTG and then aTC.

We'll re-try this FACS again tomorrow, using simultaneous IPTG and aTC induction. Because why not, just for good measure.

We got a $1,000 grant from GenScript for our CRISPR proposal! (we planned to put SynNotch onto a BioBrick backbone, and then have SynNotch expressing dCas9 on a BioBrick backbone)

Out @ 2:40 AM

160726 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-26

TUESDAY, 7/26

In @ 10:00 AM

We are setting up dilutions of overnight cultures (all of which had at least one replicate grow) of

- 34 in LB and in M9
- 34 with Broccoli in LB and in M9
- all eight RBS swaps solo in BL21 in M9
- all eight RBS swaps cotrasnformed with 16 in M9
- 57S + pACT-Tet in LG3.300 in LB

These were induced appropriately

We are also miniprepping the 34 with Broccoli LB cultures

Ethan and Andy are setting up the in vitro test of Broccoli fluorescence from the addGene bacterial plasmid
(cut at a restriction site downstream of broccoli to linearize -> purify -> in vitro trasncription kit -> activate with DFHBI)
A fire alarm during the RNA purfication step ruined the samples :(We're re-transforming the addGene plasmid into 5 alpha for more plasmid.

I'm designing primers to put T7 promoter in the 34 and 34 w/ Broccoli constructs so we can do the In Vitro Transcription tests of these transcripts.
These are P156-P158. I ordered them.

Andy and Joe FACS'd the solo RBS swaps at midlog-ish, but had poor solution quality (many events at the edge of the size gate etc.).

People tried to do many DFHBI activation protocols on the aptamers in cells in solution, but none worked. :(

We got primers to move pTet GFP and tetR onto UNS backbones, as well as to combine pTet GFP and tetR together onto one backbone.
Adam set up asemblies of these parts:

- pTet GFP on UNS backbone
- tetR on UNS backbone
- pTet GFP + tetR on non-UNS backbone

Likhitha and Joe set up transformations of

- The three assemblies Adam set up into 5 alpha
- Broccoli from addGene into 5 alpha
- 57S solo into LG3.300 (so we can characterize it in isolation.. perhaps the mCherry doesn't work or something)

Andy and I re-FACS'd the solo RBS variants. The quality of the solutions looked a lot better. A lot of the tubes simply didn't grow so it's hard to tell any distinctive pattern.

Andy and I FACS'd the pACT-Tet + 57S cotransformation with simultaenous IPTG and aTC induction (4 hours). We got no induction to happen. We put them back into the shaker to see if they need more induction time... we'll FACS them again tomorrow morning.

Andy and I FACS'd the RBS variants on 1C3 cotransformed with 16 3K3 in BL21 in M9 Glucose. The quality of the solutions looked pretty good... we decided to save FCS files for these to get final data in arbitrary units for these parts.

In order to do this, we had to forego the size gates because they were picking up too small a percentage of the cells-- in order to get a sufficiently high number of cells for the FCS saving, we would have to save data for almost a million uncounted events. File size would be limiting. However, the size gates that Matt set up were lost in the power surge anyway so it's not like these size gates were "official" or anything-- they were just something I set up afterwards by eye.

The super-close consistency across biological replicates doesn't really happen anymore. But things still look like good induction curves.

Remember you can find the results on Dropbox / FACS Data / 20160726 FACS

Overall, it's nice that the RBS variants seem to be working fine, and I'm optimistic about testing the tetO array once we get the pTet GFP with tetR combined on one backbone. It's concerning that we can't get a synthetic enhancer to work, or an aptamer to fluoresce. We need to spend more time thinking about ways to diagnose these problems.

Out @ 3:00 AM

160727 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-27

WEDNESDAY, 7/27

In @ 10:00 AM

I re-FACS'd the 57S+pACT-Tet cotransformation in LG3.300 with overnight induction of IPTG and aTc. Induction was present but mostly only at the end of the atc concentration range, and 2-fold at best. Definitely doesn't look like a staircase function.

You can see the results in Dropbox / iGEM 2016 / FACS Data / 20160727 FACS

People are setting up Colony PCRs of the transformants from last night (all five of which grew!)

All are insert checks, but the UNS pTet GFP and the UNS tetR will also be PCR'd with primers to assemble them together into a UNS-backbone pTet GFP + tetR. 20160727 LK

Adam sent out the four minipreps of WM16_051 (which is 34 with broccoli) to Macrogen for sequencing.

Adam and I set up PCRs to swap the T7 promoter into 34 and 51 with and without RiboJ. These were:

Table1

	A	B	C	D	E	F
1	**Key**	**Template**	**Primer 1**	**Primer 2**	**T_anneal**	**t_extension**
2	A	NA	P156	P157	58	0
3	B	NA	P156	P158	58	0
4	C	WM16_034 1C3 MP2 160716 (1:10 dilution)	P15	P13	68	1:45
5	D	WM16_051 1C3 MP1 160726 (1:10 dilution)	P15	P13	68	1:45
6	E	WM16_034 1C3 MP2 160716 (1:10 dilution)	P66	P13	67	1:45
7	F	WM16_051 1C3 MP1 160726 (1:10 dilution)	P66	P13	67	1:45

Eventually the following were assembled:

- WM16_053 (UNS pTet GFP + tetR) from colony PCRs
- WM16_054 (T7 - RiboJ - B0034 - mCherry - Broccoli - DT)
- WM16_055 (T7 - B0034 - mCherry -Broccoli - DT)
- WM16_054 but with Spinach
- WM16_055 but with Spinach

We transformed 20160727 LK

- the above five assemblies into 3.300 LG
- 52S Kan MP2 160716 into 3.300 LG

- 52S Kan MP2 160716 + pACT-Tet Amp MP2 160630 into 3.300 LG

I used FlowJo to convert the results of the eight RiboJ parts' inductions, the eight RBS swaps' inductions, and the Interlab Measuerment devices into absolute fluorescence units.

> **Results can be found in Dropbox / iGEM 2016 / FACS Data / Absolute Facs.xlsx**
> WM16_025 looks defective despite sequence confirmation... WM16_027 looked weird too. Recall that these were measured on 160712 and were based on the transformations done on 160710... see 20160710 LK
> Still need to measure FL1 300 gain absolute beads to see if single-trasnformation RBS characterization levels match up with the maximally-induced cotrasnforation-with-respressor RBS characterization parts.

We inoculated WM16_023, WM16_022 (UNS ptet gfp and UNS tetr) into LB, as well as the non-UNS pTet GFP + tetR combo into LB, as well as the addGene Broccoli into 400mL LB for midiprep tomorrow for in vitro testing. 160727 CEM 160727-EMJ

160728 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-28

THURSDAY, 7/28

In @ 10:00 AM

All seven transformation plates grew extremely well!

Inoculations for miniprep didn't grow :(addGene broccoli for midiprep also didn't have enough DNA.

Likhitha and Christine are setting up Colony PCRs of the transformations. 20160728 LK

We assembled WM16_040 (J23100 Broccoli) and transfomred it 160728 AJR

We sent the RBS swaps (36, 44-49) to Macrogen for sequencing

We put the 57S + pACT-Tet in the plate reader after induction to measure.

WM16_035 promoter-swap PCR failed on the PCR that amplifies 35 + backbone without RiboJ. The others worked--- these were assembled and transformed. WM16_057 (WM16_035 with J23100) and WM16_058 (WM16_035 with J23115) [both having RiboJ]. 160728 AJR

WM16_025 (mp2 160614) AND wm16_027 (mp3 160608) were cotransformed with WM16_016 160709 "from MP2" into BL21 to re-get FACS data. 160728 AJR

We re-did inoculations of the UNS pTet GFP, UNS tetR, and non-UNS combo plasmids which didn't grow today. We also inoculated colony PCR results (52S, 52S + pACT-Tet, T7 promoter characterization variants for midiprep, addgene broccoli for midiprep)

Met with more people from Development office to try and establish iGEM's relationship with alumni network-- empahsized educators/teachers for spreading outreach materials. 11:30 AM - 1:30 PM

Worked on math model. I think I got the full lacI subsystem working with lacI flux from production from the upstream circuit and degradation using the rapid buffer approximation. Need to establish more confidence in the result but it reduces down to two ODEs tracking free lacI and free pLac.

Out @ 9:30 PM ish
In @ 11:00 PM ish
Out @ 11:30 PM

160729 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-29

FRIDAY, 7/29

In @ 10:00 AM

Looked into the PCR from yesterday that tried to amplify up the WM16_035 backbone-- WM16_P066 binds to mCherry so it's natural it didn't work. I designed WM16_P159 which is equivalent, but binds to sfGFP instead. Ordered it.

All the transformants grew! Likhitha set up Colony PCRs 20160729 LK and made glycerol stocks for the cultures that we are going to measure today.

Macrogen results came back for WM16_051... all of them looked bad in the same way as the original broccoli construct-- the first half of the broccoli region was there, but after that everything fell apart.

Talked with Dr. Saha about layout of equipment for the BioMaker Space

Ethan is setting up in vitro transcription tests for addGene broccoli

Out @ 1:30 PM
In @ 2:30 PM

52S in LG3.300 grew to midlog in LB. I FACS'd the three replicates at this point. Callan resuspended the rest of the culture with the same protocol as with the 52S + pACT-Tet cotransformation cultures to serve as a control FACS later on. 160729 CEM

The solo parts were extremely weak... <10 au at FL3 600 gain. Seems like autofluorescence. This is good because NRII is expressed in the pACT-Tet plasmid, so the enhancer shouldn't be able to fold over.

Adam is assembling pTet GFP + tetR combo onto 1A3 with a three-part assembly using all sequence-confirmed parts.

UNS versions of pTet GFP and tetR were minprepped, as were the pTet GFP + tetR combo (no UNS and UNS) on 1C3 and the T7 promoter characterization variants. 160729 CEM

Adam and Likhitha are transforming pTet GFP + tetR combo and cotransforming pTet GFp + tetR combo with addGene 85x tetO array, in many backbone combinations
20160729 LK

People inoculated the results of the colony PCRs. 20160729 LK

Out @ 7:00 PM
In @ 8:00 PM

Ethan and I FACS'd the 52S + pACT-Tet cotransformation... it worked!!!!!!

The induction curve looks a lot like the staircase function expected for having 2 tet binding sites, despite the fact that Orna Atar's sequence spreadsheet says it has 3.

Following this pattern, Ethan thinks the 57S part sholud have only one tet binding site (which would explain the lack of staircase induction), and that the 55AS part should have 3 binding sites.

Out @ 11:00 PM

160730 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-07-30

SATURDAY, 7/30

In @ 7:40 AM

WM16_035 + WM16_016 inoculation didn't grow... everything else did.

Diluted cultures for FACS; also inoculated more 52S + pACT-Tet from plate for plate reader.

LK is miniprepping the cultures. I made glycerol stocks with callan.

Building with Biology!

I set up PCRs of pACT-Tet into UNS 1A3 and 52S + DT into UNS 1C3.

Table1

	A	B	C	D	E
1	52S MP2 160716	P042	P043	63C	1:30
2	WM16_014 1C3 MP3 160605	P013	P040	68C	1:30
3	pACT-Tet MP2 160630	P044	P045	59C	1:30
4	WM16_014 1A3 MP1 160603	P013	P019	67C	1:30

Joe is DPNI ing the PCRs and running gels.

Gels looked great! 160730 JLM

Callan and Ethan are setting up inductions for IPTG of the 27 + 16 cotransformation in BL21.

In @ 3:30 PM

I'm FACSing the solo 57 and 58 transformations at midlog, just to confirm that they're fluorescent.

Things look as expected-- 57 is an order of magnitude brighter than 58, and both are quite bright. 57 MP3 has no fluorescence so that one will probably get disconfirmed.

52S + pACT-Tet inoculations grew to midlog but we decided not to run them through the plate reader as Joe pointed out that we do not have the final for-measurement 96-well plates. We'll order those and put that in tomorrow.

Ethan is checking Macrogen sequences of the RBS swap variants of 14 which we want to send to our collaborators.

WM16_048 is sequence disconfirmed. Everything else has at least one confirmed replicate.

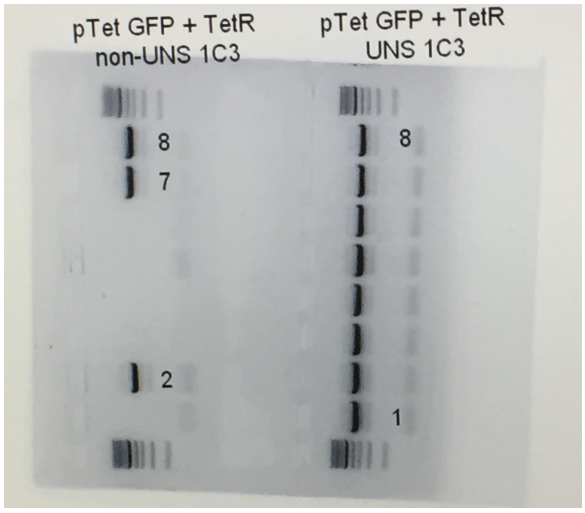
Out @ 6:00 PM

In @ 7:30 PM

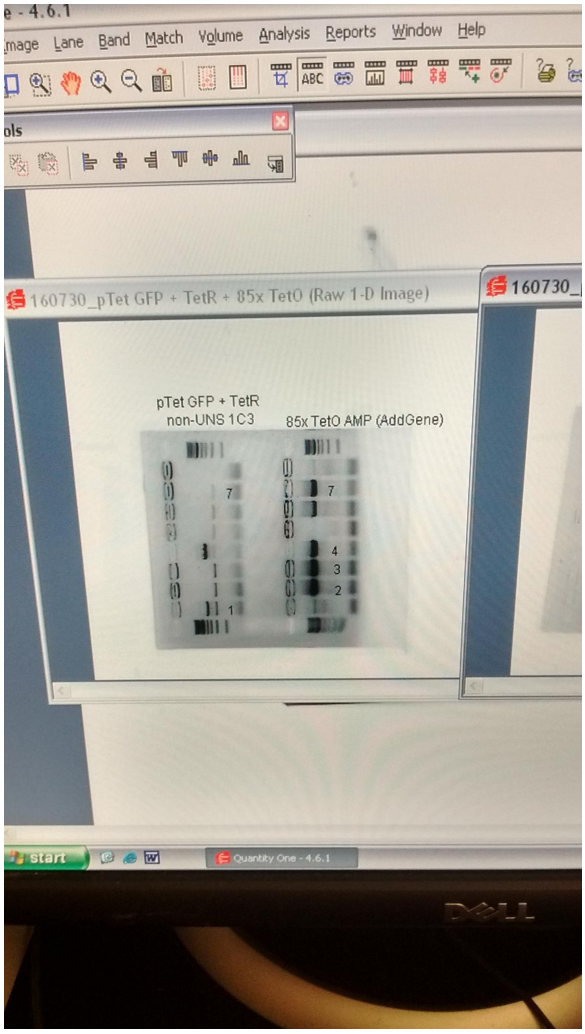
I FACS'd the WM16_027 MP3 1C3 + WM16_016 3K3 and got an actual induction curve when converted to absolute units--RiboJ graphs look less extreme (hugging one axis) now, but we can't know for sure about the replicating Fig. 3b until we get proper induction on WM16_025.

Callan and Ethan set up colony PCRs and inoculated the good colonies from teh three transformants that grew. pTet GFP + tetR combo on UNS 1C3, this part with tetO array (on amp from addgene), and pTet GFP + tetR combo on non-UNS 1C3. The rest of the combinations didn't grow on the plate. 160730 CEM160730 EMJ

File_000.jpeg



IMG_20160731_142129398_HDR.jpg



Out @9:00 PM
In @ 9:45 PM

> The fact that WM16_025 didn't grow is weird. 20160710 LK160710 AJR say only that they used WM16_025 "MP2 from Box 3", of which there are two (one disconfirmed, one confirmed). Perhaps they used the disconfirmed one? We should give the confirmed on (MP2 160614) a shot.

Out @ 10:10 PM

160731 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-07-31

SUNDAY, 7/31

In @ 1:00 PM

All three inoculations from last night grew. Adam is diluting them for induction and FACS today. 160731 AJR

Likhitha is making Chlor plates

I set up a PCR of:

- (1) pTet GFP + tetR (UNS) 1C3 MP2 160729 [this is WM16_053] with P008 P009, 64C, 1:00
- (2) WM16_014 1A3 MP1 160603 with P013, P019, 67C, 1:00

Likhitha is setting up Colony PCRs of the two plates which were plated last night (pACT-Tet UNS 1A3 and 55AS Kan + pACT-Tet amp) 20160731 LK

> *pACT-Tet looks great. the 55S + pACT-Tet cotransformation looks like it worked on one colony out of four, and for some reason the 55S insert only amplified with the non-DT overhang primers and not with the DT-overhang primers. Strange.*

Likhitha is setting up more colony PCRs on the cotransformation to get more successful colonies.

Joe and Adam did DPNI and PCR Purification of the WM16_053 insert + UNS 1A3 backbone PCRs

Joe set up Gibson Assembly of WM16_053 1A3

Joe and Likhitha transformed the assembly, as well as the glycerol streak of WM16_025 (to colony PCR to see if it grew properly) and WM16_048 to miniprep to sequence to get a confirmed MP to send to collaborators.

Out @ 7:00 PM ish
In @ 11:00 PM

Andy and I FACS'd the WM16_053 noUNS 1C3, WM16_053 noUNS 1C3 + 85x tetO addgene Amp, and WM16_053 1C3. All of the sequences looked like poor quality and weird induction. :(
Adam also set up aTc inductions of 52S + pACT-Tet cotransformation to repeat the staircase result, but we think we never added IPTG or something because nothing induced so we didn't record those.

We found out that the way we've been converting to absolute units is not correct, as the same sample measured under two different gains yields wildly different abs. unit results. Also, the line-of-fit from two different days of the same gain is different as well. Probably will have to repeat all our old samples' measurements.

Out @ 12:40 AM

160802 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-02

TUESDAY, 8/2

In @ 10:00 AM

Out @ 5:30 PM
In @ 7:00 PM

Out @ 8:00 PM
In @ 9:00 PM

Joe and Andy and I FACS'd the 55AS + pACT-Tet inductions (21 increments of aTc). None of them looked like they induced. At FL3 gain 500, we had

Table1

	A	B
1	1.a	3.7
2	1.u	4.7
3	2.a	4.5
4	2.u	5.7
5	3.a	4
6	3.u	5.2

which seems like none of the samples had proper alleviation of the repression-- all of them are at max-ish level of how the 52S sample looked. :(

160809 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-09

TUESDAY, 8/9

In @ 10:00 AM

We are beginning the two weeks! Joe and Andy are going to take care of wetlab and Andy is going to be the primary record-keeper for that. He's also going to be tracking the various project-level decisions and realizations we'll arrive at during this time. 160809 ADHI'm going to be working on the math modeling.

Worked through more of the rapid buffer approximation in the lacI submodel. Was running into issues doing proper phase plane analysis in the simple system (lacI + pLac only) after applying the approximation, as I wasn't sure how to treet [pLac] (as a state variable or as a defined function of [lacI]).

Out @ 2:00 PM
In @ 3:00 PM

Met with Smith for an hour and a half-- part of using the approximation is to treat [pLac] as a function of [lacI] (although it's still its own state variable...).

The general flow of the whole-system model seems fine according to Smith, although care should be placed in making these timescale-based approximations in different places within one model.

Continued working though thing with new insights, but now running into the problem of getting [pLac] to spike down to 0 often. This is a phenomenon that often occurred for Jmitch's whole-system numerical solutions, which I'm not getting when I numerically solve the single [lacI] ODE and apply the RBA to determine [pLac]. The problem is that the RBA expression for [bound pLac] is such that at small values of K_D, the expression will end up being very close to P_tot regardless of [lacI]. Terrible!

I'm concerned because the RBA expression for [bound pLac] is really coming from the mass-action ODEs... it's only an RBA expression because there's a [lacI] state variable in there, rather than some function of a conserved L_tot. The structure driving [bound pLac] toward P_tot is almost invariant to that. So that's a problem.
Why does this happen? There are only a few parameters involved in the simplified system so everything is BioNumbers-certified (a few nuances here that I may have misinterpreted, but.). I'm hoping that this is just a function of the fact that there are no competitors for lacI binding than the pLac promoter, but I'm a little afraid that perhaps the use of continuous equations to model a process with relatively small molecular counts (~200 pLac sites per cell on pSB1C3 backbone) might be suppressing important dynamics in processes like unbinding through its bulking of system dynamics.

Out @ 9:30 PM
In @ 10:45 PM

I transformed the nine gibson assemblies from today (testing promoter insertions into various RBS-variants), as well as a WM16_053 1C3 with and without the addGene 85x tetO array 160809 ADH

I inoculated colonies for plate reader tomorrow--

- WM16_014 1c3 160603 *for positive control*
- Interlab Device #1 160606 *for positive control*
- Restreak of Interlab Negative Control GS1 fgrom 16 JPM 160605 *for negative control*
- 20160728 52S MP2 (Kan) + pACT-Tet MP2 (Amp) *to measure*

in the incubator @ 12:00 AM

160810 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-10

WEDNESDAY, 8/10

In @ 11:30 AM

Good to note from an abstract Andy found (http://cnls.lanl.gov/q-bio/wiki/images/e/e8/10_039_abstract.pdf):

> Degradation of TF when bound to DNA is variable... some are degraded actively in their bound state (VP16 in yeast) whereas others are almost immune to degradation (p53, MyoD).

I wrote a MATLAB script to calculate dilution conditions for plate reader on the sigma 54 parts. The atc concentrations are evenly log-spaced betwen 1 and 10,000 ng/mL atc.

> These are done by putting 100 uL of culture-containing Phillps broth solution with 2 mM IPTG into each well of the plate, and creating 100 uL of various-concentration 2X aTc solution in Phillips broth. The 100 uL of aTc are added to each corresponding well in the plate, creating a final volume of 200 uL containing 1X variable aTc concentration and 1 mM IPTG concentration in the well, as required.

The script is called aTC diluter and is in iGEM 2016/workspace/john

Out @ 1:45 PM

In @ 2:30 PM

Realized that it doesn't make a lot of sense to have pLac be a constant term in the model if lacI's degradation is governed by cell division... the same process is affecting lacI and pLac equally, basically, so lacI flux should be complemented with pLac flux. RBA is going to be a little trickier but should still be possible, with lacI+pLac complex equilibrating rapidly to the slower timescale dynamics of fluxing lacI and pLac.

Out @ 4:00 PM

In @ 5:00 PM

Worked more on pursuing this thought.

Out @ 8:00 PM

160811 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-11

THURSDAY, 8/11

In @ 10:30 AM

Met with Dr. Smith about the following questions:

- In the new fully-fluxing model, rapid equilibration only accounts for a portion of each species' dynamics, as there is a slow-timescale flux for each species now. How do I properly make the RBA on the fast-timescale dynamics only?
- From the original simple model where only lacI was fluxing, we had that our initial conditions (0 lacI and P = P_tot) placed us on the P nullcline from the beginning of every trajectory... given that, shouldn't the RBA be exactly equal to a full model run?
- The use of alpha_L to lump the upstream circuit's protein production dynamics together seems problematic to me. Where are the holes in this method (the timescale-collapse of protein production seems problematic, and I don't feel comfortable about multiplying the empirical GFP transfer function by deg_GFP / deg_lacI to convert to a functionally empirical lacI transfer function) and how can we fix it?

Talk notes:

- RBA involves obtaining an equlibrium expression for only the fast-timescale dynamics... then this expression is used to obtain other relevant expressions. Basically I just ignore the slow-timescale portions of the relevant ODEs when getting this equilbrium expression.
  - A bigger point, though, is properly accounting for "degradation" via dilution via cell division... even ignoring the discrete nature of division we talked through a lot of the nuances involved in tracking state variables as concentration when volumes are changing underneath them and how to appropriately model that.
- Didn't reach this point
- Didn't reach this point
- Additional point: The fact that the pLac seems to be completely sequestering the lacI in the simple open model is a deterministic function of the KD of pLac-lacI binding, and the nature of the expression says that bound pLac at steady-state is going to be veyr close to P_tot (this is from yesterday). However, Dr Smith pointed out that in an open system lacI is going to continue fluxing into the system to occupy all 'buffers' before free lac reaches its steady state value... hence adding additional decoy sites in an open system won't solve this problem. Maybe letting P_tot flux as well might do something, but it's hard to tell before doing it.
  - The bigger thing might be that the KD I've inputted into the model might be misinterpreted, or my steady-state lacI concentration isn't right (it might be too high given that I really need to be thinking about *L_tot* at steady state, not free L).
    - Actually now that I'm writing this, this probably is the right answer. So what's the proper way to incorporate L_tot steady-state value into the model this way? I need an expression that can go from L_tot_ss (which comes from the empirical function) to L_ss (by exlucding out the binding affinity) in the proper way.

Out @ 6:30 PM

-

160812 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-12

FRIDAY, 8/12

In @ 11:00 AM

So far we know that the pLac suppression phenomenon is bound to happen given the small K_D value for lacI - pLac affinity we're using, and that this can only be counteracted by having a low value of L_{ss} to drive up the relative size of the K_D term in the LP and P expressions. I pursued the idea that a more accurate L_{ss} could be obtained by tuning Ltot_{ss} to the observed empircial transfer function, and determining L_{ss} as a fraction of this Ltot_{ss} value. I obtained this expression but it still yields a value of L_{ss} that, in the neighborhood of our current parameters, is still much too high to rescue the system from the pLac -> 0 phenotype.

I am now pursuing the alternative option of introducing flux to non-free-lacI elements. I'm starting by restricting flux to lacI only, and simply allowing degradation to occur to DNA-bound lacI as well as free lacI. This formulation assumes that lacI is being degraded at a rate faster than cell division-induced dilution (which is definitely valid for the LVA-tagged tetR which we'll do later... but I don't know about lacI) because I'm not introducing any degradation terms for the DNA elements.

160813 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-13

SATURDAY, 8/13

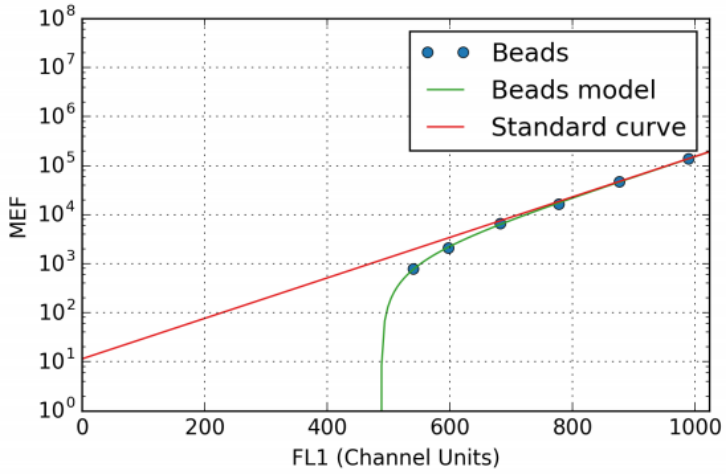
In @ 3:00 PM

We forgot to turn in our Project Title, Abstract, and Track Selection yesterday. I filled that out and sent an email to HQ. Seems okay though since the iGEM system let me modify the information.

How does one convert arbitrary fluorescence units to absolute units? Looking into how to properly convert relative fluorescence masurements to absolute units using the Spherotech beads. Reading up in the FlowCal documentation... Fig. S9 of the FlowCal paper, though, suggests that our initial linear-fitting method was incorrect (instead of a nonlinear green line, we were simply fitting a linear function to our beads and calling that our standard curve):

clipboard_2016-08-13_17:03:14.png

Figure S9. Standard Curve



A typical plot showing the calculated standard curve, as generated by FlowCal as part of the Excel UI workflow. Each blue dot represents the fluorescence in channel units (x axis) and in MEF units (y axis) of each subpopulation of the calibration particles. The green line shows the fitted beads fluorescence model with bead autofluorescence. The red line shows the resulting standard curve, used later to convert cell samples to MEF.

From "**FlowCal: A User-Friendly, Open Source Software Tool for Automatically Converting Flow Cytometry Data from Arbitrary to Calibrated Units**"

Their github documentation doesn't describe their "Beads Model" in detail, but the significant point seems to be that they fit their bead data to a model which accounts for autofluorescence. After this fitting, a standard curve is somehow drawn from the fitted curve (I imagine I could go into the code to figure out what's going on, but it's not clear from their documentation).

Turns out the beads model is fairly straightforward:

m*log(flx_refbeads_au[i]) + b = log(flx_refbeads_mef[i] + flx_auto_mef)

to yield the standard curve

fl_mef = exp(m*log(flx_refbeads_au + b)

which apparently ignores autofluorescence in cellular samples, thus requiring the user to "use an appropriate white cell sample to account for cellular autofluorescence if necessary". So we'll have to do that.

Regardless, it seems like one simply needs to adopt this new procedure and the MEFL conversion will be easy enough.

A bigger issue is that according to the actual FlowCal paper, ***"if microbead and cell fluophores have different spectra, calibrated cell fluorescence data are instrument-dependent"***. And at the time of publication, there apparently weren't any reliable / good calibration beads for sfGFP. This instrument-dependence is supposed to be linear when MEFL (instrument 1) vs. MEFL (instrument 2) is plotted, so one "simply" needs to measure the same biological sample in each instrument and calibrate accordingly. This effect is illustrated in Fig. 2F, reproduced below:

clipboard_2016-08-13_17:34:14.png

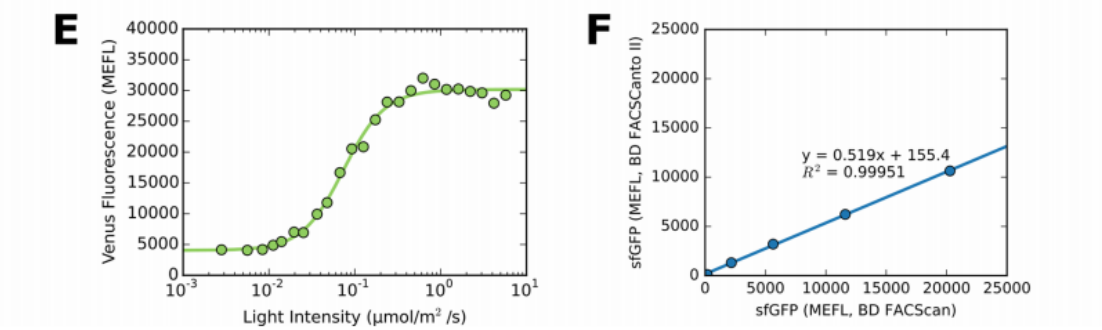


Figure 2. FlowCal histogram plots of a single sample of *E. coli* BW29655 expressing sfGFP from the IPTG-inducible promoter P_{tac}[21] when fully induced, measured at 10 different detector gain settings in (A) a.u., and (B) MEFL. Histograms of fluorescence values (geometric mean of each population) of *E. coli* expressing the CcaS-CcaR v2.0 system[22] in the dark, taken from 54 experimental replicates carried out over eight months in (C) uncalibrated arbitrary units (left) and (D) FlowCal calibrated molecules of equivalent fluorescein (MEFL) values. Robust coefficient of variation (RCV) is shown above each data set. (E) Dose response of the light-responsive PhyB-GBD/PIF3-GAD system in *S. cerevisiae*,[24] where the yellow fluorescent protein Venus is transcribed in response to red (647 nm) light. After incubating cells under the specified intensity of light for 18 h, fluorescence was measured *via* flow cytometry, and the resulting data was analyzed using FlowCal. (F) *E. coli* BW29655 expressing sfGFP from the IPTG-inducible promoter P_{tac} were induced with 0, 81, 161, 318, or 1000 μM IPTG and measured using two different flow cytometers, along with calibration beads. The resulting data was calibrated to MEFL using FlowCal. Geometric mean fluorescence values in MEFL obtained from both instruments are plotted against one another, for each sample. A least-squares linear fit is also shown.

From "**FlowCal: A User-Friendly, Open Source Software Tool for Automatically Converting Flow Cytometry Data from Arbitrary to Calibrated Units**". Apparentliy, "MEFL" doesn't mean "absolute" in an absolute sense... it's still instrument-calibrated. In this example, their two instruments both used 488 nm lasers but had different emission filters.

So apparently, conversion to MEFL solves the problem of *(a)* switching gains/settings and *(b)* instrumental setting drift over time (which is basically the same as switching gains/settings, except that you don't do it on purpose), but not the problem of *(c)* measured units being instrument-specific.
But I think it's fine-- it seems that the conversion across instruments only needs a one-time calibration to obtain a graph like Fig. 2F, after which apparently (according to the FlowCal paper again) one can simply drop the constant term in the linear function and get a linear conversion function from instrument to instrument. Furthermore, reporting our characterization data in MEFL will still lend much greater credence to the validity of our results etc.

> I also don't know if "instrument" refers to an individual instance of an instrument, or a model of instrument. If it's the latter we're even better off. I think it's the latter.

How often should one measure the calibration particles? The FlowCal paper implies that one should really be measuring the calibration beads with each sample, but if not, with each setting configuration on a given day, meaning that a particular FL1 gain level on two different days requires two different calibration runs. However, their evidence for instrumental drift-over-time is reported only as a final net effect from an 8-month timeframe... I wonder how important smaller-timescale differences are? Obviously the safest way is to measure the calibration beads every time but I don't know if this means some of our old measurements (which don't have calibrations from *that* day) would have to be thrown out.

Since I've been referencing it so much I'm just going to attach the FlowCal paper here.

FlowCal- A User-Friendly Open Source Software Tool for Automatically Converting Flow Cytometr y Data from Arbitrary to Calibrated Units.pdf

I know FlowCal was running into problems processing our FCS files when Andy tried it earlier this summer... I think I'll give it another shot now that I've read more of the documentation. Also, FlowJo should also be able to do this with a built-in Calibration module. If FlowCal doesn't work I'll read the documentation for that and try that as well.

Interestingly it seems that FlowJo is not using any special 'beads model' in their calibration, but rather just fitting a semilog-linear curve to the calibration bead FCS file. Perhaps there is some nuance we were missing when we were doing basically the same thing in Excel? Regardless we observed the phenomenon where the same sample measured on two different gains yielded wildly different MEFL results using the Excel method, so if FlowJo ends up fixing that problem then I'm content with that.

A question remains... how to convert from MEFL to number of proteins??

I also found a great paper from Stanford Bioengineering that basically says one should use **OD700** to measure cell density, as RFPs like mCherry absorb 600nm light. Seems like an easy-to-incorporate increase in rigour for us.

When wavelengths collide- bias in cell abundance measurements due to expressed fluorescent proteins.pdf

Out @ 7:00 PM

160814 JPM

Made with Benchling
Project: iGEM 2016
Authors: Joseph Maniaci
Date: 2016-08-14

SUNDAY, 8/14

In @ 11:00 AM

I got FlowCal to work on our FCS files. I had to make modifications in FlowCal's "io.py" module and "transform.py" module in order to do this, so we are now off the beaten path. Modifications were minor, though, so I don't think it should cause any problems. Here's what I did:

- FlowCal expects the Supplemental Text portion of an .fcs file to begin with the delimiter character, which for whatever reason the portions from our .fcs files don't. I wrote exceptions to the error which is thrown here, and patched up downstream housekeeping to account for the lack.
- FlowCal expects there to be no value for acquisition settings in channels for which they're not relevant, like Amplifier Gain in a FSC channel. For whatever reason our .fcs files contain a 0 value instead. I re-wrote the exception (which defaults amplifier gain to 1) that FlowCal has for the blank scenario, so that it includes a 0-value scenario.

Additional information needed to get FlowCal to work:

- The documentation suggests that, when using the Excel UI, naming the channels as 'FL1' or 'FSC' or the like is sufficient to get the program to work. It's not. You have to specify 'FL1-AREA' or 'FSC-AREA' or the like, as our .fcs files recognize these as distinct channels from 'FL1-HEIGHT' or 'FSC-WIDTH', etc. Furthermore, you can have as many fluorescent channels recorded in the instrument as you want, but the software only supports one FSC, SSC, and TIME channel each. I think it would be most appropriate to use 'FSC-AREA' and 'SSC-AREA', as these are what we use to gate our samples in ProSort. Thankfully, 'TIME' does not have to be described further even though the .fcs file actually contains two Time channels (these are actually linked in a way that none of the other channels are).

More detailed info contained here:

IMPORTANT_README.txt

However, there are still uncertainties in how to do the au -> MEFL conversion, this time at the level of the Spherotech beads. We have been using the chart on p. 26 of their beads documentation (attached below) to determine the MEFL level for each bead peak, but there are two important things:

- The table seems to correspond to the graph below it, which explicitly is set to the FITC Channel (FL1).
- The graphs on the left hand side of p. 26 have different MEFL values for different channels
- The six peaks on the left-hand graphs are supposed to correspond to the brightest five and blank of the eight-eak rainbow beads on the right-hand side, and indeed the MELF values are in a similar neighborhood between these sets.
- Does this mean that we need to have an FL3 MEFL table to do au -> MEFL conversions in FL3? This doesn't exist in the pdf. Do we need to contact Spherotech??

Spherotech Rainbow Calibration Beads for Cyto metry Performance Verification.pdf

Joe and Andy set up Minipreps of the promoter swaps with and without RiboJ (key 1A-25A, 1B-25B) as well as the lacO array from addGene (pJD100) and the WM16_025 redo.
I set up glycerol stocks of the above miniprepped cultures. The promoter swaps are labeled only with their key ID and are stored in three dedicated boxes labeled as the 160814 promoter swap glycerol stocks.

1B-8B were not glycerol stocked as the miniprep procedure was messed up, and all the culture was used up.

Out @ 8:00 PM
In @ 9:30 PM

Joe and I set up inoculations of the transformations from today, as well as the re-plates of previosu transformations:

Table1

	A	B	C	D	E
1	**Key**	**Purpose**	**Sample**	**Strain**	**Media**
2	1	**Repeat successful synthetic enhancer measurements**	52S Kan MP2 160716 + pACT-Tet Amp MP2 160630	LG3.300	LB
3	2	**Test 55AS**	55AS Kan MP1 160716 + pACT-Tet Amp MP2 160630	LG3.300	LB
4	3	**Test UNS 52S with UNS pACT-Tet**	52S DT UNS 1C3 MP1 + pACT-Tet 3K3 from restreak [160707] MP1	LG3.300	LB
5	4A	**Repeat aTc Induction**	pTET GFP (1c3) from MP2 (Box 3 slot 58) + tetR (3k3) from MP1 (Box 7 Slot 61)	5 alpha	M9 Thiamine
6	4B	**Repeat aTc Induction**	pTET GFP (1c3) from MP2 (Box 3 slot 58) + tetR (3k3) from MP1 (Box 7 Slot 61)	10 beta	M9 Leucine
7	4C	**Repeat aTc Induction**	pTET GFP (1c3) from MP2 (Box 3 slot 58) + tetR (3k3) from MP1 (Box 7 Slot 61)	BL21	M9
8	5A	**Test combination**	pTet-GFP + TetR (UNS) 1C3 MP1 (Box 9 Slot 22)	5 alpha	M9 Thiamine
9	5B	**Test combination**	pTet-GFP + TetR (UNS) 1C3 MP1 (Box 9 Slot 22)	10 beta	M9 Leucine
10	5C	**Test combination**	pTet-GFP + TetR (UNS) 1C3 MP1 (Box 9 Slot 22)	BL21	M9
11	6A	**Test array**	pTet-GFP + TetR (UNS) 1C3 MP1 (Box 9 Slot 22) + 85x TetO addgene Amp MP1 (Box 6 Slot 64)	5 alpha	M9 Thiamine
12	6B	**Test array**	pTet-GFP + TetR (UNS) 1C3 MP1 (Box 9 Slot 22) + 85x TetO addgene Amp MP1 (Box 6 Slot 64)	10 beta	M9 Leucine
13	6C	**Test array**	pTet-GFP + TetR (UNS) 1C3 MP1 (Box 9 Slot 22) + 85x TetO addgene Amp MP1 (Box 6 Slot 64)	BL21	M9
14	1B	**J23100 w/out RiboJ**	from 160812 Gibsons	5 alpha	LB
15	2B	**J23101 w/out RiboJ**	from 160812 Gibsons	5 alpha	LB
16	3B	**J23102 w/out RiboJ**	from 160812 Gibsons	5 alpha	LB
17	4B	**J23103 w/out RiboJ**	from 160812 Gibsons	5 alpha	LB
18	5B	**J23104 w/out RiboJ**	from 160812 Gibsons	5 alpha	LB
19	6B	**J23105 w/out RiboJ**	from 160812 Gibsons	5 alpha	LB
20	7B	**J23106 w/out RiboJ**	from 160812 Gibsons	5 alpha	LB
21	8B	**J23107 w/out RiboJ**	from 160812 Gibsons	5 alpha	LB

6A didn't grow on the plate. 6B and 6C had very minimal growth-- only 2 or 3 colonies which were very large.
In the incubator at 11:00 PM

For the above inoculations, I made 100 mL of M9 Leucine by adding 1 mg Leucine to 100 mL M9 Glycerol media.
I also made the M9 Thiamine solution by adding 1 uL of 1 mM Thiamine to 10 mL M9 Glycerol.

Out @ 11:15 PM

160815 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-15

MONDAY, 8/15

In @ 10:00 AM

I got the MEF calibration excel template from Spherotech (saved in Dropbox now). For our S3e Cell Sorter I think appropriate settings are

- FL1 -> MEFL (Fluorescin)
- FL2 -> MEPE (PE)
- FL3 -> MEPETR (PE Texas Red)

I ran through the one data pair that we have of one sample measured on two gains (FL3 600, FL3 800) using FlowCal's standard curve generated from the Spherotech MEPETR calibration settings.

Note that FlowCal's Excel output will tell you the Beads Model parameters, which are given as m*log(fl_au) + b = log(fl_mef_auto + fl_mef). The Standard Curve, according to their github documentation, is fl_mef = exp(m*log(fl_au)+b). See 160813 JPM

From 8.2 au (FL3 600) and 97.4 au (FL3 800), I got that MEPETR = 0.776 (FL3 600) or 1.098 (FL3 800), which is still different.

However compared to our original values (1577.7 MEFL (FL3 600) vs. 470.8 MEFL (FL3 800)), these new values make more sense... the sample was a non-IPTG-induced 52S + pACT-Tet cotrasnformation, so there should theoretically be no mCherry present, hence a very low absolute fluorescence value would be expected. In hindsight this was a pretty bad sample to run the two-gain test on since it's so close to the edge of our measurement range... when we re-FACS sfGFP samples tonight we should measure them on two FL1 gains and do the same test.

Another issue with this sample is that we only manually recorded a mean of the distribution instead of getting a .fcs file-- I don't really even remember what the distribution looked like. FlowCal can generate statistics about the distributions (in MEF) which would make the conversion results much more interpretable.

I ran the 160711 InterLab Measurement files through FlowCal. Comparing the resulting MEFL distributions to the mean-MEFL values we got from the old FlowJo-based method. For the most part the values tend to agree at the order-of-magnitude level. Some differences arise at the <10x fold level between the values. The trend in fluorescence follows the expected trend given what the Interlab devices are. The saturated samples look closer than the midlog samples do between the two methods.

Out @ 1:00 PM
In @ 2:00 PM

Reading into the proper way to convert fluorescence measurements to protein counts. The process is... going to be difficult.

Moving into the new Bio MakerSpace!

Out @ 6:30 PM
In @ 7:00 PM

Moving into the new Bio MakerSpace!

Out @ 11:45 PM

160816 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-16

TUESDAY, 8/16

In @ 10:30 AM

Joe and Andy are prepping all of the promoter swap (w/ and w/out RiboJ) minipreps to send to Macrogen for sequencing.

Joe and Andy are also prepping minprep solution of the 8 RBS swaps to send to Pitt iGEM.

We sent 10 uL of 1:10 Miniprep:NFW solution for each of:

Table1

	A	B
1	**Sample**	**Location**
2	WM16_035 MP3	Box 7 Slot 43
3	WM16_036 MP1	Box 8 Slot 54
4	WM16_044 MP1	Box 8 Slot 57
5	WM16_045 MP1	Box 8 Slot 60
6	WM16_046 MP2	Box 8 Slot 63
7	WM16_047 MP3	Box 9 Slot 2
8	WM16_048 from GS1 RS1	Box 9 Slot 49
9	WM16_049 MP1	Box 9 Slot 6

For parts with several sequence-confirmed MPs, we used the lowest-numbered confirmed MP for all parts except WM16_035. The MP1 for WM16_035 (Box 7 Slot 42) is missing.

I'm running the 0726 RBS characterization data (with 16 3K3 and IPTG induction) through FlowCal. I used the WM16_035 samples as a trial run and the resulting MEFL distributions fit pretty closely to the mean MEFL values we'd recorded using the old method-- it seems like the agreement is pretty close between the methods in this region of the setting / measurement value space.

All FlowCal data in *Dropbox/FACS Data/FLOWCAL RESULTS*

Remember that with this dataset it like some of the samples had the 10 uM condition mixed up with the 100 uM condition. The MEFL intensities of the different RBS samples at 10 mM IPTG generally follow the same ranking as they did using the old method-- RBS values that were close on the old method have a few rank-swaps but are still close using FlowCal's MEFL conversion. Generally clusters are retained.

This concludes the FlowCal conversions that we can do right now-- we didn't save .fcs files for the RiboJ constructs, and we didn't save .fcs files for the 52S + pACT-Tet instance that we had that worked.

Out @ 2:30 PM
In @ 3:30 PM

Continued moving into the Bio MakerSpace.

Andy and Joe are inducing the three Synthetic Enhancer transformations with aTc. We are doing 24 points of aTc concentration (evenly logspaced between 10^0 and 10^4 ng/mL) each for FACS tomorrow after an overnight of induction.

aTc (ng/mL)

	A	B	C	D	E	F	G
1	1	1.492496	2.227543	3.324598	4.961948	7.405685	11.

This much aTc in addition to 1 mM IPTG in 500 uL final concentration Phillips Broth. LB cell culture was spun down and resuspended into these.

Thinking more about the transition from the empirical transfer function to our model. There seems to be two main routes one could go:

- (1) Using our model, to understand the function of each parameter change (toolbox part) as an operator that acts on input functions and produces output functions. The calculator has a method that optimizes the sequence of parametrized operations that best approximates the desired target function given the input empirical function.
- (2) Figuring out somehow the number / concentration of lacI (or other modified circuit component) which would be produced had it replaced the reporter protein in whatever assay the user used to generate the empirical transfer function. Then, given this information, running the model many times to generate final transfer functions. These final functions are then assessed to find a best-fit match to the desired target function.

Both are difficult...

My current state of mind is that the first part of (2), making the conversion from empirically reported transfer function to equivalent transfer function of lacI, is going to be too difficult. I am also afraid that noise here would throw off a lot of the efficacy of the model, and much of it can be experimental. I'd like to try (1), but there's always the possibility that we end up being unable to determine a general definition of each parameter as an 'operator' on functions. Perhaps it'd be possible to do some sort of exhaustive list, though, if we can't have some defined form...?

Even so, (1) only holds an advantage over (2) if the forms of these 'operators' end up being independent of the actual value of alpha in the model and in the functions... otherwise, we'd still need to determine it, which defeats the whole purpose.

Basically, I need to know if [d(Hill Parameter)/d(Model Parameter)]/d(alpha) = 0. Otherwise, (1) is not really worth pursuing, I think.

I might be able to get by if [d(Hill Param)/d(Model Param)]/d(alpha) is nonzero but independent of alpha... need to think more on this one.

Out @ 10:30 PM

160817 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-17

WEDNESDAY, 8/17

In @ 11:30 AM

Thinking more about how empirical functions are going to be fed into the model (and hence the calculator). I think I've convinced myself that the idealized test-case of having the empirical function be a series of ([input], alpha) values is okay, but I still need to convince myself that results from this would be generalizable if they turned out to be usable.
I need to determine how best I can formulate and interpret d Hill Param / d Model Param. I'll spend a little more time on this before settling on brute-forcing lots of model runs and going back to work on the model.

Set up Macrogen sequences of WM16_053 1A3 and WM16_053 1C3 with Joe.

Out @ 3:00 PM
In @ 4:00 PM

Joe and Andy are FACSing the Synthetic Enhancer parts in LG3.300:

Table1

	A	B
1	**Key**	**Part**
2	1	52S Kan MP2 160716 + pACT-Tet Amp MP2 160630
3	2	52S Kan MP2 160716 + pACT-Tet Amp MP2 160630
4	3	52S Kan MP2 160716 + pACT-Tet Amp MP2 160630
5	4	55AS Kan MP1 160716 + pACT-Tet Amp MP2 160630
6	5	55AS Kan MP1 160716 + pACT-Tet Amp MP2 160630
7	6	55AS Kan MP1 160716 + pACT-Tet Amp MP2 160630
8	7	52S DT UNS 1C3 MP1 + pACT-Tet 3K3 from restreak [160707] MP1
9	8	52S DT UNS 1C3 MP1 + pACT-Tet 3K3 from restreak [160707] MP1
10	9	52S DT UNS 1C3 MP1 + pACT-Tet 3K3 from restreak [160707] MP1

Sample 2 was not measured because 2.7 kept clogging the FACS

with 1 mM IPTG and the aTc concentrations described:

aTc (ng/mL)

	A	B	C	D	E	F	G
1	1	1.492496	2.227543	3.324598	4.961948	7.405685	11.

6, 7, 8, 9 did not use the 10,000 ng/mL concentration because we ran out of aTc.

I thought more about the problem of converting the empirical transfer function to a production parameter that can be used in the model.

Out @ 10:00 PM

160818 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-18

THURSDAY, 8/18

In @ 10:00 AM

Met with Development to discuss long-term stable external funding sources for iGEM at W&M. Emphasis on annual commitments by a smaller number of wealthier donors rather than crowdfunding.

Joe and Andy are checking sequence data for the promoter swap constructs.

I'm running last night's FACS data on Synthetic Enhancer constructs through FlowCal.

> I ran the samples of 9.1, 9.12, and 9.23 done on FL3 gains 400, 500, 600, 700, and 800. The distributions look basically the same when converted to MEPETR!!! It seems like the absolute-unit conversion works with FlowCal. The synthetic enhancer constructs exhibit induction, but no staircases, on MEPETR.

Met with Dr. Smith to talk through the Flx -> Concentration issue. Didn't get to work through much because the meeting was shorter-- spent some time talking about the time-scale collapse that occurs when condensing the arbitrary genetic circuit that the user has predesigned into one protein production step... haven't finished the analysis but it's looking like it's going to be an unavoidable but not-too-catastrophic assumption to make. Still need to do more thinking on the Concentration issue.

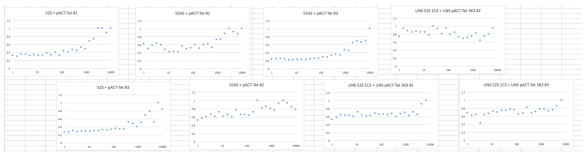
Met with Dr. Saha to talk through Honors Lab design.

Out @ 6:30 PM
In @ 7:30 PM

I converetd the synthetic enhancer data to % of Max MEPETR to see if that would change the effect... still no staircases. I haven't explicitly plotted the x axis values on a logscale, although since they were chosen to be evenly spaced on logscale then the Excel default of evenly spacing the value shouldn't affect anything. :(

They look like this:

Screen Shot 2016-08-18 at 8.31.47 PM.png



But some of them I could convince myself...

Looking into how other predictive models handle the Fluorescence -> Concentration issue. Voigt's Cello paper uses RPUs for everything, and since they have no dynamics in their models (they just compose steady-state transfer function values together) they don't need to worry about incorporating dynamics via concentration.

Out @ 9:00 PM

160819 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-19

FRIDAY, 8/19

In @ 11:10 AM

Met with Dr. Smith to talk through the Fluorescence -> Concentration conversion. Didn't make much headway. Don't think we can retain the mechanistic-modeling aspect we've been using, as well as the predictive power, using this approach.

Found a Jim Collins paper ("Diversity-based, model-guided construction of synthetic gene networks with predicted functions", Nature Biotech 2009) that uses predictive genetic circuit design modeling with RPUs. The difference between their model and ours is that they represent promoters as Markov Chains which can transition, with derived probabilities, between states of various repressor-occupancies, and that these states have empirically measured production functions associated with them. Co-opting this method of modeling will take some work as some of our toolbox components (decoy binding array in particular) are explicitly concentration-dependent, and so the idea of scaling all transfer functions to be "with respect to saturation" does not immediately translate. Working on this.

Out @ 2:00 PM

In @ 3:00 PM

Did statistics on determining confidence for observed Pearson's correlation values for calcium entropy vs. FISH score for Calcium 2 dataset for Dr. Saha.

Out @ 5:00 PM

160822 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-22

MONDAY, 8/22

Today's To-Do

Decoy Binding Array

- ☒ Diagnostic Transformation: I would like to try solo transforming the addGene 85x tetO array into the BL21 strain. Has this been done before? If not, there might be a strain-incompatibility issue with the particular part.
  - ☒ Diagnostic Transformation: When doing this I would also like to solo transform a pSB1C3 85x tetO array into the BL21 strain to see if the issue is with the part and BL21 or with the backbone and BL21.
- ☒ For-Eventual-Induction Transformation: Since WM16_053 1A3 was sequence confirmed today, we should solo transform this into BL21. We should also cotransform it with tetO arrays on 1C3 and 3C5.

  (85x tetO 3C5 turns out to not exist. Did the solo transformation and the co-transformation with 85x tetO 1C3) All transformations see 160822 *.*
- ☒ Assembly Pipeline: I would like to move the pTET GFP + tetR construct (WM16_053) onto another backbone (again)... 1A3 has been tried several times unsuccessfully. Let's try pSB1K3.

  (WM16_053 1A3 ended up being sequence-confirmed today in new Macrogen Results... however, WM16_053 on pSB1K3 was also assembled and transformed for miniprep)
- ☒ Ethan Assembly Pipeline: Let's try and move the 85x tetO array onto 1A3.

  (got to the overnight ligation step 160822-EMJ*)*

Synthetic Enhancer

- ☐ Troubleshoot by Reading and Thinking: So far we have a few instances of 52S (two tetO sites) and one instance of 55AS (three tetO sites) displaying a staircase-induction in BL21, but we also have other instances of these same constructs in the same strain in the same experiment not working as advertised. What's going on? Is it just that noisy? Are parts working in some replicates and not in others? How can we fix this?

  By the time we get to combining parts together to make a circuit modification, we need to be able to count on the synthetic enhancer working without having to go all the way to the measurement stage to determine if the construct didn't work.

Promoter Characterization

- ☒ Macrogen Sequencing: Need to send out the re-dos of the failed promoter constructs to Macrogen to sequence. See 160820-EMJ

Model

- ☐ Read and Think: The issue of Fluorescence -> Protein Concentration (see earlier instances of JPM Benchling) still needs to be resolved. I think the Collins paper's probability-based model (160819 JPM) holds promise but it needs to be modified to be able to incorporate explicitly concentration-dependent phenomena like molecular titration.

In @ 10:00 AM

Ethan is setting up sequences to send to Macrogen.

Andy is setting up PCRs to move WM16_053 (pTet GFP + tetR) 1C3 onto 1K3 backbone.

- Backbone: WM16_014 Kan MP1 160609 Box 3 Slot 19; P013 P019
- Insert: pTet GFP + TetR (UNS) 1C3 MP1 160729 Box 9 Slot 22; P008 P009

Andy is setting up DpnI of the PCR products. Will be done at 2:30 PM.

I'm reading more into the Collins model, specifically how they go from the dose-response curves they characterize for each promoter in their library to the actual efficiency parameters they use to *predict* protein level.

The gist seems to be that they infer a lot of their parameters via curve-fitting to observed dosage-response curves (we can do this), except for a few key parameters related to the actual efficiency of the production (and it is from these parameters that the conversion from Absolute Fluorescence to Concentrations that needs to happen within the model takes place). These are inferred from minimal and maximal absolute fluorescence values of each relevant promoter expressing a standardized construct... the nature of the actual inference is stilll unclear. Still-unspecified parameters are determined through an almost Monte Carlo-like procedure within a bounded region.

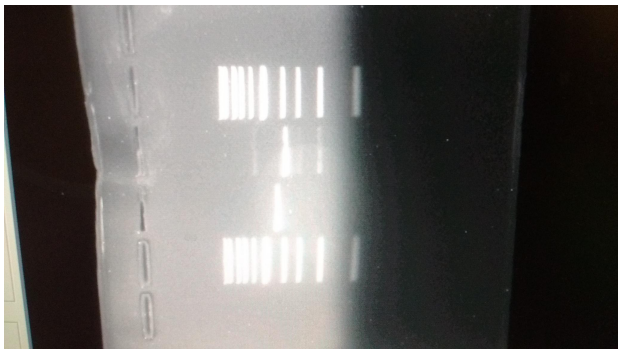
It seems like this method might have promise if we can generalize the way they're treating their individual promoters (Markov Chains with various occupancy states, each determining protein production at a different level) to the level of arbitrary genetic circuits. Inside their model things are interacting with each other as concentrations, so things like the Decoy Binding Array should be implementable fairly easily-- the two difficult spots are, I think:

I. How to generalize promoter-occupancy to circuit-activation-level in the same way
II. How to replicate their experimental measurement -> model parameter (ie. the Absolute Units of Fluorescence -> Concentration) transition with only feasibly implementable experimental data collection required.

Out 1:30-5:00 for mandatory Applied Science TA training session

We had to redo the PCR because the primers were matched incorrectly with the templates. Here's the gel:

160822_53_onto_1k3_try_2.jpg



Top: WM16_053 insert (1920 bp) [four bands! but the strongest band seems to be the right size...]
Bottom: UNS 1K3 backbone (2284 bp) [seems right]

Likhitha is checking the Macrogen results from 160817 order. They are WM16_053 on 1C3 and 1A3. They look good! This means we can try co-transforming WM16_053 1A3 (confirmed) with tetO Array 1C3 and tetO array 3C5 tonight.

It looks like the only instance of the 85x tetO array that we have is on 1C3! The 3C5 part is disconfirmed in the Miniprep Inventory (I remembered us having one...). This means we should re-construct it to get the different-backbone effect.

Out @ 7:30 PM

160824 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-23

TUESDAY, 8/23

Today's To-Do

Decoy Binding Array

- ☒ Dilute and Induce the WM16_053 1A3 BL21 cultures (with and without 85x tetO 1C3) (M9; aTc), then FACS it.
  (EMJ and KC are diluting in the morning)
  CEM & AJR induced, inductions incubated at 4:55pm (160824 CEM)
- ☒ FACS the WM16_053 1A3 and WM16_053 1A3 + 85x tetO 1C3 induction curves.
- ☒ Diagnostic PCR: 85x tetO array 1C3 miniprep (see table at bottom of160822for location of the construct) with P030 and P031 (same parameters as 160823 JPM) to see if they look like the gels for the colony PCR yesterday.
- ☐ Miniprep: The WM16_053 1K3 construct.
  (this didn't grow overnight... why??)
- ☒ Inoculate: The three glycerol streaks (see bottom of 160822-EMJ) for miniprep for restriction digest cutting.

Promoter Characterization

- ☒ Assembly Pipeline: Reconstruct the promoter constructs which failed in the latest Macrogen sequencing batch. This would more solidly suggest that the assembly failed, so before we start we need to really examine the primers / templates involved before we start.
  - **EMJ and I finished checking sequences. See my notebook (**160824 ADH**) for details. Seems like we should just re-do the same attempted Gibsons, but I cannot find the backbone template we originally used. We should be able to use any sequence confirmed RiboJ swap we have, but we should discuss this.**

- ☒ **PCRs started at 11:30, 1% agarose gel is in the hood waiting for products to be run.**

RBS Characterization

- ☒ Send the eight RBS construct plasmids (see todya's notes for constructs) to Alverno High School.
  JPM set up tubes on a rack in the fridge to send tomorrow.

Misc.

- ☐ Make more 1X TAE
- ☒ Obtain more NFW
- ☒ Make more Kan plates and Amp plates!
  CEM and AJR are doing this - KC is finishing this up

In @ 12:50 PM

Ethan and Andy went through the sequences from Macrogen. At this point, we have at least one MP confirmed of (table from 160824 ADH)

Table1

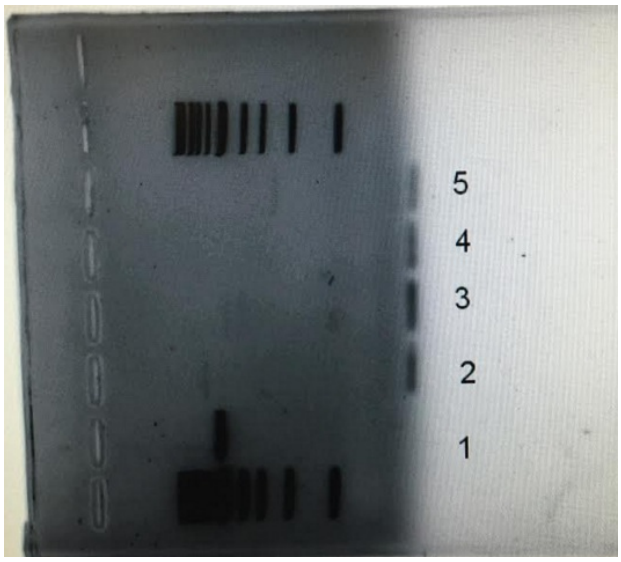
	A	B	C
1	Construct	W/ RiboJ (at least one MP successful	W/O RiboJ (at least one MP successful
2	J23100	Yes	Yes
3	J23101	Yes	Yes
4	J23102	No	Yes
5	J23103	Yes	Yes
6	J23104	Yes	Yes
7	J23105	Yes	Yes
8	J23106	Yes	Yes
9	J23107	Yes	Yes
10	J23108	Yes	Yes
11	J23109	Yes	Yes
12	J23110	Yes	Yes
13	J23111	Yes	Yes
14	J23112	Yes	Yes
15	J23113	Yes	Yes
16	J23114	Yes	Yes
17	J23115	Yes	Yes
18	J23116	Yes	Yes
19	J23117	Yes	Yes
20	J23118	Yes	Yes
21	J23119	No	Yes
22	R0040	No	Yes
23	R0010	Yes	Yes
24	R0011	Yes	Yes
25	J23150	Yes	Yes
26	J23151	No	Yes

Andy set up PCRs to re-do the Gibsons of the four nonconfirmed promoters, but using a different template from before (see 160824 ADH).

Ethan and Kalen diluted the cultures of 53 1A3 and 53 1A3 + 85x tetO 1C3 into M9 to grow up to midlog to induce to FACS

Likhitha imaged the gel of the PCRs that Andy set up, and then DpnI'd the samples.

Screen Shot 2016-08-24 at 2.13.01 PM.png



2-5: Promoter PCRs
1: Backbone template.
Gel from 20160824 LK
PCR from 160824 ADH

Ethan realized that the 85x tetO 1C3 construct doesn't have a correct Prefix/Suffix region. This means we should colony PCR with VF2 and VR instead of P030 P031. He is setting these PCRS up, going off of the colonies that Joe chose to inoculate from the cotransformations of WM16_053 1A3 and 85x tetO 1C3.

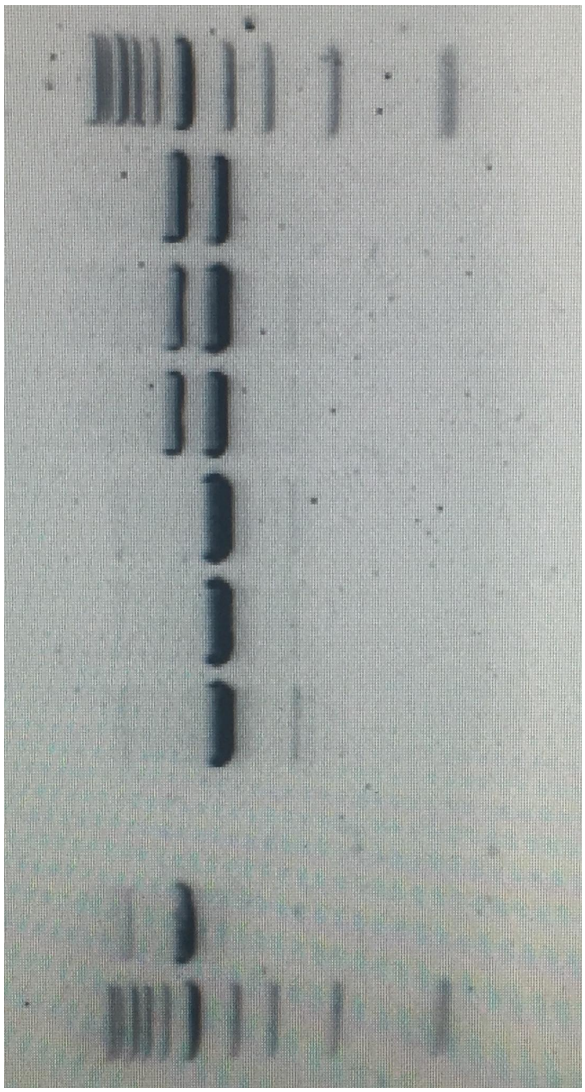
Ethan is also re-doing the backbone PCR that Andy set up earlier this morning, now that we found the correct template (WM16_053 RS1), with the same parameters.

I set up minipreps and tubes for Alverno, but FedEx is done for the day. We'll send them tomorrow.

Out @ 3:10 PM
In @ 5:00 PM

Andy and Ethan imaged the gel of the Colony PCR of the WM16_053 1A3 transformation and the WM16_053 1A3 + 85x tetO 1C3 cotransformation. This time we used VF2 and VR to PCR up the tetO array. Beautiful!!

File_000.jpeg



Colony PCRs from last night's transformations (these are the colonies that ended up getting inoculated and will be FACS'd today) :
Top 3: WM16_053 1A3 + 85x tetO 1C3 (VF2 VR)
Middle 3: WM16_053 1A3 (VF2 VR)
Bottom: Backbone
Gel from 160824-EMJ

Out @ 6:30 PM
In @ 7:30 PM

Sam said he wanted 1 ug of plasmid per construct for cell-free extract. I set up (labled 1 - 8 in descending order):

Table2

	A	B	C	D	E
1	**Sample**	**Location**	**Concentration (ng/uL)**	**Volume Sent (uL)**	**Total Plasmid (ng)**
2	WM16_035 MP3	Box 7 Slot 43	334.6	3	1003.8
3	WM16_036 MP1	Box 8 Slot 54	132.6	8	1060.8
4	WM16_044 MP1	Box 8 Slot 57	165.6	8	1324.8
5	WM16_045 MP3	Box 8 Slot 62	197.8	6	1186.8
6	WM16_046 MP2	Box 8 Slot 63	168.2	6	1009.2
7	WM16_047 MP3	Box 9 Slot 2	111.3	9	1001.7
8	WM16_048 from GS1 RS1	Box 9 Slot 49	419.8	3	1259.4
9	WM16_049 MP1	Box 9 Slot 6	91.8	11	1009.8

Note that WM16_045 is MP3 instead of MP1 (as sent to Pitt) because there was insufficient volume in the MP1.

I am FACSing the atc inductions of WM16_053 1A3 with and without 85x tetO 1C3. The population without 85x tetO display a continuous gradient of fluorescence down to a very low level-- sufficiently large that I can't fit the entire range into the measurement window at any gain level for some of the samples.

Out @ 12:00 AM

160823 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-23

TUESDAY, 8/23

Today's To-Do

Decoy Binding Array

- [x] Examine Diagonstic Transformations: We transformed solo tetO array on various backbones into BL21. Did some work while others did? What does this imply?
- [x] Colony PCR: Cotransformations of WM16_053 and tetO array so we can inoculate to FACS tomorrow.
- [ ] Inoculate: The aforementioned WM16_53 1A3 with / without 85x tetO 1C3 transformants (BL21 -> M9 media)
- [ ] Restriction Digest Assembly: We don't have a sequence-confirmed tetO 3C5, so we need to make one.
- [ ] Re-Streak glycerol stock containing 3C5 plasmid so that we can cut it tomorrow to put the 85x tetO array on it.

Promoter Characterization

- [ ] Analyze Macrogen Sequences: We should receive sequencing results of re-dos of failed promoter characterization constructs tonight. We need to analyze these and assess the failure/success tendencies compared to the last time.

In @ 11:00 AM

Met with Dr. Saha about Calcium II paper

All plates grew from transformants last night! This suggests the cotransformation problems are not due to incompatibility between the array or the addGene backbones and BL21.

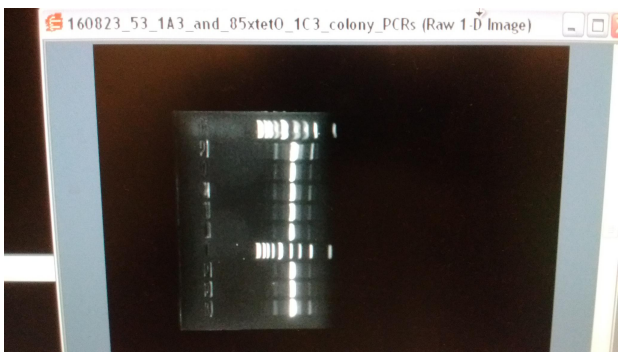
Joe set up Colony PCRs of the WM16_053 1A3 with/without 85x tetO 1C3 transformations using P030 and P031.

Andy and I heat-killed the overnight ligation at 1:00 PM by heat-shocking at 65C for 10 min.

Out @ 12:30 PM
In @ 3:30 PM

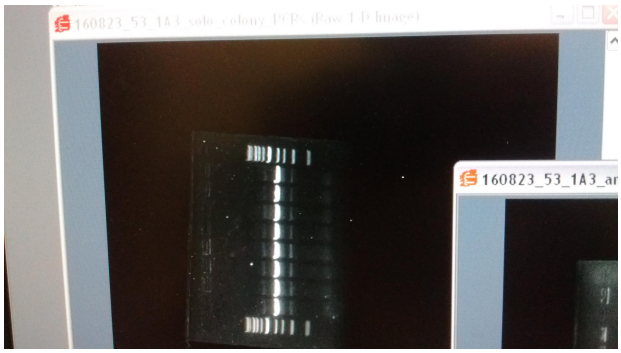
I imaged the gel of the colony PCRs that Joe set up. It looks like the cotransformation didn't work.

160823_53_1a3_with_85x_tetO_1c3.jpg



Cotransformation. Colonies #1-8 from bottom to top. Bright band is correct size of WM16_053 insert. Maybe there's a faint band at ~3kb for the tetO array?
Transformation #6

160823_53_1a3.jpg



Solo transformation. Colonies #1-8 from bottom to top. Birght band is correct size of WM16_053 insert, but has multiple banding... why?
Transformation #5

This raises the question of how the actual array is behaving... I set up additional colony PCRS fo the 85x tetO array 1C3 solo transformation into BL21 (Transformation #2), as well as the WM16_053 1K3 transformation (Transformation #1).

- tetO array transformation had four colonies (2.1, ..., 2.4) using P030 and P031. 70C at 1:30 extension. Maser Mix (for 25 uL reactions :( ) was:
  - 62.5 uL Q5
  - 6.25 uL P030
  - 6.25 uL P031
  - 45 uL NFW
- WM16_053 1K3 transformation had four colonies (1.1, ..., 1.4) using P008 and P009. 64C at 1:00 extension. Master Mix (for 25 uL reaction :( ) was:
  - 62.5 uL Q5
  - 6.25 uL P030
  - 6.25 uL P031
  - 45 uL NFW

These PCRs went in around 4:30 PM

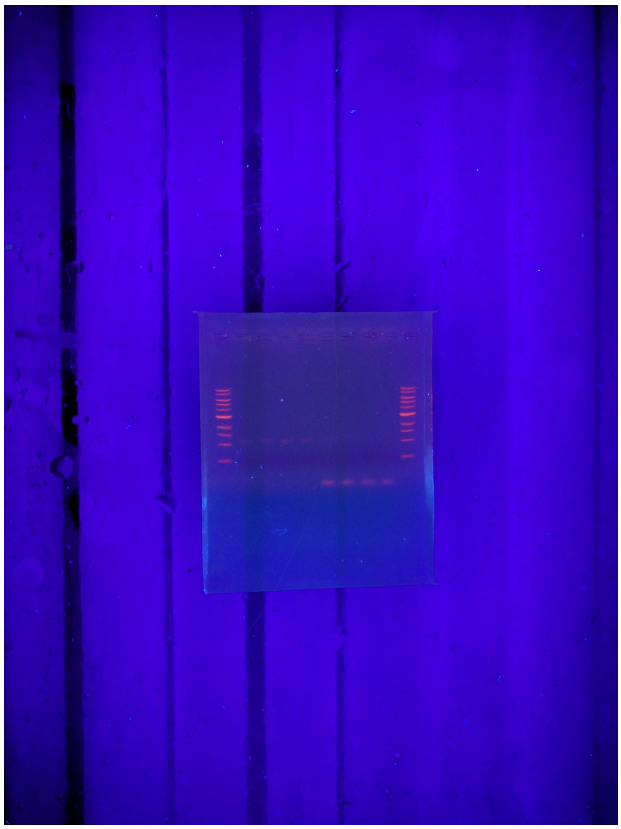
Out @ 5:00 PM
In @ 8:20 PM

Joe is transforming the ligation of 85x tetO on 1K3.

Ethan is re-streaking 85x tetO array, part for 1K3 backbone, and part for 3C5 backbone (see bottom of160822-EMJ)

Joe ran the gel of the colony PCRs that I did.

IMG_20160823_215855.jpg



Left: WM16_053 1K3 colonies with P008 P009. The band is about 1 kb too low.
Right: 85x tetO array 1C3 with P030 P031. The lack of band is consistent with the lack of an additional band on the cotransformation colony PCR above....

^ Could it be that the tetO array does not actually contain the array? Or perhaps the Prefix and Suffix aren't present / unmodified... And why is the 1K3 band so low? Should be 1920bp, but it's only slightly above 1kb.

160825 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-24

WEDNESDAY, 8/24

Today's To-Do

Competition Requirements

- [ ] **Make Part Pages!!** There isn't a lot of wetlab today but we have to make comprehensive part pages for every part we intend to submit. This takes a while!! See Joe's instructions on how to make one.
    How-to guide on Dropbox/iGEM 2016/Drylab/Parts

Promoter Characterization

- [x] Colony PCR and Inoculate the four re-assemblies of the promoter characterization constructs
    - [x] Colony PCR (JPM moved the plates onto the bench 10:20 AM)

    AJR put colony PCRs in, making gels at 1300 hours

    - [x] Gel

    Gel pics in 160825 AJR

    - [x] Inoculation of colonies - ***see gel pics for which colonies to use***

Decoy Binding Array

- [x] Miniprep the backbone / insert re-streaks so they can be used for restriction digest.
    CEM nanodropped & inventoried (MP Box 11) 160825 CEM
- [ ] If possible, follow up with Restriction Digest protocol to move 85x tetO array onto 3C5 backbone. **EMJ says, I'll need ~3ish hours to do this, probably will be transforming on Saturday.**
- [x] Convert FACS data from last night into Absolute Units. Interpret.

RBS Characterization

- [x] Send RBS parts (in Fridge) to Alverno iGEM **(before 2:00 PM)**

Misc.

- [ ] Make more 1X TAE

In @ 9:50 AM

I took the transformation plates from last night out of the incubator and put them on the bench to prevent overgrowth.

Setting up conversion of last night's FACS data to absolute units.
Got the software to run. As I noted yesterday, the with-array samples had a consistent and long lower tail of the fluorescence distribution which streteched so low that I couldn't capture the entire dynamic range with any one given gain setting. I thought that FlowCal would automatically chop off values near the edges of the fluorescence detection, but this does not seem to be the case... reading into this.

Sent parts to Alverno.

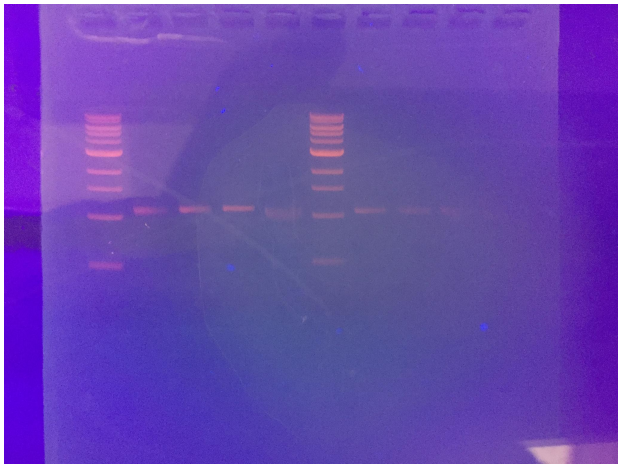
Registered everyone except John Mitchell and Christine for the conference.

Adam's gel pictures (copied from 160825 AJR):

IMG_8241.JPG

Gel #1: Use Colonies 1.1, 1.3, 1.4; 2.1-2.3

IMG_8240.JPG



Gel #2: Use colonies 3.1-3.3; 4.1-4.3 (I know the third band on plate 4 looks super faint - it was brighter in real life than in the picture)

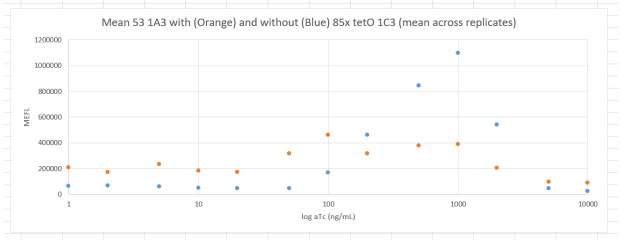
Out @ 1:50 PM
In @ 4:00 PM

John Mitchell got the forms from EXTREEMS to cover his travel costs to the conference.

Talked to Christine about her using her Beckman Travel funds to cover the Jamboree. She was willing to do it.

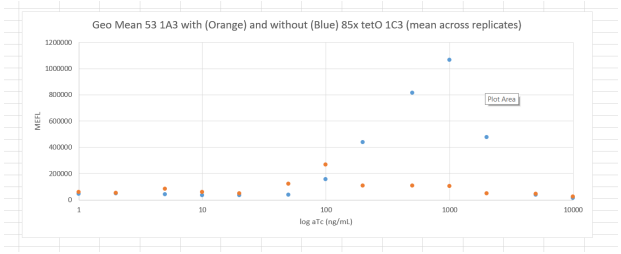
Turns out that the summary stats (mean, etc.) that FlowCal exports in the spreadsheet are calculated from the gated data, hence the huge peak 0 in the histograms on the cotrasnformations is not included in the means. I put together summary stats and they don't look the best... (full data on Dropbox/FACS/Flowcal Data). However one can convince oneself that you see a leftward shift in the induction curve when the array is added (as it's supposed to be... I think)

clipboard_2016-08-25_16:21:46.png



Mean Flx. of Population, average of 3 replicates

clipboard_2016-08-25_16:22:11.png



Geometric Mean Flx. of Population, average of 3 replicates

Met with Dr Saha and Joe and Andy to discuss equipment for the new MakerSpace.

Out @ 6:40 PM
In @ 8:00 PM

Ethan and I talked through the interpretation of the FACS plots-- it seems like the leftward shift is the correct phenomenon when introducing a decoy binding array when the inducer molecule concentration is the horizontal axis. When the horizontal axis is total amount of repressor, it would be a rightward shift.

Andy and I set up inoculations of the four promoter swaps into LB Chlor. I followed the colony key in the legend of the gel pictures above, with tube #1, 2, 3 corresponding to the colony numbers in increasing order.

In incubator @ 8:45 PM

Out @ 9:00 PM
In @ 10:00 PM

Ran through Travel Authorization Form and Conference Registration with JMitch.

Out @ 10:45 PM

160826 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-25

THURSDAY, 8/25

Today's To-Do

Competition Requirements

- ☐ **Make Part Pages!!**

Interlab

- ☒ Inoculate Interlab Constructs from plate (use the glycerol re-streaks that already exist) (all of them in LB Chlor) *(KPC is doing this) (in @ 9:00 PM)*
- ☒ Also Streak Out the Interlab Constructs from Glycerol Stock

Promoter Characterization

- ☒ Miniprep the Four Promoter Constructs (J23102, J23119, J23151, R0040)
  (EMJ and KPC)

Sequencing

- ☒ Prepare for Macrogen Sequencing the Four Promoter Constructs above, and also the three 85x tetO 1A3 minipreps from 160825 (see 160825 CEM) , and also WM16_025 1C3 (Box 10 Slots 62-64). (18 tubes, Fwd Rev everyone)
  (LK)
  (The WM16_025, if confirmed, needs to be measured with WM16_016 to characterize the IPTG induction curve for the RiboJ project)
- ☒ Dilute Tubes and Send Sequences before 2:00 PM! See 20160826 LK

Decoy Binding Array

- ☒ Transform the WM16_053 1C3 into BL21 and WM16_053 1C3 + 85x tetO 1A3 into BL21. *(EMJ is starting this at 8:00 PM)*
- ☐ Design Primers to make a combo part (like WM16_053) but with lacI and pLac, so we can test our new lacO array.
- ☐ Design Primers to move the lacO array onto BioBrick backbone

In @ 12:00 PM

Kalen is setting up the miniprep solution intubes for Macrogen.

Ethan is thinking about primer design to do the following (no rush on ordering because it's the weekend):

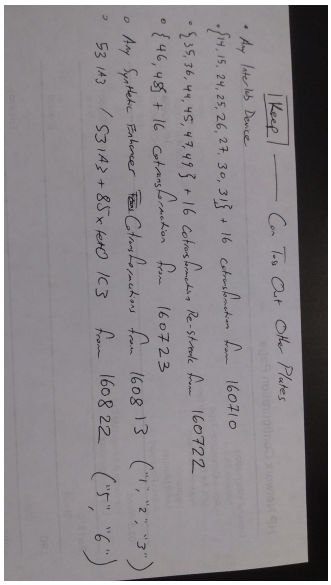
- the lacO array does not have BioBrick-compatible restriction enzyme sites. Ethan wants to clone the construct UNS2 - lacO enzyme cut site 1 - lacO enzyme cut site 2 - UNS3 on BioBrick backbone, so we can restriction enzyme into an actually biobrick-compatible construct
- the lacO array is 48x so we can also attempt to do typical Gibson assembly by amplifying starting a long ways out from the array on each end
- Also we need to construct the lac analogue of WM16_053.

Joe looked into the requirements for the interlab study and found that they are extremely specific. Need to inoculate tonight the interlab constructs again.

Out @ 2:00 PM
In @ 3:20 PM

Dr. Saha told us to throw out all of our excess plates. Made a list of the thing we need to keep (these are constructs which have been measured. Glycerol stocks also exist for these things (presumably), but the plates are an orthogonal backup):

plates_to_keep.jpg



- Any Interlab Device
- 160710: {14, 15, 24, 25, 26, 27, 30, 31} + 16 cotransformations
- 160722: {25, 26, 44, 45, 47, 49} + 16 cotransformations
- 160723: {46, 48} + 16 cotransformations
- 160813: Any Synthetic Enhancer cotransformations (may be labeled "1", "2", or "3")
- 160822: 53 1A3 and 53 1A3 + 85x tetO 1C3 (may be labeled "5" or "6")

Adam, Kalen and I tossed out the plates from the cold room that did not meet the above criteria. We also kept all of the original Orna Atar transformation plates, any additional cotransformations, and some particularly red and green plates.

Out @ 6:15 PM
In @ 8:00 PM

Ethan is setting up transformations of WM16_053 1C3 and WM16_053 1C3 + 85x tetO 1A3 in BL21. See 160826-EMJfor samples.

Kalen is setting up inoculations of the glycerol streaks of the Interlab Devices so we can dilute and FACS them tomorrow. They are:

- Interlab #1 GS1 160618 JLM
- Re-Streak of Interlab Device #2 GS2 from 16 JPM 160605
- IMP #3 GS1 Chlor 160618 JLM
- Interlab Pos. Control GS1 160618 JLM
- Re-Streak of Interlab Device Negative Control GS2 from 16 JPM 160605

I'm plating out the two transformations, as well as streaking out glycerols of Interlab Parts in case the inoculations-from-plate don't work (we are doing this because Joe was worried that the plates are too old and that the colonies contained would be dead). The parts are (ignore what 20160826 LKsays) : They went in @ 10:35 PM

Table2

	A	B
1	Sample	Location
2	Interlab #1 GS1	Box 2 Slot 52
3	Interlab #2 GS2	Box 1 Slot 17
4	Interlab #3 GS1	Box2 Slot 55
5	Positive Control GS1	Box 1 Slot 49
6	Negative Control GS2	Box1 Slot 11

Replicates were chosen to match the replicates which were measured on 0711.

Out @ 10:45 PM

160827 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-26

FRIDAY, 8/26

Today's To-Do

Interlab

- [ ] Dilute inoculations of Interlab Constructs according to Interlab instructions *(LK and JPM are doing this at 11:00AM)*
- [ ] FACS Interlab Constructs according to Interlab instructions
- [ ] Inoculate the glycerol streaks if the inoculations or FACS results don't look good.

Decoy Binding Array

- [x] Heat Kill Ethan's overnight ligation of tetO array on 3C5 backbone and on 1K3 backbone. Do this by putting the tubes in 65C for 10min. These tubes are in the heat plate in the cold room. Thermocylcer 4
- [ ] Inoculate the 53 1C3 and 53 1C3 + 85x tetO 1A3 for atC induction and FACS tomorrow
- [ ] Transform the ligated products of 85x tetO 3C5 and 85x tetO 1K3

  (Each of these should be plated on both Kan and Chlor because EMJ is skeptical about the samples being labeled correctly by backbone)
- [x] Design Primers to make a combo part (like WM16_053) but with lacI and pLac, so we can test our new lacO array.
- [ ] Design Primers to move the lacO array onto BioBrick backbone-- do this by either:
  - Gibson assembling the 48x lacO array with primers that bind to very-up/downstream flanking regions, or
  - Gibson assembling the requisite restriction enzyme cut sites into UNS backbone for restriction digest of lacO array into the BioBrick standard (w/out destroying the Prefix and Suffix this time)

Thinking about the Project

- [ ] We can almost kind of claim that we have demonstrated function for each of the tools in our toolbox. We need to seriously think about how we're going to get to the same stage for parts working in concert, before we're forced by time to do said thinking.
- [ ] Also, how are we going to get the synthetic enhancer to a more convincing proof-of-concept stage?

Misc.

- [x] Make more Chlor plates, Kan/Amp plates, and Amp/Chlor plates *(Callan made Chlor, Amp/Chlor plates)*

In @ 10:50 AM

Inoculations: Device #1 grew to turbidity, but the others have not. This suggests that either the plates are too old, as Joe suggested, or that the glycerol stocks that were re-streaked on that day weren't good.

Transformations:

- The two transformations have a few tiny colonies-- need more time.
- The glycerol stocks grew up to lawn growth. I put in too much solution (30 uL) last night. I'm re-doing the streaks now (using pipette tip to streak) since all we need to do is inoculate them tonight. This time I'm doing all the glycerol stocks just in case:

Table2

	A	B
1	Sample	Location
2	Interlab #1 GS1,2,3	Box 2 Slot 52,53,54
3	Interlab #2 GS1,2	Box 1 Slot 16,17
4	Interlab #3 GS1,2,3	Box2 Slot 55,56,57
5	Positive Control GS1,2,3	Box 1 Slot 49,50,51
6	Negative Control GS1,2	Box1 Slot 10,11

See the table on 160826 JPMfor the samples which correspond to the previously measured IMP parts.

These went in the incubator at 11:30 AM.

Model: I'm looking back into what Beal & Weiss do in their EQuIP framework and seeing if we can use their methodologies.

I need to read about Spectral Overlap more, for the future

Likhitha is designing primers to make the WM16_053 lac analogue out of WM16_014 and WM16_016.

Out @ 1:15 PM
In @ 1:45 PM

Found an old 2009 Drew Endy paper about standardizing promoter measurements to RPUs which talks about the Fluorescence -> RPU -> AU transitions. Looking into this. Some nice takeaways:

- GFP Synthesis rates from two standard constructs (J23101, J23150) were sensitive to strain but not carbon source or tempearture. They were also sensitive to antibioitic marker and plasmid copy number.
-

Out @ 4:25 PM
In @ 5:00 PM

I checked on the transformation plates that I let grow longer this morning. The solo 53 1C3 transformation has a fair number of medium-sized colony. The 53 1C3 + 85x tetO 1A3 cotransformation has only one medium-sized colony.

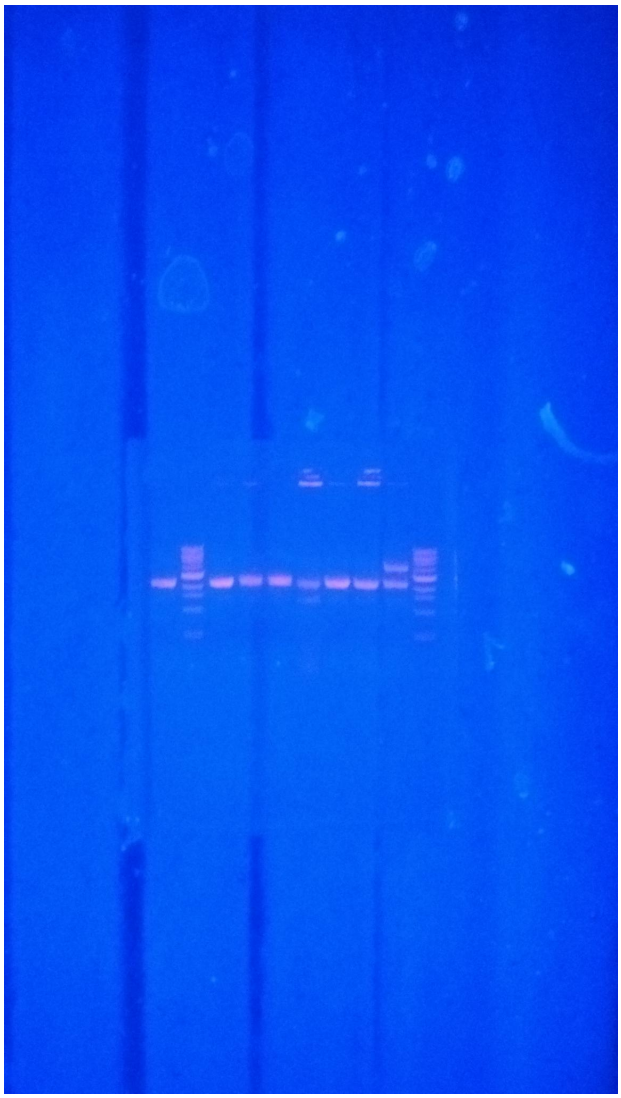
Looked into the Flx -> Concentration conversion more.

Out @ 6:30 PM
In @ 7:30 PM

Ethan set up Colony PCRs of the 53 1C3 + 85x tetO 1A3 and the 53 1C3 transformations.

I'm setting up transformations of 85x tetO 1K3 and 85x tetO 3C5. These will each be plated onto Kan and Chlor plates because Ethan is not confident that they are labeled correctly. Outgrowth in at 9:00 PM.

0827_colony_PCRs.jpg



#1-7: WM16_053 1C3
#8: WM16_053 1C3 + 85x tetO 1A3

The size of the bands don't seem to align with what they're supposed to be-- everything sems to be shifted up ~1kb.

Ethan and I are talking through the Fluorescence -> [Protein] conversion.

Out @ 10:00 PM

iGEM Transformation

Introduction

This is how you insert your plasmid(s) into cells. Please be sure you know **which strain** you are using and you know the **appropriate amount of time** to heat shock your **specific strain**.

Materials

- Comp Cells
  - (5alpha one tube)
- Plasmid DNA
  - (85x tetO 1K3 ligation product)
  - (85x tetO 3C5 ligation product)
- SOC
-
  -

Procedure

Thaw Cells

1. Take out enough cells so that you can have at least 15 uL of cells per thing you are trying to transform. There is about 45-50 uL of competant cells per NEB tube of cells.
2. Thaw cells on ice
3. Transfer appropriate amount of cells to appropriately labelled Eppendorf tube (I would suggest using the same key as you used for the gibson assemblies).

Transform

4. Add 2.5 uL of plasmid DNA to each aliquot of cells.
5. Ice for 30 minutes. Prewarm heatblock to 42 degrees C.

   I would strongly recommend that you take this time to prelabel your plates and place them in the incubator to prewarm.

Heat Shock

6. Heat shock cells for appropriate amount of time. This varies based on which strain you are using.

   BL21 gets heat shocked for 10 seconds
   10Beta and 5alpha get heat shocked for 30 seconds
7. Ice for five minutes.
8. Pipette in SOC based on the amount of cells you used. 50 uL of cells get 950 uL of SOC, for reference.

Outgrow

9. Place in shaking incubator 250 rpm 37C for 1 hour (chlor, amp, or tet) or 2 hours (kan)
10. Remove bacteria from shaking incubator.
11. **INVERT EVERY TUBE 4-6 TIMES. IF YOU DONT DO THIS YOU WILL NOT HAVE A SUCCESSFUL TRANSFORMATION.**

Plate

12. Plate out 100 uL of bacteria.

    (We have been having lawn growth for a lot of constructs; you may want to do 50 uL if you have experience with this part overgrowing. Likewise, if you are doing a double transformation or a low copy number, do 150 uL).
    Use glass beads in a bunsen burner sterile field. Dispose of glass beads into ethanol.
13. Put plates in incubator upside down (agar side up, lid down). Let grow overnight.

    Do not be alarmed if you do not see colonies for up to 18 hours.

160829 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-28

SUNDAY, 8/28

Today's To-Do

General Lab Maintenance

- ☒ Order:
  - ☒ More gloves (2 boxes of S, M, and L)
  - ☒ Concentrated GFP product (Ethan)
  - ☒ More 0.2 mL Tubes
  - ☐ More 1.5 mL Tubes *(these were not ordered as they had been ordered last week-- they just haven't arrived yet).*

Misc.

- ☒ Miniprep: *(adam is doing this)*
  - ☒ 85xTetO constructs (1K3, 3C5); may need to dilute first
    - there is an 85xTetO 3C5 construct that grew on a Kan plate for some reason (hence why it's labeled "weird"), but it looks like it didn't grow in Kan media, so no need to miniprep that obviously

Decoy Binding Array

- ☒ Run a Gel of the Colony PCR re-dos of 85x tetO 1K3, 85x tetO 3C5, and 85x tetO 3C5 that grew on Kan plate.
  see 160829 AJRfor gel pics; they justify our choice for innoculation
  except for the weird chlor that grew on the Kan plate; luckily this construct did *not* grow in LB Kan media
- ☐ Assembly Pipeline: Swapping the RBS on tetR on WM16_053 to be weaker.
  - ☒ PCR:

Table1

	A	B	C	D	E
1	**Template**	**Location**	**Primers**	**Annealing Temp**	**Ext. Time**
2	WM16_053 1C3 MP1 160817	Box 11, Slot 4	P143, P008	66	0:30
3	WM16_053 1C3 MP1 160817	Box 11, Slot 4	P144, P013	68	1:30
4	WM16_053 1A3 MP2 160817	Box 11, Slot 7	P144, P013	68	1:30

see 160829 AJRfor gel pics of these PCRs

  - ☒ DpnI
  - ☒ PCR Purification *(adam is doing this)*
  - ☒ Gibson Assembly:
    - 53 1C3 insert (P143 P8) + 53 1C3 backbone (P144 P13)
    - 53 1C3 insert (P143 P8) + 53 1A3 backbone (P144 P13)
  - ☐ Transformation into 5 alpha
    LK is doing this
- ☒ FACS the overnight aTC inductions

Interlab

☒ Dilute the inoculations of the Interlab overnight cultures, according to the Interlab Protocol found on the iGEM website HERE (https://docs.google.com/forms/d/e/1FAIpQLSeTnsOXQWs2j-bMTjL28QsFYkHkCL6DBua4DF56ekE0Ym1nIQ/viewform).

- NOTE: The protocol mention an Excel worksheet. I have downloaded and saved this as Dropbox/iGEM 2016/FACS Data/TeamName_iGEM2016_Flow_Cytometry_Workbook.xls and I will attach another copy just in case here:

TeamName_iGEM2016_Flow_Cytometry_Workbook.xls

I think the following is the Excel sheet they refer to in the protocol for "volume of preloading culture and media in Excel (normalisation) sheets" . I saved it in Dropbox/iGEM 2016/Interlab along with the protocol pdf. (KPC)

TeamName_iGEM2016_Interlab_Sheet_1_updated.xls

Dilutions went in at 3:50PM (160829)

☒ FACS the Interlab parts

Measuring

☐ FACS sort the WM16_014 construct and measure it in the plate reader to see if we can get a feasible sorted cell count to be visible on plate reader.

Registration

☒ John Mitchell and Christine need to register for the conference via their respective funding sources!

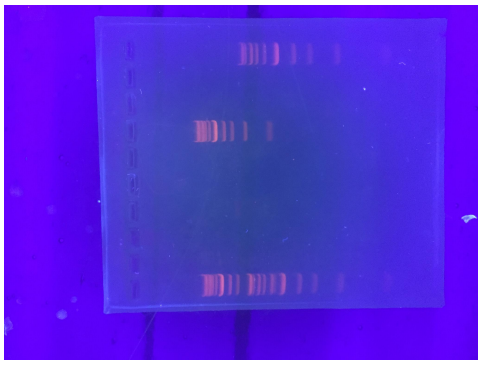
In @ 1:00 PM

The following indented block is excerpted from 160829 AJRfrom earlier this morning:

Imaged colony PCR gels run by CWG

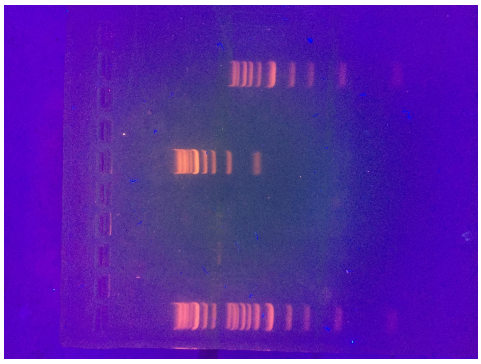
- bad quality pics but there were visible bands at around 3kb for each construct (even the weird 3C5 that grew on Kan)

IMG_8246.JPG



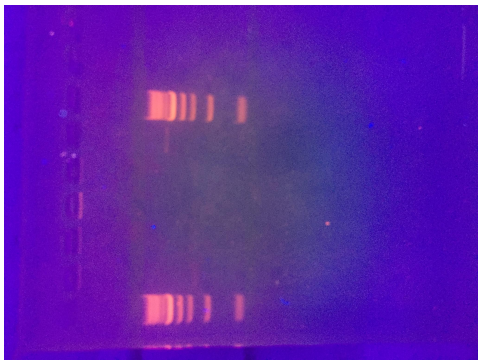
K (85xTetO 1K3) - all bands visible (not in picture but in real life)

IMG_8247.JPG



C (85xTetO 3C5) - bands 1-4 were visible

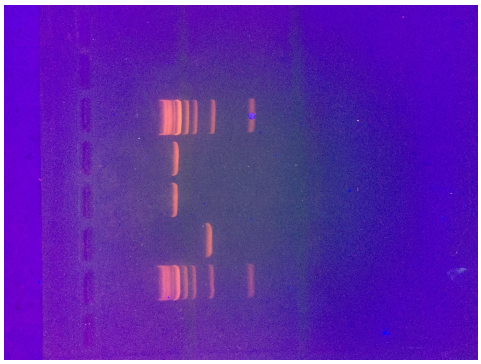
IMG_8248.JPG



?? (85xTetO 3C5 grown on Kan plate) - ?5 had band of right size, others had weird smaller bands around 1.4 kb

- DPNI'd the PCRs ran by CWG, also running a gel of those PCR products (See 160829 CWG)

IMG_8249.JPG



From Bottom to Top: A, B, C (again, see CWG); all bands look good

Checked through the primers Ethan designed for primer-dimering a restriction-cut-site construct to insert into UNS backbone, which we could then restriction digest the lacO or tetO arrays into. We realized that the enzymes cut the same way on the DNA, which apparently poses a problem-- holding off on ordering these today to think the process through.

I did order the primers to Gibson PCR the lacO array from flanking regions, to make the 53 lac analogue, and to swap the RBS on the constitutive lacI.

Adam is setting up PCR Purification and Gibson Assembly of the B0031-WM16_053 construct.

Adam is setting up Minipreps of 85x tetO 1K3 and 85x tetO 3C5. Both grew well in liquid culture-- the 85x tetO 3C5 which grew on the Kan plate failed to grow in Kan LB, which is good.

Out @ 3:00 PM

In @ 5:00 PM

Joe, Andy and I looked through Joe's first draft of LearnSynBio intro video. We decided someone else needs to do the actual drawing of the video's word and images, but otherwise the thing was quite good!

Out @ 6:00 PM
In @ 8:00 PM

I FACS'd the aTC inductions of 53 1C3 in BL21, the 53 1C3 + 85x tetO 1A3 in BL21 (only one replicate from the one colony... there was basically nothing in the samples :( ), and the Interlab parts.

Interlab Device #1 did not grow, and fluorescent cells were not visible on the FACS for this device. We need to re-grow Device #1 and the two Controls to get those measurements again.

But Device #2 and Device #3 yielded great measurements! FL1 was set to 550.

160828 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-28

SUNDAY, 8/28

Today's To-Do

Decoy Binding Array

- ☒ Colony PCR the 85x tetO 3C5 and 85x tetO 1K3 using VF2 and VR *(AJR is doing this)*
- ☒ Inoculate the above two arrays for miniprep tomorrow *(in addition we will inoculate the 85x tetO 3C5 into Kan LB to see if it can grow in liquid Kan)*
- ☒ Dilute the 53 1C3 and 53 1C3 + 85x tetO 1A3 transformations for aTc induction (overnight) tonight. (make 4 mL media for each. M9, as all are BL21.)
  AJR did this - dilutions are in shakinbator at 1510 hours
- ☐ Induce the above two samples with aTc (14 stages) in the evening for overnight induction. (As in 160824 CEM)

Interlab

- ☒ Inoculate the glycerol re-streaks of the Interlab devices *(The CEM versions-- see* 160826 JPM*for the specific replicates to inoculate.)*

Measuring

- ☒ Inoculate the WM16_014 glycerol re-streak so we can try out the FACS -> Plate Reader idea tomorrow.

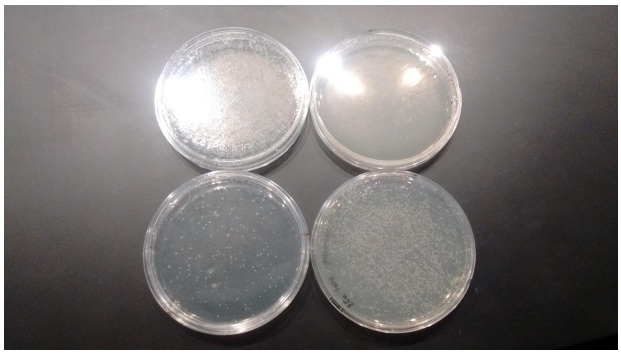
In @ 5:40 PM

Adam diluted the inoculations of 53 1C3 and 53 1C3 + 85x tetO 1A3 into 4 mL M9 media (in @ 3:00 PM)

The glycerol streaks grew properly. These will be inoculated tonight.

The ligation product plates looked like this:

0828_ligations.jpg



Top: "85x tetO 3C5"
Bottom: "85x tetO 1K3"
Left: Kan plates
Right: Chlor plates

This suggest that the labels were swapped, as Ethan suspected. There was no growth of the Kan construct on the Chlor plate, but there *was* growth of the Chlor construct on the Kan plate.... we are going to inoculate the Chlor construct into Kan media to see if it can persist there as well. Hopefully the issue is that this particular plate batch is bad, and not that our diluted antibiotic is bad.

Adam is setting up Colony PCRs, 5 colonies each of 85x tetO 1K3, 85x tetO 3C5, and 85x tetO 3C5 that grew on Kan plate, using VF2 VR. These went in around 6:30 PM.

Reviewing Likhitha's primer (P160) to make the lac analogue of WM16_053. Decreased the length but otherwise looks good.

Out @ 7:10 PM

In @ 9:45 PM

I think that swapping the RBS on the tetR of Wm16_053 to a weaker one will make the Decoy Binding Array results look more like what we expect to see--

- this will mean that the maximum repression-relief (the high plateau of the induction curve) will occur at a lower value of aTc, which means we can better avoid the toxicity effects we see at high [aTc].
- this might also let the binding array be relatively more present with respect to tetR in the cell, so that the array would have a more noticeable effect.
- It will mean that the max repression level (the low plateau of the induction curve) will be weaker than before, though.

I set up Adam's colony PCRs on a gel:

Left gel: 1-5 is 85x tetO 1K3, 6-8 and...

Right gel: 1-3 is 85x tetO 3C5, and 4-8 is 85x tetO 3C5 that grew on Kan plate.

I'm inoculating the Interlab parts from Callan's glycerol streaks that she did last night. I chose replicates according to the table on the bottom of 160826 JPM. These will be overnight inoculated for 16-18 hours before being diluted according to the Interlab protocol tomorrow. In @ 10:40 PM

It turns out Adam set up the PCRs with P008 and P009 instead of VF2 and VR... He is re-setting up the colony PCRs.

Since it is late, we are going to go ahead and inoculate three random colonies each of 85x tetO 1K3, 85x tetO 3C5, and 85x tetO 3C5 that grew on Kan plate. We'll then retroactively associate them with the gel results in the morning. The colonies are #1-3 for each of the three construct samples. 160828 AJR

I designed primers P165, P166 to swap the RBS on the lacI on the new combo part from B0034 to B0031.

Likhitha is desgning primers P161-164 to primer-dimer enzyme-site-containing inserts to gibson into the UNS backbone, so that we can restriction assemble the lacO and tetO arrays into a BioBrick-compatible backbone.

She is also desgining P167, P168 to Gibson the LacO array conventionally, annealing to flanking regions around the array.

Callan is inducing the 53 1C3, 53 1C3 + 85x tetO 1A3 cultures with 14-step aTC inductions. 160828 CEMThese will be induced overnight.

In @ 12:30 AM

Out @ 12:30 AM

160830 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-08-29

MONDAY, 8/29

Today's To-Do

Interlab

- [x] Dilute the Interlab Device #1 and Positive and Negative Controls according to the FACS protocol from yesterday (160828 JPM) [This needs to happen between 3:30 - 5:30 PM] ***(JPM will do this. In @ 3:54 PM)***
- [ ] FACS the Interlab devices.

Decoy Binding Array

- [x] Colony PCR the 53 w/ B0031 1A3 and 53 w/ B0031 1C3 transformations using P008 and P009 *(adam is doing this)*
  - [ ] Inoculate appropriate colonies into LB for miniprep tomorrow
- [ ] Assembly Pipeline: (putting lacO array on UNS backbone)
  - [ ] PCR:
    - P167 P168 on pJD100 (lacO array)
    - P019 P013 on a 1C3 UNS backbone template (your choice)
    - P019 P013 on a 1A3 UNS backbone template (your choice)
  - [ ] DpnI
  - [ ] PCR Purfication
  - [ ] Gibson Assembly
  - [ ] Transformation
- [ ] Assembly Pipeline: (combining pLac sfGFP with constitutive lacI)
  - [ ] PCR:
    - P160 + P013 on WM16_016 1C3
    - P008 + P140 on WM16_014 1C3
  - [ ] DpnI
  - [ ] PCR Purification
  - [ ] Gibson Assembly
  - [ ] Transformation

Macrogen

- [ ] Check Sequences of the results *(andy did this for everything except the 85x tetO 1A3)*

General

- [x] Make LB

  Joe made it and put it in the autoclave... will be done around 12:45 PM

In @ 11:00 AM

Met with Joe and talked about how to do the Interlab OD measurement and dilution for Device #1, Pos control, and Neg control

Out @ 11:10 AM
In @ 3:30 PM

Measured the ODs of the Interlab Constructs on the plate reader and obtained volumes to enter into 3mL LB (according to chart from 160829) :

Table1

	A	B	C
1	Sample	OD	uL to add
2	1.1	1.045	57.4162679426
3	1.2	1.152	52.0833333333
4	1.3	1.242	48.309178744
5	Positive 1	0.859	69.848661234
6	Positive 2	0.872	68.8073394495
7	Positive 3	1.162	51.6351118761
8	Negative 1	1.238	48.465266559
9	Negative 2	1.21	49.5867768595
10	Negative 3	1.313	45.69687738
11			

Dilution went into the incubator at 3:54 PM

Andy assessed the Macrogen sequences for everything except 85x tetO 1A3, and found that everything (the 4 promoter construct re-dos and the WM16_025 redo) was disconfirmed. He designed geneBlock fragments to Gibson Assemble the parts with, given that now each of these have undergone multiple PCR-based cloning attempts and failed.

For WM16_025 in particular, the cI sequence seemed to be the source of the issues for the disconfirmation-- I double-checked old sequences for WM16_015 to make sure cI was intact (yes) and WM16_027 to make sure a Gibson Assembly going through cI would be properly assembled (it can be done).

WM16_027 MP3 160608 was marked as confirmed despite being disconfirmed.

In @ 9:00 PM

Set up the FACS

Talked with Dr Saha about Honors Lab, BwB Forum, and the Sep. 23 Bio Dept Seminar Talk

Interlab Device #1 didn't grow after 6 hours post-dilution, just as before! This time we are doing two things:

(1) We left Device #1 and its controls in the shaker for overnight growth. We'll FACS them tomorrow and see what they looked like.

(2) We inoculate glycerol stocks of Device #1, but from glycerol stocks 2 and 3 (the past two times we've been using glycerol stock 1).

Ordered P169-173 and the gBlocks to construct WM16_025 and the four promoter constructs we're missing.

Ethan inoculated WM16_109 (this is WM16_053 but with B0031 RBS) 1A3 into LB. Colonies #1,2,3 from the colony PCR.

Out @ 11:00 PM

160831 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-30

TUESDAY, 8/30

Today's To-Do

Interlab

- [x] Dilute the Interlab Device #1 and Positive and Negative Controls according to the FACS protocol from yesterday (160828 JPM) [This needs to happen at 1:45 PM]. Honors Lab happens at 2:00 PM!!
- [x] FACS the Interlab devices 6 hours after dilution.
- [x] Also FACS the Interlab Device #1 which stayed in the shaker from last night.

Decoy Binding Array

- [x] Miniprep the WM16_053 (with B0031) (which we are calling WM16_109) 1A3 inoculations.
  waiting on honors lab to finish so I can nanodrop these -AJR

~~Below this line we need IDT primers to arrive~~

- [ ] Assembly Pipeline: (putting lacO array on UNS backbone)
  - [ ] PCR:
    - P167 P168 on pJD100 (lacO array)
    - P019 P013 on a 1C3 UNS backbone template (your choice)
    - P019 P013 on a 1A3 UNS backbone template (your choice)
  - [ ] DpnI
  - [ ] PCR Purfication
  - [ ] Gibson Assembly
  - [ ] Transformation
- [ ] Assembly Pipeline: (combining pLac sfGFP with constitutive lacI)
  - [ ] PCR:
    - P160 + P013 on WM16_016 1C3
    - P008 + P140 on WM16_014 1C3
  - [ ] DpnI
  - [ ] PCR Purification
  - [ ] Gibson Assembly
  - [ ] Transformation

~~~~~~

In @ 1:15 PM

Did the Interlab dilutions via OD:
~~~~~~

Table1

	A	B	C
1	Sample	OD	uL to add
2	1.1 GS2	1.086	55.2486187845
3	1.2 GS2	1.007	59.5829195631
4	1.3 GS2	1.006	59.6421471173
5	1.1 GS3	1.111	54.0054005401
6	1.2 GS3	1.217	49.3015612161
7	1.3 GS3	1.214	49.4233937397
8	Positive 1	0.99	60.6060606061
9	Positive 2	1.396	42.9799426934
10	Positive 3	1.063	56.4440263405
11	Negative 1	1.369	43.8276113952
12	Negative 2	1.269	47.2813238771
13	Negative 3	1.275	47.0588235294
14			

In @ 2:00 PM

Out @ 2:00 PM
- Honors lab 2:00 - 3:30 -
In @ 8:00 PM

> FACS'd the Interlabs. GS2 and GS3 each had 1/3 replicates with visibly turbid growth whereas the others did not-- however, GS3 had sufficient cells (albeit with low density) to get 10,000 measurements on Device 1 following the protocol. This completes raw data collection for the Interlab!

Out @ 9:30 PM

160901 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-08-31

WEDNESDAY, 8/31

Today's To-Do

General

- [ ] **Part Sheets!!**

Decoy Binding Array

- [ ] Assembly Pipeline: (putting lacO array on UNS backbone)
  - [x] PCR:
    - P167 P168 on pJD100 (lacO array)
    - P019 P013 on a 1C3 UNS backbone template (your choice)
    - P019 P013 on a 1A3 UNS backbone template (your choice)

    Done by CEM 160901 CEM
  - [x] DpnI
  - [x] PCR Purification **(don't continue pJD100 (PCR #3) because it was gel-disconfirmed, but *do* continue the backbones)**
  - Gibson Assembly
  - Transformation
- [ ] Assembly Pipeline: (combining pLac sfGFP with constitutive lacI)
  - [x] PCR:
    - P160 + P013 on WM16_016 1C3
    - P008 + P140 on WM16_014 1C3

    Done by CEM 160901 CEM
  - [x] DpnI
  - [x] PCR Purification
  - [x] Gibson Assembly: [P160 P13 16 1C3] + [P8 P140 14 1C3]
  - [x] Transformation
- [ ] Assembly Pipeline: (making Primer Dimers that contain Cut Site Inserts to put the arrays into UNS backbone)
  - [x] PCR: *(these inserts will go with the backbone PCRs from above. UNS 1A3 and UNS 1C3)*
    - P169 P170 no template (for the lacO array)
    - P171 P172 no template (for the tetO array)
  - [x] DpnI
  - [x] PCR Purfication
  - [x] Gibson Assembly:
    - [P169 P170] + UNS 1A3 from above
    - [P169 P170] + UNS 1C3 from above
    - [P171 P172] + UNS 1C3 from above
  - [x] Transformation

Macrogen Sequencing

- [x] Send out 85x tetO 1K3, 85x tetO 3C5 (both of these VF2 VR), and WM16_109 1C3 (P008 P009)

  (adam is doing this)
- [x] Check sequence of 85x tetO 1A3 from 160826 Macrogen Sequencing

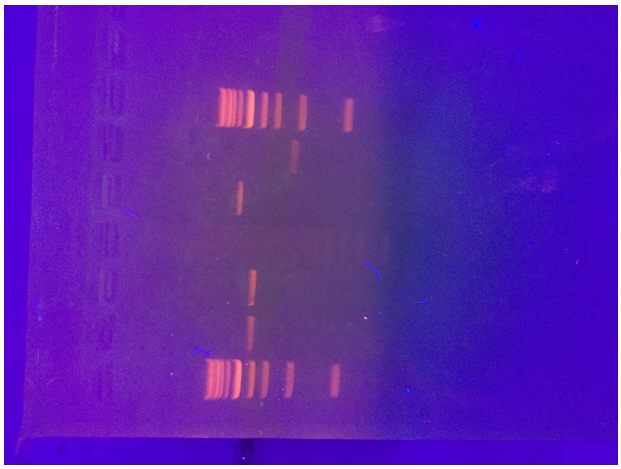
  (EMJ determined that the Fwd sequence has ~30 tetO sites. The reverse had nothing...???)

In @ 12:00 PM

All ordered primers have arrived now.

Gel for the PCRs from this morning:

IMG_8255.JPG



5: pLac sfGFP insert form 14 1C3. ~1200 bp
4: lacI + backbone from 16 1C3. ~4126 bp
3: lacO array insert . ~1700 bp
2: UNS backbone from 14 1A3. 2778 bp
1: UNS backbone from 14 1C3. 2778 bp
From 160901 CEMPCRs

Out @ 1:00 PM

I ran Interlab smaples through FlowCal-- the FSC/SSC adjustment seems to make FlowCal's auto-gating feature behave strangely. The distributions are capturing a lot of 'non-cellular' events, which means the distribution are weighted down by autofluorescent events.

The Interlab form does not require FlowCal calibration, but simply dividing means of sample peaks by means of bead peaks... for this I think the better move would be to open up the .fcs files on ProSort again and manually record the means to follow the Interlab protocol explicitly.

In @ 5:30 PM

Went back to ProSort-- turns out you can explicitly save .fcs files that contain only a selected subset of post-gate events! I feel embarassed I didn't look into this before, but this should get around FlowCal picking up non-cellular events. Re-running FlowCal. The new plots look amazing! Crisp, single-peak distributions that follow the MEFL levels that would be expected from the promoters driving the different constructs.
Unfortunately I forgot to obtain .fcs files for the Negative Control and so can't complete the FlowCal analysis... I set up inoculations from-plate of all the Interlab Devices again, in one go, to finish it off once and for all.

In @ 7:00 PM means that they should be diluted via OD around 11:00 AM - 1:00 PM tomorrow

Out @ 7:20 PM
In @ 8:40 PM

Talked to Dr Saha about equipment for the BioMakerSpace... need to think about the future of Synthetic Biology and what future iGEM teams here would want to do but cannot because of a lack of equipment.

Out @ 10:50 PM

160902 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-02

FRIDAY, 9/2

Today's To - Do

General Project

- ☐ Construct a gBlock to express the GFP which we ordered in purified form (ppluGFP1 http://getentry.ddbj.nig.ac.jp/getentry/na/AY268071/?filetype=html (http://getentry.ddbj.nig.ac.jp/getentry/na/AY268071/?filetype=html))
- ☐ Part Pages!!

Decoy Binding Array

- ☒ Colony PCR the transformants from last night. [VF2 VR for the arrays and P8 P9 for the other constructs] *(christine did this)*
- ☒ Inoculate them into LB for miniprepping tomorrow *(Kalen's doing this)*
- ☒ Assess the Macrogen sequences that we will get back this evening *(EMJ checked them)*

Interlab

- ☒ Dilute the cultures via OD around 12:30 PM ***(JPM will do this)***
- ☒ FACS the parts at 7:00 PM!

In @ 12:30 PM

Diluted the Interlab parts according to OD:

Table1

	A	B	C
1	Sample	OD	uL to add
2	Device 1 1	1.033	58.0832526621
3	Device 1 2	1.138	52.7240773286
4	Device 1 3	1.124	53.3807829181
5	Device 2 1	1.156	51.9031141869
6	Device 2 2	1.115	53.8116591928
7	Device 2 3	1.235	48.5829959514
8	Device 3 1	0.941	63.7619553666
9	Device 3 2	1.052	57.0342205323
10	Device 3 3	1.076	55.7620817844
11	Positive 1	1.068	56.1797752809
12	Positive 2	1.083	55.4016620499
13	Positive 3	1.244	48.231511254
14	Negative 1	1.138	52.7240773286
15	Negative 2	1.198	50.0834724541
16	Negative 3	1.176	51.0204081633
17			

These went back into the incubator at 1:00 PM so FACS should happen at 7:00 PM.

Out @ 2:00 PM
In @ 5:00 PM

Loaded the gels of the Colony PCR that Christine loaded:

A1 A2 A3 A4 B1 B2 B3 B4
C1 C2 C3 C4 D1 D2 D3 D4

Out @ 6:15 PM
In @ 7:00 PM

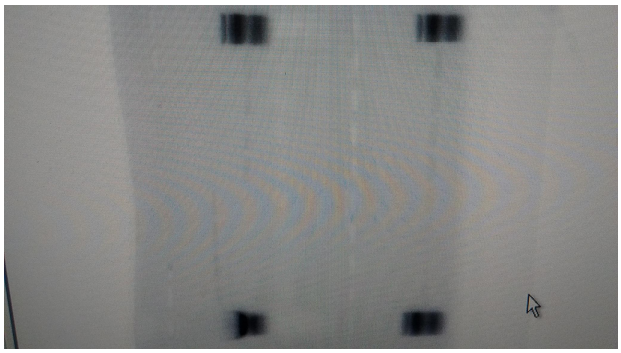
Ran the Interlab samples through the FACS (taken out of the incubator at 7:00 PM). They all grew properly and looked perfect on the FACS! Filled out the FACS spreadsheet in the way that the Interlab form specifies.

It is in Dropbox/iGEM 2016/FACS Data

Ethan checked the Macrogen sequences-- everything was confirmed!!!

The ladder I used last time wasn't correct-- should've used the 50bp ladder. Kalen re-ran the gel with the proper ladder:

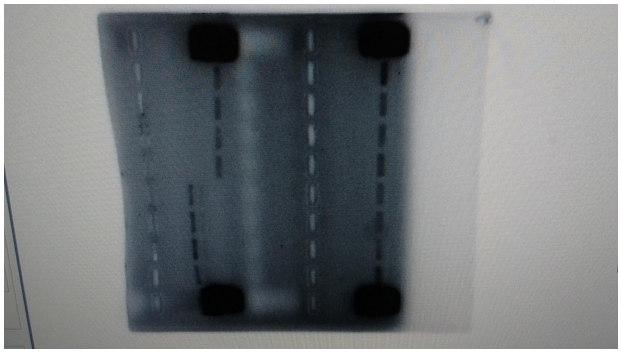
IMG_20160902_204358085.jpg



lacO restriction insert 1A3 --- tetO restriction insert 1C3

WM16_110 1C3 --- lacO restriction insert 1C3

IMG_20160902_204424745.jpg



Same gel as above but with more clarity on bands.

Kalen inoculated colonies #1, 2, 3 of each of the four samples above. In ~9:15 PM

Out @ 9:15 PM

160903 JPM

Made with Benchling

Project: iGEM 2016

Authors: Callan Monette

Date: 2016-09-02

FRIDAY, 9/2

Today's To-Do

Decoy Binding Array

- [x] Miniprep the four constructs (lacO landing pad 1C3 and 1A3, tetO landing pad 1C3, WM16_110 1C3) around 11:00 AM
  Done by CEM 160903 CEM
- [ ] Transform WM16_109 1A3 with and without 85x tetO 1C3 into BL21

General

- [ ] Fill out order form for KpnI !
- [ ] PAT PAGES
- [ ] Construct a gBlock to express the GFP which we ordered in purified form (ppluGFP1 http://getentry.ddbj.nig.ac.jp/getentry/na/AY268071/?filetype=html (http://getentry.ddbj.nig.ac.jp/getentry/na/AY268071/?filetype=html))
- [ ] Wash Bottles :(

September Outline

Made with Benchling

Project: iGEM 2016

Authors: Kalen Clifton

Date: 2016-09-06

TUESDAY, 9/6

Decoy Binding Array

- [ ] Thursday Sep. 8: Co-Transform 110 with the addGene lacO array
  - [ ] Friday Sep. 9: Inoculate
  - [ ] Saturday Sep. 10: Induce and FACS
- [ ] Move lacO array onto BioBrick Backbone
  - [x] Thursday Sep. 8: Cut and Ligate
  - [x] Friday Sep. 9: Transform
  - [ ] Saturday Sep. 10: Inoculate *(it failed at Colony PCR)*
  - [ ] Monday Sep. 12: Cut and Ligate again
  - [ ] Tuesday Sep. 13: Transform
  - [ ] Wednesday Sep. 14: Inoculate
  - [ ] Thursday Sep. 15: Miniprep and send to Macrogen
- [ ] Saturday Sep. 17: Co-Transform 110 with the UNS lacO array
  - [ ] Sunday Sep. 18: Inoculate
  - [ ] Monday Sep. 19: Induce and FACS
- [ ] Swap the RBS on the lacI of WM16_110 into a weaker one
  - [x] Saturday Sep. 10: PCR -> Gibson Assembly -> Transform
  - [x] Sunday Sep. 11: Inoculate
  - [x] Monday Sep. 12: Miniprep AND Send to Macrogen

Synthetic Enhancer

- [x] Wednesday and Thursday, Sep. 7, 8: Design and Order Primers to:
  - Swap the mCherry coding region for a sfGFP coding region in 52S and 55AS
  - Remove the NRII coding region from pACT-Tet and clone it into 52S and 55AS
  - Put the NRII coding region on the UNS backbone
- [ ] Create GFP-coding combos of NRII on 52S and 55AS:
  - [x] Friday, Sep. 9: PCR -> Gibson Assembly -> Transform
  - [x] Saturday Sep. 10: Inoculate
  - [x] Sunday Sep. 11: Miniprep
  - [x] Monday Sep. 12: Send to Macrogen
  - [ ] Wednesday Sep. 14: PCR -> Gibson Assembly -> Transform (the second step of the two-step sequence to get a GFP-coding combo with NRII on 52S and 55AS)
  - [ ] Thursday Sep. 15: Inoculate
  - [ ] Friday Sep. 16: Miniprep AND send to Macrogen (to arrive on Saturday)

Toolbox

- [ ] Diagnostic test of 28 and 29 (pBad lacI-mCherry and pBad tetR-mCherry)
  - [x] Tuesday, Sep. 6: Streak Glycerol Stocks of 28 and 29
  - [x] Wednesday, Sep. 7: Inoculate
  - [x] Thursday, Sep. 8: Induce
  - [x] Friday, Sep. 9: FACS
  - [x] If good:
    - [ ] Friday, Sep. 9: Cotransform 28 and 29 with a pLac GFP / a pTet GFP
    - [ ] Saturday, Sep. 10: Inoculate
    - [ ] Sunday, Sep. 11: Induce and FACS
    - [ ] If bad:

- [ ] Construct lacI + pLac version of tetR-mCherry...

- [ ] Determine the Conversion curve from MEFL to [GFP]
  - [ ] Wednesday, Sep. 7: Inoculate a GFP and a Neg. control for tomorrow's OD600 serial dilution in the Plate Reader
  - [ ] Thursday, Sep. 8: Determine the minimal OD600 at which the FACS can detect cells vs. autofluorescence from blank
  - [ ] ...

160906 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-09-06

TUESDAY, 9/6

Today's To-Do

Decoy Binding Array

- ☒ Dilute 109 1A3 w/ and w/out 85x tetO 1C3 (BL21) into 4 mL M9 each at 11:00 AM
- ☒ Run Gel of Colony PCRs of the above (co)transformations using P8 P9

  Because if you look at the VF2 VR Colony PCR gels from 160905-EMJyou see questionable double banding on the solo transformation. EMJ thought this was due to off-target amplification so we diagnosed that with P8 P9 test.
- ☒ Image Gel
- ☒ Induce the (co)transformation dilutions with 14-step aTc induction at approx. 3 or 4 PM ***(CEM is doing this)***
- ☒ FACS the 109 w/ w/out 85x tetO cotransformations... hopefully the induction curve shift is more distinct with the weaker RBS.
- ☒ SEND to Macrogen the four most recently-miniprepped constructs:
  - WM16_110 1C3 MP 1 - 3 Forward AND Reverse
  - lacO cut site insert 1A3 MP 1 - 3 Forward Only
  - lacO cut site insert 1C3 MP 1 - 3 Forward Only
  - tetO cut site insert 1C3 MP 1 - 3 Forward Only

  All of these sequences are P008 P009 since they are flanked by UNS2 and UNS3.

General

- ☒ Make more M9 media
- ☐ Wash Bottles!!
- ☐ Part Pages

In @ 10:40 AM

Ordered KpnI-HiFi
Glycerol arrived from Genesee.

Made gel to image P8 P9 colony PCRs in.

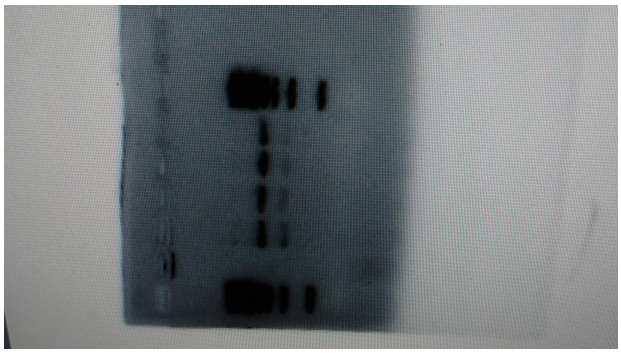
Overnight inoculations of the (co)transformations' status:

- Only 1/3 of the cotransformations (#1) grew.
- 2/2 of the solo transformations grew.

I inoculated the three successful inoculants into M9 w/ appropriate low-dosage antibiotic. 1:50 dilution of 80 uL into slightly over 4mL.
They went into the incubator around 11:15 AM.

Kalen loaded the gel with the P8 P9 colony PCRs of the (co)transformations. 160906 KPC

IMG_20160906_115202558.jpg



All with P008 P009 (array should not amplify):

109 1A3 + 85x tetO 1C3 #3 (didn't grow)
109 1A3 + 85x tetO 1C3 #2 (didn't grow)
109 1A3 + 85x tetO 1C3 #1
109 1A3 #2
109 1A3 #1

compare with gel from 160905-EMJwhich is the same colonies, just with VF2 VR amplification.

Why is there that lower band?? Seems like perhaps 109 may have been constructed incorrectly... but the sequence says confirmed on the miniprep log. Middle area is unreachable by Macrogen sequencing... perhaps there's a UNS2 or UNS3 in there somehow??

Kalen and I set up Macrogen sequencing of the constructs on the to-do list, according to their specifications.

Out @ 12:15 PM
In @ 5:40 PM

Planned out the next two weeks with Ethan and Andy.

Out @ 6:20 PM
In @ 7:40 PM

Made the September Outline. Started designing primers with Ethan.

Out @ 9:15 PM
In @ 10:45 PM

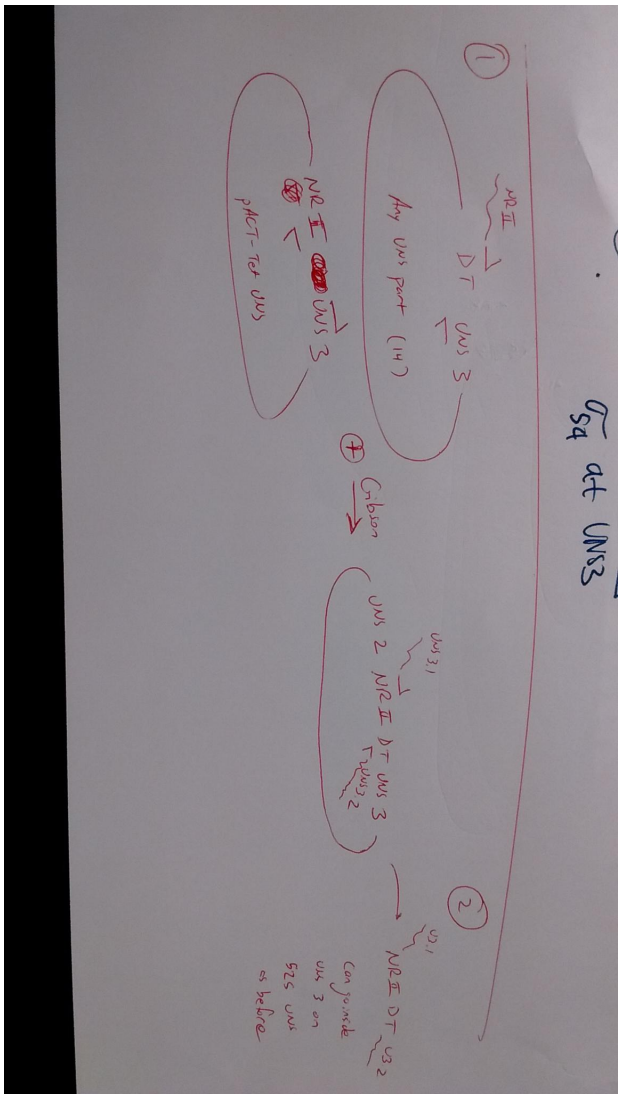
FACS'd the 109 solo and cotransformations. The solo induced but the co didn't look great....

Likhitha is designing primers from the September Outline to move Sigma 54 parts together.

Talking with Likhitha about the primer designs... turns out everything we thought was wrong!!! ahhh

- Issue 1: How to properly replace mCherry with sfGFP when we aren't sure of the directionality of the promoter and RBS in the original construct
- Issue 2 (resolved): How to put DT onto the end of the NRII sequence appropriately (requires this becoming a two-step assembly, making the whole thing three-step). See image below for schematic of new idea:

help.jpg



Out @ 2:00 AM :(:(:(:(:(

160907 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-09-07

WEDNESDAY, 9/7

Today's To-Do

Synthetic Enhancer

- [ ] Design Primers to:
    - Swap the mCherry coding region for a sfGFP coding region in 52S and 55AS
    - Remove the NRII coding region from pACT-Tet and clone it into 52S and 55AS
    - Put the NRII coding region on the UNS backbone

  See 160906 JPMfor preliminary designs

Decoy Binding Array

- [ ] Check Macrogen Sequences when they arrive tonight

General Project

- [x] Inoculate the WM16_028 and WM16_029 glcyerol streaks into *M9 Glycerol* for Arabinose Induction and FACS tomorrow.
- [ ] Determine the minimal OD600 at which the Plate Reader can distinguish a GFP sample from autofluorescence, using the inoculated Interlab construct overnight. ***(EMJ)***

Misc.

- [ ] Wash Bottles :(

  They're in the back... the Incubator room...

In @ 11:00 PM

We forgot to inoculate a Negative control last night for the OD600 test so we're doing that again.

Had an iGEM meeting at 9:00 PM to discuss the course of the project in the next two weeks. Brief.

Likhitha and Ethan figured out how to solve the sfGFP orientation issue, and Likhitha designed primers to perform that operation.

I am desgining primers to move NRII with Double Terminator onto the UNS3 region of a Sigma 54 plasmid.

Ethan is inoculating:

- WM16_028 1C3 glycerol streak,
- WM16_029 1C3 glycerol streak,
- Interlab Device #1 Glycerol Streak,
- Interlab Neg. Control Glycerol Streak

into M9 + Thiamine Chlor. In the incubator around 12:15 AM.

Out @ 12:30 AM

Designed Primers P179 - P181.

160909 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-09-08

THURSDAY, 9/8

Today's To-Do (courtesy of Ethan Jones)

Misc Info

There is now a form to fill out if a supply is running low. It is preferred that you write something down, even if it might turn out that we actually have it.

Additionally there is a checkbox thing on the freezer. Please check a box whenever you use up a tube of DPN1, HiFi MM or Q5.

Lastly, the plate area of the fridge is now organized. Main bags of plates are arranged in order of use from left to right on the lower shelf. Triples have there own spot, and kan+amp plates are on the door (why do we have so many of these). All plates with any tet in them have been moved into a drawer. We still have the little part above where we put chlor and amp plates unbagged.

As a note, please **USE THE OPENED AND SMALLEST BAGS FIRST**, I have placed them on top (you know cause stacking) for convenience. In the future when making a full bag of plates please put at the bottom of the stack, it will cost a small amount of effort, but will pay off in preventing our fridge from making no sense.

Tasks:

- [x] Autoclave the stuff on the cart

- [x] FACS the overnight inductions of 28 and 29 in 5 alpha at around 2:00 PM ***(JPM will do this)***

- [ ] Transform:
  - **WM16_014 3K3 into BL21**
  - **28 1C3 and WM16_014 3K3 into BL21**
  - **ptet GFP (WM16_023) 3K3 into BL21**
  - **29 1C3 and pTet GFP (WM16_023) 3K3 into BL21**

  the purpose of these transformations is to induce them (with lots of Arabinose and a gradient of IPTG) to make sure the repressor function of lacI-mCherry and tetR-mCherry are preserved. We are putting them onto 3K3 because pTet GFP only exists on 3K3 (other than 1C3), and 14 is also placed there for consistency across repressors.
  - **25 1C3 [MP2 160814 Box 10 Slot 63] and 16 3K3 into BL21**

  which is a straggler RiboJ part that needs to be IPTG-induction-tested

- [ ] Convert FACS data from 109 solo and w/ 85x tetO into MEFL

- [ ] GIBSON ASSEMBLY:
  - [x] PCRs:
  - Move functional NRII onto UNS backbone
    - P180, P182 on pACT-Tet *(not UNS version!)* [Box 9 Slot 36] - 1
    - P181, P013 on WM16_014 1C3 (using Box 7, 51: 014 1C3 from MP2, 160709 AJR) - 2
  - Swap mCherry -> sfGFP in the Synthetic Enhancer construct 52S
    - P175, P178 on WM16_014 1C3 - 3
    - P019, P174 on 52S DT UNS 1C3 MP1 160802 (Box 9 Slot 45) - 4
  - Move the Synthetic Enhancer construct 55AS onto the UNS backbone

        - P042, P043 on 55AS Kan (Box8 Slot 32) - 5
        - P040, P013 on WM16_014 1C3 - 6
    - ☒ Run Gel
    - ☒ DpnI
    - ☒ PCR Purification
    - ☒ Gibson
    - ☒ Transform into 5 alpha

- ☒ Colony PCR WM16_028, WM16_029, WM16_011, WM16_016 (1C3 and 1A3), WM16_110, and WM16_110 + pJD.
  - plate B(WM16_110+pJD) didn't grow
    - Use p008 and p009 for all parts
    - In addition use p167 and p168 for WM16_110 +pJD

- ☐ Make the following things *(lowest priority)*
    - ☐ 2x LB (.5 liters)
    - ☐ 5x M9 salts (.5 liters)
    - ☐ CaCl2 1 M (.25 liters)
    - ☐ Sterile ddH2O (1 liter)

- ☒ Innoculate the things above **(see Gel pictures below for which colonies to use)**, *as well as something with sfGFP (medium strength) in BL21* **(EMJ)**
    - LB for WM16_011, WM16_016 (1C3 and 1A3).
    - The others are going into the FACS pipeline, so people who know about induction should know the media.
        - WM16_028 and WM16_029 are going to be induced with arabinose (fusion mcherry repressor under pBAD)
        - WM16_110 w/ and w/o pJD are going to be induced with IPTG
- ☐ Transform Ligation (ligation labeled "lig" and is in thermocycler 4) [this is the ligation of lacO array into UNS bakcbone 1A3 (1C3 doesn't work because it has a SacI cut site... need to gibson the array over after 1A3 works to get it on 1C3)]
    - Use about 1µl ligation per 10µl cells

- ☒ Think about/look at sequencing and primer design to determine why yesterdays gibson PCR failed (see160908 CEM for PCR details)
    - Note, I repeated the same PCR but with miniprep 3 and it still failed. The only alteration I made was raising the annealing temp of one PCR to 67 from 66.
    - ***Turns out it's because the PCR should have been [P166 + P008] + [P165 + P013].***

In @ 1:00 PM

The cotrasnformation of WM16_110 1C3 with the addGene lacO array (pJD100) did not grow!! Maybe we should have grown it at 30C as recommended.

Setting up FACS.

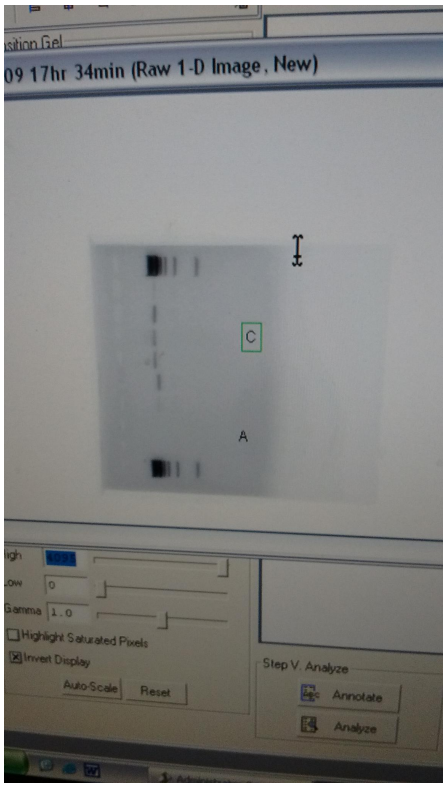
FACS'd the Arabinose inductions of 28 and 29 in 5 alpha. They induced quite well based on au (didn't need to change gain within a replicate)!!!

I looked into yesterday's PCR primers. Turns out I incorrectly wrote [P165 + P008] + [P166 + P013] instead of the correct version, [P166 + P008] + [P165 + P013]. Ouch.

Bio Seminar 4:00 - 5:00 PM

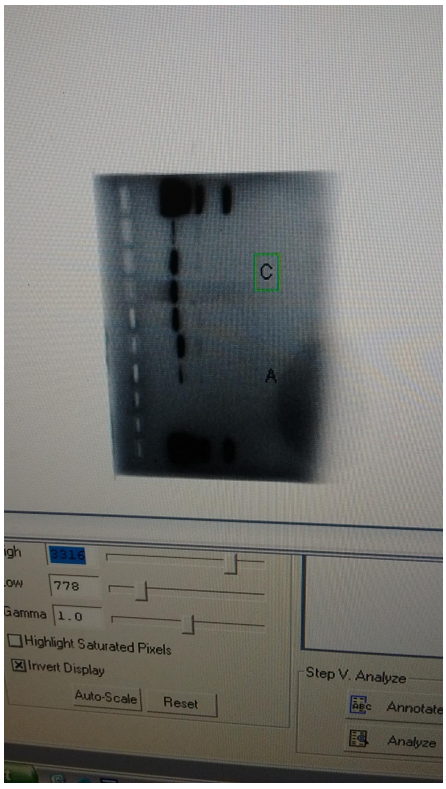
Kalen and I imaged the gels of colony PCRs (160909 KPC) . Lengths all looked good to the extent that we could tell, although 28 and 110 had weak double-banding. 110 isn't transforming well.

IMG_20160909_171518885.jpg



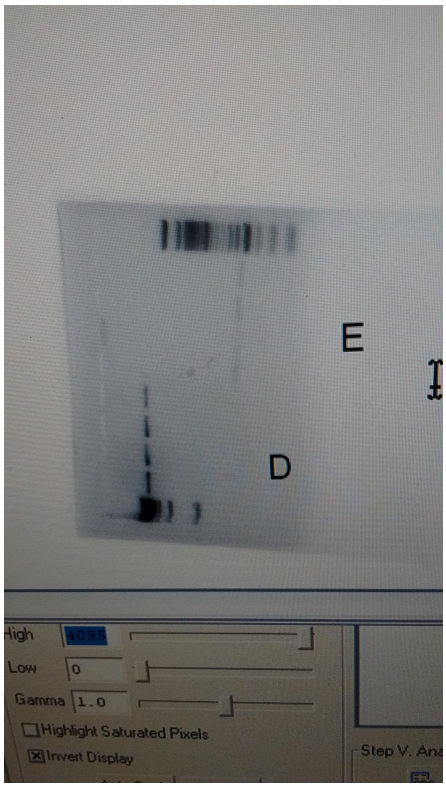
Top 4: WM16_028 1C3 in BL21 ***(use #1,2,3)***
Bottom 4: WM16_110 1C3 in BL21 ***(use #3, 4)***

IMG_20160909_171614477.jpg



Same as above picture, but darkned to highlight double-banding.

IMG_20160909_171755753.jpg



Top 4: WM16_011 (ICA part) in BL21 ***(use #1, 3, 4)***
Bottom 4: WM16_029 1C3 in BL21 ***(use #1,2 ,3)***

IMG_20160909_171859225.jpg

WM16_016 1A3 in BL21 ***(use #1,3,4)***

Kalen is running Colony PCR of F (16 1C3 in BL21) on a gel.

Callan PCR Purified.

It turns out that nobody had run the PCR products on a gel yet!! Kalen is doing that now. Many of the PCR Purfication nanodrop values are near zero. :(

Gibson Calculator ended up being:

Table1

	A	B	C	D	E	F	G
1	160909						
2	**Backbone**	**2: P181 P013 on WM16_014 [1C3]**	**0.06**	**2299**	**88.7**	**1.026385569**	
3	Insert	1: P180 P182 on pACT-Tet	0.18	1237	119.3	1.231815591	2.741
4	**Backbone**	**4: P019 P174 on 52S DT UNS [1C3]**	**0.06**	**4100**	**28.9**	**5.61799308**	
5	Insert	3: P175 P178 on WM16_014	0.18	889	1.7	62.12541176	-62.743
6	**Backbone**	**6: P040 P013 on WM16_014 [1C3]**	**0.06**	**2279**	**100.2**	**0.9006826347**	
7	Insert	5: P042 P043 on 55AS Kan	0.18	2798	2.5	132.96096	-128.86

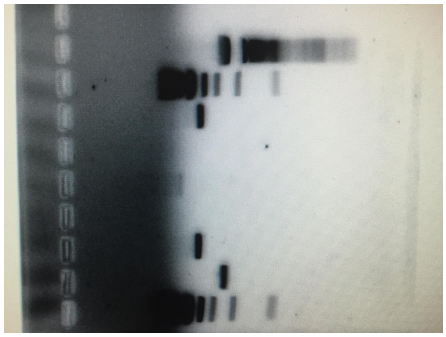
We are proceeding with the Gibson to make UNS functional NRII 1C3 but not the other two.

It turns out that Adam miscalculated the extension times for PCRs #4 and #5, which explains their low yields (see 160909 AJR). However, the annealing temp. and extension time for PCR #3 was correct, so I'm not sure why it didn't work... looked over primers again and found no issues. Possibly could be a fluke. Tomorrow we'll do it again, but on a different template (should have been WM16_014 1A3 anyhow to get the two backbones involved in the Gibson to have different antibiotics)

Out @ 7:00 PM
In @ 8:40 PM

Adam imaged the gels: *(the following verbatim from* 160909 AJR*)*

IMG_8288.JPG



From bottom to top: #1-#6
#3 and #5 didn't work - this was apparent from nanodrop values. We will need to redo these. #4 looks weak, but it had a low nanodrop value so that makes sense. The extension time for that one was also a little off -> should'e been 2:00 instead of 1:30. We can try that one again if need be.

IMG_8289.JPG

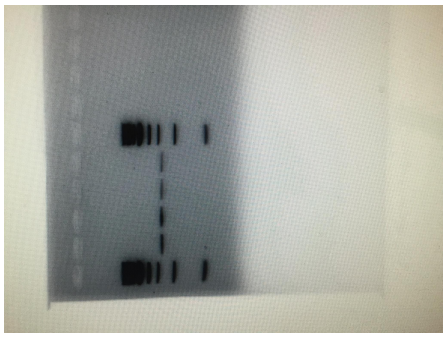


Plate F, colony PCRs: all look great, will innoculate first three colonies

Adam set up the transformation of the Gibson part (functional NRII UNS 1C3) into 5 alpha. He forgot to transform the UNS 48x lacO 1A3 into 5 alpha and the five BL21 transformations so Ethan is starting that now.

Kalen is setting up the inoculations of the colony PCRs. In the incubator around 10:00 PM

Out @ 10:00 PM

160908 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-08

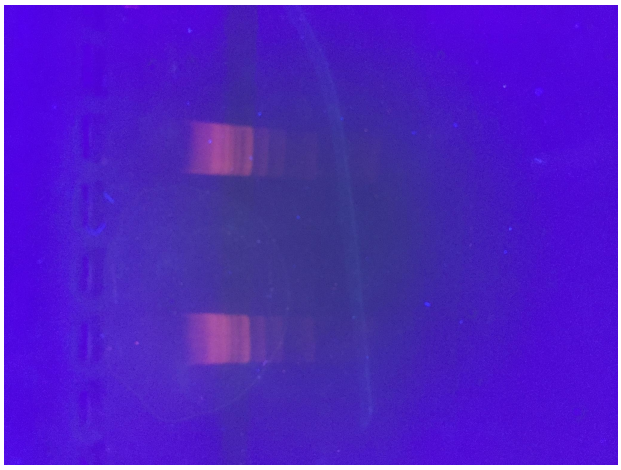
THURSDAY, 9/8

Today's To-Do

Decoy Binding Array

- [x] Cut and Ligate lacO array and tetO array into UNS backbone. Want LacO 1A3, LacO 1C3, tetO 1A3 ***(EMJ + JLM)***
- [ ] Assembly Pipeline to swap the RBS in the lacI of WM16_110 (to make WM16_111)
  - [x] PCR:
    - [P008 + P165 on WM16_110 1C3 MP2]
    - [P166 + P013 on WM16_110 1C3 MP2]
  - [x] Run Gel

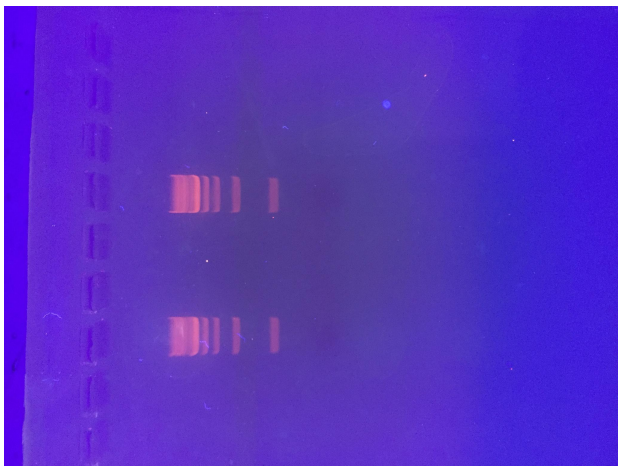
IMG_8277.JPG



Product 2
Product 1
Will run gel again on DPNI products, since the gel looked weird to begin with

  - [x] DpnI

IMG_8279.JPG



PCR of DPNI products didn't show anything either - need to redo PCR

  - [x] Redo PCR (see above) -failed EMJ
  - [x] DPNI
  - [ ] PCR Purficiation
  - [ ] Gibson Assembly (of the above two PCR products)

- [ ] Transformation

- [x] Transformations:
    - WM16_110 1C3 with addGene lacO array (pJD100) Amp into BL21
    - WM16_110 1C3 solo into BL21

        This is where we could cotransform WM16_110 with a non-coding Amp plasmid to control for its presence in the cotransformation, but the origin of replication of the pJD100 has unknown copy number and so we can't know to what extent using a 1A3 will actually "control" the difference... will save this control for when we have a UNS lacO 1A3.

Table1

	A	B	C
1	A	WM16_110 1C3 MP2 160903	11.2
2	B	WM16_110 1C3 MP2 160903 + pJD100 amp mp1 160814	11.2 + 11.1
3	C	WM16_028 1C3 MP3 160629	6.34
4	D	WM16_029 1C3 MP3 160626	6.3
5	E	WM16_011 1A3 MP3 160603	1.61
6	F	WM16_016 1C3 MP1 160608	2.53
7	G	WM16_016 1A3 MP2 160614	4.3

Done - EMJ

Synthetic Enhancer

- [x] Check Primers that Likhitha and I designed (P174 - P182)
- [x] **ORDER THEM BY 3:00 PM!!!!!!!**

General

- [x] Induction of 28 and 29 (pBad lacI-mCherry and pBad tetR-mCherry) (these are in 5alpha!!)
    - [x] Dilute into 5mL *M9 Glycerol + Thiamine* to grow up to midlog (around 12:00 PM - ish)
    - [x] Induce with Arabinose at midlog Overnight, so we can FACS tomorrow
        - 14-step induction
            - Lowest non zero point was removed due to lack of tubes
- [ ] Determine minimal OD600 for comparison of GFP construct vs Neg Control on Plate Reader ***(EMJ)***
- [x] Transform 28 and 29 into BL21 (in case the FACS don't work)

Misc.

- [ ] Resuspend gBlocks (they are by the Mac in the back) ***(JPM)***
- [x] Wash Bottles
- [ ] Make a Part Page for WM16_110 and WM16_111

In @ 10:50 AM

Designed and Checked primers. P174-P182.

Calcium meeting 1:00 - 1:45

Ordered Primers.

Out @ 2:00 PM
In @ 12:00 AM

To-Do List.

Out @ 12:30 AM

160910 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-09

FRIDAY, 9/9

Today's To-Do

- **Gibson Assembly** to swap the RBS in the lacI of WM16_110 from B0034 to B0031 (creating WM16_111)
  - ☒ PCR:
    - P008 + P166 on WM16_110 1C3 MP2 (Box 11 Slot 25) (ext. time 0:30) (anneal at 65C)
    - P165 + P013 on WM16_110 1C3 MP2 (Box 11 Slot 25) (ext. time 1:30) (anneal at 66C)
  - ☒ Run Gel
  - ☒ DpnI
  - ☒ PCR Purification
  - ☒ Gibson Assembly
  - ☒ Transformation into 5 alpha
- **Gibson Assembly** to re-do the failed Synthetic Enhancer Assemblies from yesterday:
  - ☒ PCR
    - *(to swap mCherry -> sfGFP in the Synthetic Enhancer construct 52S)*
      P175, P178 on WM16_014 1A3 (Box 9 Slot 50) [ext. time ~0:30] (anneal at 67C)
      P019, P174 on 52S DT UNS 1C3 MP1 160802 (Box 9 Slot 45) [ext. time ~2:00] (anneal at 66C)
    - *(to move 55AS onto the UNS backbone)*
      P042, P043 on 55AS Kan (Box 8 Slot 32) [ext. time ~1:30] (anneal at 64C)
      P040, P013 on WM16_014 1C3 *(<- this has already been done yesterday. See yellow rack in fridge, PCR Purification #6, and use this for the Gibson Assembly)*
  - ☒ Run Gel
  - ☒ DpnI
  - ☒ PCR Purification
  - ☒ Gibson Assembly
  - ☒ Transformation into 5 alpha
- **Inoculate** the transformants from yesterday
  - ☒ Colony PCR all seven transformants with P008 P009
    - UNS 48x lacO 1A3 -- 1980 bp = 1:00 extension
    - 14 3K3 -- 1100 bp = 0:30 extension
    - 28 1C3 + 14 3K3 -- 3234 bp and 1100 bp = 1:30 extension
    - 23 3K3 -- 1017 bp = 0:30 extension
    - 29 1C3 + 23 3K3 = 2775 bp and 1017 bp = 1:15 extension
    - 25 1C3 + 16 3K3 = 1803 bp + 1348 bp = 0:45 extension
    - UNS functional NRII 1C3 = 1366 bp = 0:45 extension
  - ☐ Inoculate the UNS 48x lacO 1A3 into LB for Miniprep
  - ☒ Inoculate the BL21 transformations into M9 Glycerol for Induction:
    - WM16_014 3K3
    - 28 1C3 + WM16_014 1K3 with 5 mM arabinose
    - ptet GFP (WM16_023) 1K3
    - 29 1C3 + pTet GFP (WM16_023) 1K3 with 5 mM arabinose
    - 25 1C3 + 16 3K3

      These will be induced with a high [Arabinose] to activate the pBad overnight, after which they will be induced as we normally do with an IPTG / aTC gradient. 14 and 23 are used as reference points for spectral compenstation of GFP / mCherry in the same cell
  - ☒ Inoculate the Synthetic Enhancer Transformations into LB for Miniprep
    - UNS functional NRII 1C3

- **Dilute** the 28 and 29 solo BL21 inoculations to put them on the same pace as the 28, 29 cotransformations
  - [ ] Dilute into more M9 Glycerol with 5 mM arabinose for Induction tomorrow

    These will be induced with a high [Arabinose] to activate the pBad, but otherwise untouched. Used as a reference point for spectral compensation of GFP / mCherry in the same cells.
- **Induce and FACS** WM16_110 in BL21
  - [ ] Dilute the overnight inoculants in the morning into more M9
  - [ ] Induce them with aTc (14-step) once they reach midlog
  - [ ] FACS them
- **Do the Thing** for the Plate Reader and OD600 ***(EMJ)***
- **Make:**
  - [ ] 2x LB (.5 liters)
  - [ ] 5x M9 salts (.5 liters)
  - [ ] CaCl2 1 M (.25 liters)
  - [ ] Sterile ddH2O (1 liter)

In @ 11:30 PM

General upkeep and received debriefing of day's events from Ethan, Kalen, and Callan.

Modification to original plan for today was that we added 5 mM Arabinose to the 28 and 29-containing inoculations and dilutions tonight, rather than tomorrow, because arabinose induction is an overnight timescale whereas IPTG/aTc is shorter.

Inoculations went in around 1:00 AM

Out @ 1:15 AM

160912 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-11

SUNDAY, 9/11

Today's To-Do

Minipreps and Macrogen

- ☒ Miniprep: around Noon
  - UNS 52S + DT with sfGFP 1C3 *(pick 3 tubes with growth out of the 8 inoculants)*
  - UNS 55AS + DT *(pick 3 tubes with growth out of the 8 inoculants)*
  - WM16_111 1C3 *(there are only 3 inoculants so miniprep them all)*
- ☒ Send to Macrogen: *(all of them P008 and P009) before 2:00 PM!! (spreadsheet in dropbox, pre-labeled tubes on bench, aliqoutted primers in fridge in orange rack, started online form on mac-KPC)*
  - The above three samples' minipreps
  - UNS NRII + DT 1C3 160911

Colony PCRs and Inoculations

- ☒ Colony PCR: *(all of them P008 and P009... you can determine extentsion times from part pages) around 2:00 PM*
  - WM16_029 1C3 MP3 160626 [Box 6 Slot 3] + WM16_023 3K3 MP1 [Box 5 Slot 15] in BL21
  - WM16_023 3K3 MP1 [Box 5 Slot 15] in BL21
  - WM16_109 1A3 MP2 160831 [Box 11 Slot 23] + WM16_011 1C3 MP1 160605 [Box 2 Slot 40] in BL21
  - WM16_053 1A3 MP1 160817 [Box 11 Slot 7] + WM16_011 1C3 MP1 160605 [Box2 Slot 40] in BL21
- ☒ Inoculate them for Induction tomorrow:
  - 29 1C3 + 23 3K3 -- inoculate into M9 Glycerol with 5 mM Arabinose (tomorrow we will aTC-induce)
  - 23 3K3 -- inoculate into M9 Glycerol
  - 109 1A3 + 11 1C3 -- inoculate into M9 Glycerol (tomorrow we will aTC-induce)
  - 53 1A3 + 11 1C3 -- inoculate into M9 Glycerol (tomorrow we will aTC-induce)

Cuts and Ligations

- ☒ Cut to make UNS 48x lacO again
- ☒ Ligate overnight

General

Autoclave:

- ☒ More Glass Tubes for Inoculations *(tubes are in bin, with autoclave tape, ready to go)*
  - ☐ If someone wants to load a third rack of tubes that'd be greaaaaaaat
- ☒ Trash *(trash bag is in bin, with autoclave tape, ready to go)*

In @ 1:00 PM

Miniprepped following the Monarch protocol. Sent to Macrogen.

Started setting up Glycerol stocks

Out @ 3:00 PM

In @ 6:00 PM

Loaded the gel of the Colony PCRs that Likhitha did. 1kb A1 - A4 B1 - B4 1kb ; 1kb C1 - C4 D1 - D4.1kb

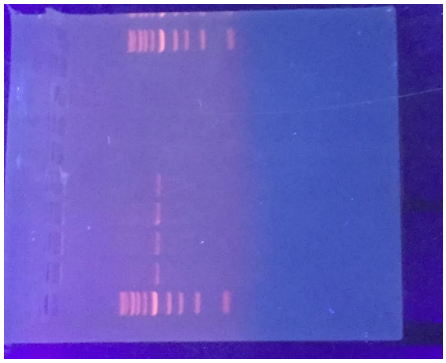
Ethan determined that the restriction cut on UNS 48x lacO was insufficiently cut... will try again tonight.

Out @ 6:25 PM
In @ 9:45 PM

The 29 + 23 cotransformation and the 23 solo transformation did not amplify on the colony PCR. 20160912 LK

GEL 1: A1--> A4, B1-->B4

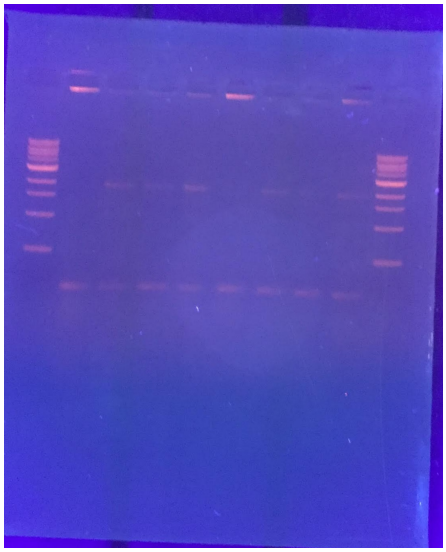
Screen Shot 2016-09-12 at 7.34.43 PM.png



Top: 23 3K3
Bottom: 29 1C3 + 23 3K3

GEL 2: c1--> c4, d1-->d4

Screen Shot 2016-09-12 at 7.34.50 PM.png



Left: 53 1A3 + 11 1C3
Right : 109 1A3 + 11 1C3

I got the feeling that perhaps 23 does not contain the UNS sequence but that it might still be functional.... to confirm this I traced back the sequence and found that we had sequence with VF2 and VR and found that the UNS regions were not present in the sequence (which was otherwise confirmed at the beginning and end, but due to length could not reach the middle).

Kalen set up redos of the Colony PCRs, but using P030 and P031 instead of P008 and P009. 160912 KPC

I inoculated all four colonies of 23 3K3 and 29 1C3 + 23 3K3, with the cotransformation containing 5 mM Arabinose, all in M9 Glycerol. These went in around 10:30 PM.

We imaged the gel and there were less bands than before... and definitely no bands on the 23 3K3 :(However we'll proceed as-is because maybe the issue is with P030 P031, given that there were less bands on the cotransformation than there were on the P008 P009. In hindsight we should have colony PCR'd with VF2 VR. But since Ethan already diluted out aTc for the cotransformation induction, we'll proceed.

Out @ 12:40 AM

160911 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-11

SUNDAY, 9/11

Today's To-Do

Inductions and FACS

Don't just blindly dilute everything because some inoculations are for Miniprepping!

Yesterday we were intending to induce and FACS WM16_110 solo. For some reason they took forever to grow and we missed the midlog. We're going to need to catch them at midlog again today and induce them.

- ☒ Dilute WM16_110 in the morning in M9 so that they can reach midlog
- ☒ Induce them at midlog with a 14-step IPTG induction
- ☒ FACS them around 7:00 PM

We also inoculated BL21 transformations used to test the efficacy of 28 (pBad lacI-mCherry) and 29 (pBad tetR-mCherry)'s repressor functions. These parts are:

14 3K3 28 1C3 + 14 3K3 23 3K3 28 1C3 29 1C3

And there was supposed to be a 29 + 23 cotransformation but it didn't grow.

- ☒ Dilute the above in the morning in M9 so that they can reach midlog
  - 14 3K3 -- dilute in M9
  - 28 1C3 + 14 3K3 -- dilute in M9 with 5 mM Arabinose
  - 28 1C3 -- dilute in M9 with 5 mM Arabinose
  - 23 3K3 -- dilute in M9
  - 29 1C3 -- dilute in M9 with 5 mM Arabinose
- ☒ Induce them at midlog:
  - 14 3K3 -- no induction, leave in shaker
  - 28 1C3 + 14 3K3 -- 14-step IPTG induction
  - 28 1C3 -- no induction, leave in shaker
  - 23 3K3 -- no induction, leave in shaker
  - 29 1C3 -- no induction, leave in shaker
- ☒ FACS them around 7:00 PM

We also inoculated the RiboJ part WM16_025 with WM16_016 to get a missing induction curve from way back in the day.

- ☒ Dilute the 25 + 16 cotransformation in the morning in M9 so that it can reach midlog
- ☒ Induce it at midlog with a 14-step IPTG induction
- ☒ FACS it around 7:00 PM

Inoculations

Yesterday we transformed Gibson assemblies to do the following things:

- *Move 55AS onto the UNS backbone (synthetic enhancer)*
- *Replace mCherry with sfGFP in UNS 52S (synthetic enhancer)*
- *Swap the RBS in the lacI of WM16_110 from B0034 to B0031 to make WM16_111 (Decoy Binding Array)*

- ☒ Colony PCR the transformants around 3:00 PM for 111, and late at night for the Synthetic Enhancer constructs, using P008 and P009 for all three above.
  - UNS 55AS ext. time ~1:30
  - UNS 52S sfGFP ext. time ~1:30
  - WM16_111 ext. time ~1:15
- ☒ Inoculate them, all into LB for miniprep tomorrow.
  - only able to innoculate WM16_111 1C3 - in shakenbator at 2020 hours

Minipreps

Yesterday we inoculated UNS NRII 1C3 (synthetic enhancer). This is a functional NRII-expressing construct under the original pACT-Tet promoter and nothing else.

- [x] Miniprep the UNS NRII 1C3 around 3:00 PM

Transformations

*Yesterday we tried to cotransform WM16_029 with WM16_023 in BL21 so that we can test 29's repressor function. The solo transformations worked but the cotransformation did not. The inoculation failed because it didn't grow on plate. There is only one good miniprep of WM16_023 3K3, and there are no minipreps of WM16_023 in any other non-chlor backbone. Meanwhile, there is basically only one good WM16_029 (besides it was already confirmed to induce with arabinose). I think it's worth re-doing it once. If we can't get 23 to work (which might be the case, given that even the plate that grew had 0/4 successful colony PCRs yesterday (*160910 KPC*), we will probably have to try and reconstruct it.*

- [x] Transform
    - WM16_029 1C3 MP3 160626 [Box 6 Slot 3] + WM16_023 3K3 MP1 [Box 5 Slot 15] into BL21
      Taking care to add 2 uL each of Miniprep solution to the tube of cells
    - WM16_023 3K3 MP1 [Box 5 Slot 15] into BL21

After converting the 109 + 85x tetO data to MEFL, it seems that the presence of the 85x tetO plasmid induces some significant strain on the cell, heavily impacting the fluorescence readout from the reporter construct. Also the 109 induction curves looked pretty sketchy in general. We should re-measure these with control blank plasmids (WM16_011):

- [x] Transform
    - WM16_109 1A3 MP2 160831 [Box 11 Slot 23] + WM16_011 1C3 MP1 160605 [Box 2 Slot 40] into BL21
    - WM16_053 1A3 MP1 160817 [Box 11 Slot 7] + WM16_011 1C3 MP1 160605 [Box2 Slot 40] into BL21

  The 53 is the same miniprep that was used in the 160824 FACS with 85x tetO, and the 109 is the same miniprep that was used in the 160906 FACS with 85x tetO. This means these results should be comparable with the old data as long as we use the Spherotech beads properly.

General

Make**:**

- [x] 2x LB (.5 liters)
- [x] 5x M9 salts (.5 liters)
- [x] CaCl2 1 M (.25 liters)
- [x] Sterile ddH2O (1 liter)

Autoclave:

- [ ] More Glass Tubes for Inoculations
- [ ] Trash - will do tomorrow (trash bag is in bin, with autoclave tape, ready to go)

Wash:

- [ ] Bottles

Analyze FACS Data:

- [x] Convert 109 + 85x tetO to MEFL
- [x] Convert 28 and 29 to MEPTR

Ethan Thing for Lonely OD's

Ethan has developed a protocol that will determine using serial dilutions in a plate reader what the minimum cell density of fluorescent cell is required in the plate reader to detect compared to a negative control.

- [ ] You got it man

In @ 11:20 AM

The synthetic enhancer gibson transformations had been plated on Chlor Kan and Chlor Amp instead of just Chlor as they were supposed to be. I re-plated from the transformation products in 4C. In @ 11:45 AM.

Inoculants that need inductions are at approx. midlog (by eye) now. Given the variability in midlog growth time, I'm going to go ahead and induce them now.

Inductions went in at 1:00 PM:

Protocol:

23 was not inoculated last night because it failed at colony PCR.

- I removed all inoculants from the shaker and put them on the bench
- I made 5 mM Arabinose M9 Glycerol (no antibiotic) solution by adding 150 uL 100 mM Arabinose M9 Glycerol solution into 3 mL M9 Glycerol.
- I made 1 mM IPTG + 5 mM Arabinose M9 Glycerol (no antibiotic) solution by adding 10 uL stock 100 mM IPTG solution into 990 uL of the above 5 mM Arabinose M9 solution. This is my 1 mM IPTG for the following dilution table:

Table2

	A	B
1	**Key**	**Induction Condition**
2	1	Add 0uL to 250uL culture to make 0uM IPTG
3	2	Add 1.25uL 1mM IPTG to 250uL culture to make 5uM
4	3	Add 2.5uL 1mM IPTG to 250uL culture to make 10uM
5	4	Add 5uL 1mM IPTG to 250uL culture to make 20uM
6	5	Add 12.5uL 1mM IPTG to 250uL culture to make 50uM
7	6	Add 25uL 1mM IPTG to 250uL culture to make 100uM
8	7	Add 50uL 1mM IPTG to 250uL culture to make 200uM
9	8	Add 1.25uL 100mM IPTG to 250uL culture to make 500uM
10	9	Add 2.5uL 100mM IPTG to 250uL culture to make 1mM
11	10	Add 5uL 100mM IPTG to 250uL culture to make 2mM
12	11	Add 12.5uL 100mM IPTG to 250uL culture to make 5mM
13	12	Add 25uL 100mM IPTG to 250uL culture to make 10mM

- ...which followed the key

Table1

	A	B
1	**Key**	**Sample**
2	A.1, A.2	WM16_110 1C3 in BL21 #1, 2
3	B.1, B.2, B.3	28 1C3 + 14 3K3 in BL21, #1, 2, 3
4	C.1, C.2, C.3	25 1C3 + 16 3K3 in BL21, #1, 2, 3

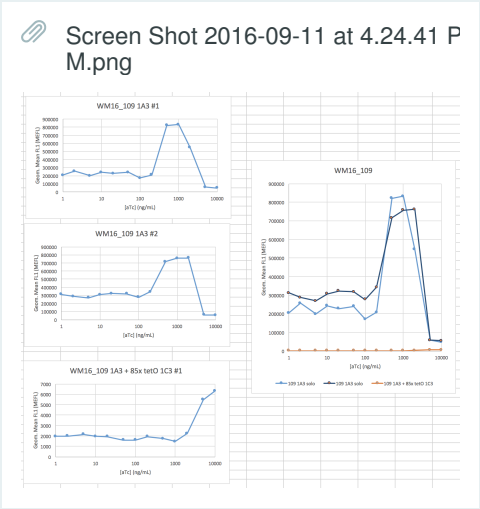
where LETTER.NUMBER1.NUMBER2 follows SAMPLE.REPLICATE.INDUCTION_CONDITION

- And then placed everything back in the shaker, including the un-induced tubes of 14 3K3, 28 1C3, and 29 1C3.

Out @ 1:20 PM
In @ 2:20 PM

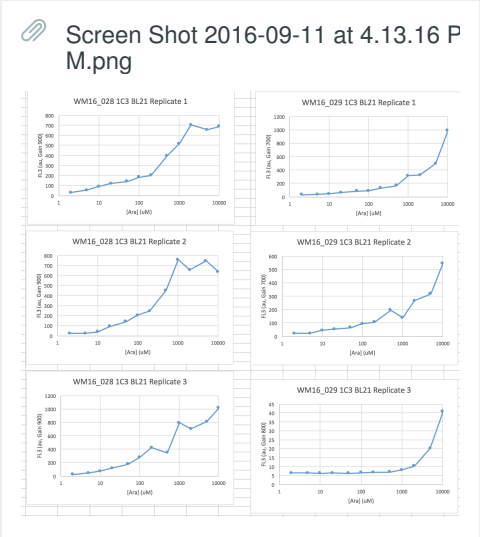
Converting FACS data to Absolute Units (109 w/ w/out 85x tetO and 28, 29).

- Seems like WM16_109 exhibits switch-like behavior in induction, by an order of magnitude... need to make a summary induction curve.



The two solo 109's look fairly consistent... and it looks like the cotransformation is really suffering. Having the blank-plasmid control is probably going to be for the best.

- FlowCal is crashing on the bead samples from the 28 29 tests! It seems that FlowCal doesn't like having the beads be measured at the FSC SSC settings that were used (in the Interlab style of increasing them to ~500 each to put the cell distribution in the center of the FSC SSC scatterplot). Measuring the beads on the ungated original FlowCal settings (FSC 339, SSC 292) seems to be the correct procedure. But this means we don't have absolute fluorescence values for 28 and 29's induction...
  - Thankfully, the gain did not have to be changed across the replicates in a given construct (with the exception of the third replicate of 29) so we can make arbitrary-unit induction curves. Doing that now.



28 looks fairly consistent.
29 looks like it could handle a higher level of [Ara] before it saturates... however note that the max of Replicate 1 is twice the max of Replicate 2 and 10-fold higher than the max of Replicate 3.

But we are in arbitrary units, too.

Likhitha came in to set up Colony PCRs. She did these at 3:00 PM, when there were only three colonies on the WM16_111 plate. We put the plate back in the incubator to see if more will grow.

Likhitha did Minipreps of UNS NRII + DT 1C3

Ethan came in at 4:00 to dilute his OD600 constructs.

Out @ 4:55 PM
In @ 7:40 PM

Reading on how to do spectral compensation using our FACS machine. ProSort has an autocompensation wizard. It seems like things will be okay as long as we have single-color controls for each combination with at least 5,000 relevant events. There also needs to be a global negative control. Autocompensation wizard seems to be able to be run retroactively on saved FCS files.

Set up and started FACSing. Settings are:

- A (WM16_110) -- FSC 600 SSC 550 FL1 750
- B (28 + 14) -- FSC 600 SSC 550 FL1 800 FL3 700
- C (25 + 16) -- FSC 600 SSC 550 FL1 800
- D (29) -- FSC 600 SSC 550 FL3 700
- E (28) -- FSC 600 SSC 550 FL3 700
- F (14) -- FSC 600 SSC 550 FL1 400 FL3 700

Out @ 12:00 AM

160913 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-12

MONDAY, 9/12

Today's To-Do

General

- ☒ Autoclave more glass tubes!!
  All uncapped tubes have been bleached-- caps can be put on tubes-to-autoclave
- ☒ Take them out of Autoclave around 11:30 AM

Transform

- ☒ UNS 48x lacO 1A3 into 5 alpha for eventual Miniprepping

Colony PCRs

- ☒ Diagnostic colony PCR using *VF2 and VR* on A1-4, B1-4 from 160912 Colonies using the same protocol as 160912 KPC
- ☒ Image Gel around 12:30

Inductions and FACS

- ☒ Dilute so that the overnight inoculations can reach midlog:
  - 29 1C3 + 23 3K3 -- dilute 150 uL culture into 4 mL M9 Glycerol with 5 mM Arabinose* *(pick three)*
  - 23 3K3 -- dilute 150 uL into 4 mL M9 Glycerol *(pick three)*
  - 109 1A3 + 11 1C3 -- dilute 150 uL into 4 mL M9 Glycerol
  - 53 1A3 + 11 1C3 -- dilute 150 uL into 4 mL M9 Glycerol

  ** ~ Make 5 mM Arabinose M9 Glycerol by adding 150 uL 100 mM Arabinose M9 Glycerol (in 4C Fridge, in a 5 mL Eppendorf tube) into 3 mL M9 Glycerol. ~*
- ☒ Induce with aTC at midlog
  - A: 29 1C3 + 23 3K3 -- 14-step aTC induction* *(pick three)*
  - B: 23 3K3 -- no induction *(pick three)*
  - C: 109 1A3 + 11 1C3 -- 14-step aTC induction
  - D: 53 1A3 + 11 1C3 -- 14-step aTC induction

  ** ~ aTC dilution protocol: ~*
  - ☒ Make 20,000 ng/mL aTc (1:100 dilution) *(This is in the fridge wrapped in aluminum foil!) (aTC is light-sensitive)*
    - ☒ 10 uL of 2mg/mL aTc
    - ☒ 990 uL of M9 Glycerol w/ appropriate low-dosage Antibiotic
  - ☒ Make 200 ng/mL aTc (1:10,000 dilution)
    - ☒ 10 uL of 20,000 ng/mL aTc
    - ☒ 990 uL of M9 Glycerol with Appropriate low-dosage Antibiotic
  - ☒ Follow the table below *(if there isn't enough media for 14 steps, cut the 1 ng/mL then the 2 ng/mL steps then the 10,000 ng/mL step, in that order, as needed)*:

Table2

	A	B
1	14	Add 250 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 10000 ng/mL aTc
2	13	Add 125 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 5000 ng/mL aTc
3	12	Add 50 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 2000 ng/mL aTc
4	11	Add 25 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 1000 ng/mL aTc
5	10	Add 12.5 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 500 ng/mL aTc
6	9	Add 5 uL of 200000 ng/mL aTc to 250 ul diluted culture to make 200 ng/mL aTc
7	8	Add 250 uL of 200 ng/mL aTc to 250 ul diluted culture to make 100 ng/mL aTc
8	7	Add 125 uL of 200 ng/mL aTc to 250 ul diluted culture to make 50 ng/mL aTc
9	6	Add 50 uL of 200 ng/mL aTc to 250 ul diluted culture to make 20 ng/mL aTc
10	5	Add 25 uL of 200 ng/mL aTc to 250 ul diluted culture to make 10 ng/mL aTc
11	4	Add 12.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 5 ng/mL aTc
12	3	Add 5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 2 ng/mL aTc
13	2	Add 2.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 1 ng/mL aTc
14	1	Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc

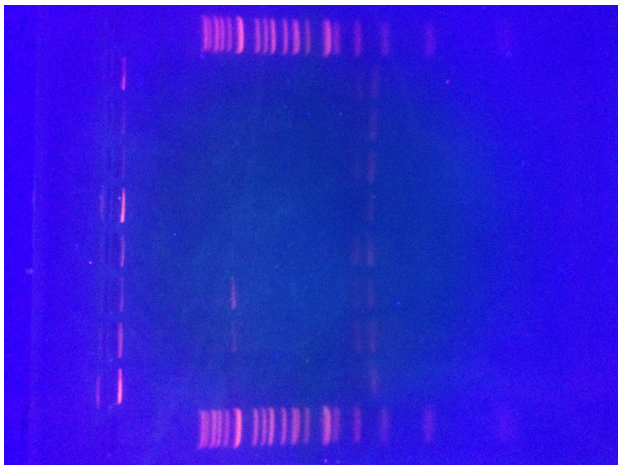
- [ ] FACS after 6-8 hours

In @ 11:45 AM

Had meeting 11:00-11:45 with Annie, Gerald, and Dan from advancement about long-term iGEM fundraising and relationship between iGEM and advancement office.

Loaded gel of Colony PCRs-- Kalen will image them.

160913AB.jpeg



Out @ 12:00 PM
In @ 5:40 PM

Setting up for FACSing the inductions.

A (29 1C3 + 23 3K3) had no growth in the induced cultures... left them in the shaker for now and prepped B, C, D.
C and D had 12, 13, 14 have visibly little growth in their replicates.

FACS:

Settings:

- D (53 1A3 + 11 1C3) : FSC 700 SSC 600 FL1 500
- C (109 1A3 + 11 1C3) : FSC 700 SSC 600 FL1 500

The FACS decided to stop behaving after three samples. Troubleshooting.....

During a wait step in the troubleshooting I converted the final 160902 Interlab Data to MEFL using FlowCal (rather than through the iGEM-endorsed spreadsheet). FlowCal says Device #3 looks a lot closer to Positive Control than being much lower than Pos. Control as it is in the spreadshet but generally the results are otherwise close enough. This suggests that recording the Spherotech beads under the FSC SSC settings of the original "iGEM E coli protocol" is indeed the correct way to record bead data. Saving only the gated portion of the events is also fine.

by the way alignment QC has FSC 354 SSC 301. Didn't notice that before, might be good to know.

The second time in a row that D.1.4 has caused a system clog. Something is wrong with this sample.

Samples were fine until C.2.8....

C.2.10 also broke it....

Finished C. B's are all just autofluorescence (you can tell because there is no bleedthrough from FL1 into FL2 channel). pTet GFP 3K3 is just a dud part... didn't FACS A because the reporter is broken. :(:(:(

Out @ 12:15 AM

160914 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-09-13

TUESDAY, 9/13

Today's To-Do

☒ Check Macrogen Results

(they are now available for download from the Macrogen website!)
(Part Pages are defined NRII : WM16_112 , UNS 55AS : WM16_113, UNS 52S w/ sfGFP : WM16_114)

☒ Get the Plate out of the Incubator

If it's grown sufficiently-- UNS 48x lacO 1A3.

Gibson Pipeline

- ☒ PCR: *(depending on what is confirmed, pick the best minipreps for the Gibsons which are possible)*
  - (1) UNS 55AS + DT 1C3 MP 2 [Box 11 Slot 46] P019, P174 [ext. time ~2:00 because 4348 bp] (anneal at 66C)
  - (2) WM16_014 1A3 from GS1 [Box 9 Slot 50] P175, P178 [ext. time ~0:30 because 889 bp] (anneal at 67C)
  - (3) UNS NRII + DT 1C3 MP 2 [Box 11 Slot 37] P176, P177 [ext. time ~0:45 because 1366 bp] (anneal at 70C)
  - (6) UNS 52S with sfGFP 1C3 MP _ [Box 11 Slot _] P178, P179 [ext. time ~2:30 because 5104 bp] (anneal at 64C)
  - (4) UNS 55AS + DT 1C3 MP 2 [Box 11 Slot 46] P178, P179 [ext. time ~2:30 because 5104 bp] (anneal at 64C)
  - (5) 52S DT UNS 1C3 MP1 160802 [Box 9 Slot 45] P178, P179 [ext. time ~2:30 because 5104 bp ish] (anneal at 64C)
- ☒ Make and Run Gel
- ☒ DpnI
- ☒ PCR Purfication
- ☒ Gibson Assembly:
  - (1) + (2) makes "UNS 55AS with sfGFP" 1C3
  - (3) + (6) makes "UNS 52S with sfGFP and NRII" 1C3
  - (3) + (4) makes "UNS 55AS and NRII" 1C3 <- canceled due to PCR failure of (3) and (4) :(
  - (3) + (5) makes "UNS 52S and NRII" 1C3 <- canceled dude to PCR failure of (3) :(
- ☐ Transform into 5 alpha for Miniprep eventually

Colony PCRs and Inoculations

- ☒ Colony PCR
  - UNS 48x lacO 1A3 with P008 P009 *and* VF2 VR.
    (ext. time 1:00)
    (since this is just diagnostic I'll keep 1:00 for the ~2kb insert, but I wonder if we should go longer if this were for a Gibson over a repetitive sequence?)
- ☐ Inoculate
  - UNS 48x lacO 1A3 into LB for miniprep tomorrow <- canceled due to failed ligation

In @ 11:00 AM

Removed plate of UNS 48x lacO 1A3 transformation from incubator. A small but decent number of small colonies, as expected.

Recall that:
WM16_111 = lacI + placO-sfGFP with B0031 on the lacI
WM16_112 = UNS functional NRII + DT

WM16_113 = UNS functional 52S + DT with sfGFP instead of mCherry
WM16_114 = UNS functional 55AS + DT

Checked Macrogen sequences. 111, 112, and 114 were all confirmed (114 confirmed to the extent that we can, given there is no consensus sequence for 114.)

113 were all disconfirmed because the sequences all had an alignment gap around the region that is supposed to code for sfGFP. I get a complete match when I align to an mCherry-coding version of the sequence... so it looks like the assembly didn't work here.

Out @ 11:30 AM
In @ 11:40 AM

I set up PCRs (1), (2), (3), (4), (5). 25 uL volume, 1:10 miniprep dilutions. Went into Thermalcylers 1-4 at 12:00 PM ish.

Kalen set up colony PCRs

Out @ 12:10 PM
In @ 1:35 PM

Loaded gels

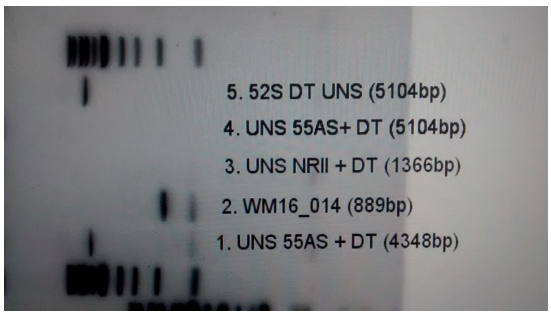
Out @ 1:55 PM
In @ 5:10 PM

The following from 160914 CEM:

Imaged gel of the following Gibson PCRs:

I. UNS 55AS + DT 1C3 MP 2 [Box 11 Slot 46] P019, P174 [ext. time ~2:00 because 4348 bp] (anneal at 66C)
II. WM16_014 1A3 from GS1 [Box 9 Slot 50] P175, P178 [ext. time ~0:30 because 889 bp] (anneal at 67C)
III. UNS NRII + DT 1C3 MP 2 [Box 11 Slot 37] P176, P177 [ext. time ~0:45 because 1366 bp] (anneal at 70C)
IV. UNS 55AS + DT 1C3 MP 2 [Box 11 Slot 46] P178, P179 [ext. time ~2:30 because 5104 bp] (anneal at 64C)
V. 52S DT UNS 1C3 MP1 160802 [Box 9 Slot 45] P178, P179 [ext. time ~2:30 because 5104 bp ish] (anneal at 64C)

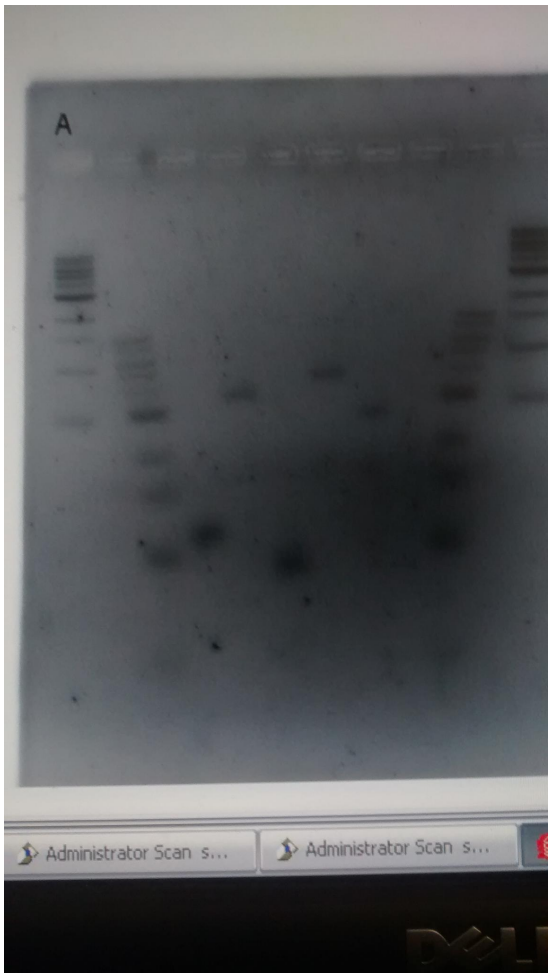
IMG_20160914_151226557.jpg



Bands 1, 2, 5 are appropriate sizes--will proceed with 1, 2

Also imaged gels for colony PCRs done by KPC:

IMG_20160914_152949556.jpg



UNS 48x lacO 1A3 with P008 P009 (~2kb)

IMG_20160914_153143484.jpg

UNS 48x lacO 1A3 with VF2/VR (~2kb)

Given that the gels were so weird for UNS 48x lacO, I re-ran the gel of the p008 p009 colony PCRs. Making a gel to re-run the VF2 VR ones as well.

Kalen is setting up Colony PCRs of the (1) and (2) PCR products.

Kalen set up the Gibson assembly of (1) + (2).

Imaged A1-A8 gel again. There were no bands. (p008 p009)

Loaded B1-B8 gel again.

Out @ 6:20 PM
In @ 8:10 PM

Imaged the B1-B8 gel again. There were solid bands across all 8 colonies at the <500 bp level. (vf2 vr)
So it looks like the UNS 48x lacO array didn't work.

:(

Out @ 9:15 PM
In @ 10:00 PM

Plated the Transformation.

Out @ 10:10 PM

160915 JPM

Made with Benchling

Project: iGEM 2016

Authors: Ethan Jones

Date: 2016-09-14

WEDNESDAY, 9/14

Today's To-Do

General

- ☒ Make Chlor Plates!! We are out!!
- ☒ Bleach tubes
- ☒ Wash bottles

Gibson Pipelines

- ☒ PCR:
  - (1) UNS NRII + DT 1C3 MP 1 [Box 11 Slot 36] P176, P177 [ext. time ~0:45 because 1366 bp] (anneal at 70C)
  - (2) UNS NRII + DT 1C3 MP 2[Box 11 Slot 37] P176, P177 [ext. time ~0:45 because 1366 bp] (anneal at 70C)
  - (3) UNS NRII + DT 1C3 MP 3 [Box 11 Slot 38] P176, P177 [ext. time ~0:45 because 1366 bp] (anneal at 70C)
  - (4) UNS 55AS + DT 1C3 MP 1 [Box 11 Slot 45] P178, P179 [ext. time ~2:30 because 5104 bp] (anneal at 64C)
  - (5) UNS 55AS + DT 1C3 MP 2 [Box 11 Slot 46] P178, P179 [ext. time ~2:30 because 5104 bp] (anneal at 64C)
  - (6) UNS 55AS + DT 1C3 MP 3 [Box 11 Slot 47] P178, P179 [ext. time ~2:30 because 5104 bp] (anneal at 64C)
  - (7) WM16_014 1A3 from GS1 [Box 9 Slot 50] P175, P009 [ext. time ~0:30 because 909 bp] (anneal at 64C)
    - *Only do (7) if the transformation from last night doesn't grow*
- ☒ Run Gel
- ☒ DpnI
  - ADD PCR (5) FROM YESTERDAY INTO THE PIPELINE IN THE DPNI STEP!
- ☒ PCR Purification
- ☒ Gibson Assembly
  - (1, 2, 3) + (4, 5, 6) *[depending on which end up working at the Gel level... no need to carry redundancy through the Gibson step]*
  - (7) + [PCR purification (1) from yesterday] <- canceled because yesterday's transformation looked good
  - [PCR purification of PCR (5) from yesterday] + (1, 2, 3) *[depending on gel]*
- ☒ Transformation
  - All Chlor into 5 alpha

Colony PCRs and Inoculations

- ☒ Colony PCR the UNS 55AS w/ sfGFP 1C3 using P008 P009
- ☒ Inoculate into LB for miniprep tomorrow (any three colonies)

Thinking

- ☐ Why doesn't UNS 48x lacO work????
- ☒ Order HiFi MM 2x

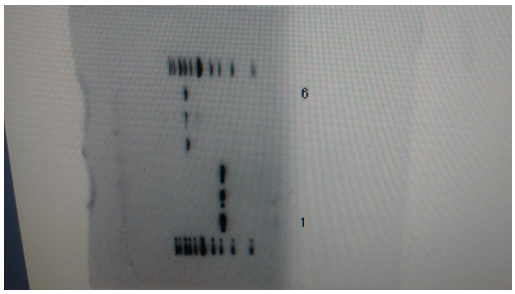
In @ 11:00 AM

Finished making LB plate broth-- put into autoclave at 11:30 AM

Made 0.5g agarose gel for PCRs which callan set up

Ran gels. Everything worked except for one double band. I don't know why it didn't work before-- guess it was a fluke?

0915_givsons.jpg



6
5
4
3
2
1

Proceeding with NRII MP2 and 55AS MP3 to DpnI, as well as PCR (5) from yesterday.

Set up DpnI of (2), (6), and (5 from yesterday).

In @12:45 PM

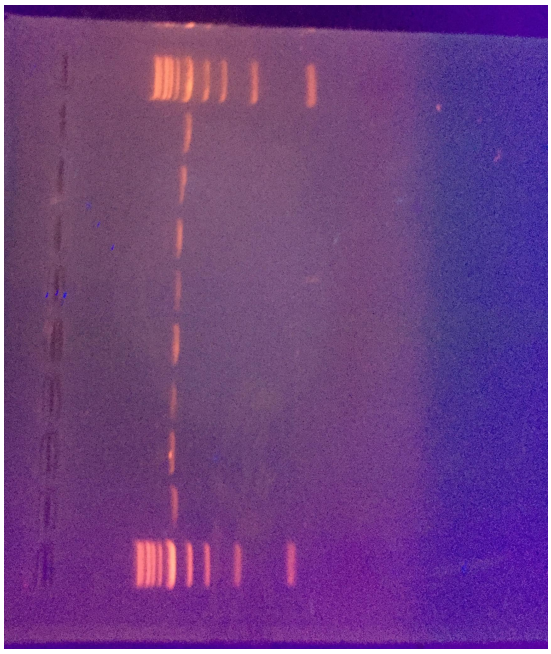
Out @ 12:45 PM
In @ 1:30 PM

Plate broth was sufficiently solidfied that I didn't think Abx would diffuse through the media. Put back in autoclave at 1:45 PM

Out @ 1:50 PM

During this time LK did PCR Purification and someone ran colony PCRs on a gel.

IMG_0604.JPG



This is UNS 55AS w/ sfGFP 1C3. It's the correct length.

In @ 3:30 PM

Set up Gibsons:

Table1

	A	B	C	D	E	F
1	160915					
2	**Backbone**	**6- 55AS + DT 1C3 P178 P179**	**0.06**	**5104**	**173.4**	**1.165619377**
3	Insert	2- NRII P176 P177	0.18	1366	273.7	0.5929148703
4	**Backbone**	**5- 52S + DT 1C3 P178 P179**	**0.06**	**5104**	**188.9**	**1.069975648**
5	Insert	2- NRII P176 P177	0.18	1366	273.7	0.5929148703

I'm highly skeptical of the yields on the PCR purifications but what can you do.
A is the 55AS and B is the 52S version.

Gibsons went in at 4:00 PM

Added 1:1000 [Chlor] into the LB Agar broth and made 1L of Chlor Plates

Out @ 4:30 PM
In @ 4:45 PM

Started transformation of Gibsons into 5alpha.

Key:

- A = UNS 55AS with NRII 1C3
- B = UNS 52S with NRII 1C3

I bleached the tubes by the sink.

Transformation outgrowth started at 5:45 PM

Cleaned up around the lab

Out @ 6:20 PM

iGEM DpnI Digestion

Introduction

DpnI Digestion allows you to specifically eliminate methylated DNA. This is useful becuase it allows you to eliminate template plasmid from your PCR, so you can be sure that your transformants did not take up old plasmid.

Materials

- DpnI Enzyme
- Cutsmart
- PCR Product
  -

Procedure

1. 24 uL of PCR product (this is assuming you did a 25 uL PCR and ran 1 uL on a gel).

   We recommend adding other reagents directly into your PCR tube to save time and money.
2. 2.7 uL 10X cutsmart buffer
3. 0.5 uL DpnI enzyme
4. Thermal Cycler on DpnI program

   37 for 60 min, 80 for 20 min, hold at 4.

iGEM Transformation

Introduction

This is how you insert your plasmid(s) into cells. Please be sure you know **which strain** you are using and you know the **appropriate amount of time** to heat shock your **specific strain**.

Materials

- Comp Cells
- Plasmid DNA
- SOC
-

Procedure

Thaw Cells

1. Take out enough cells so that you can have at least 15 uL of cells per thing you are trying to transform. There is about 45-50 uL of competant cells per NEB tube of cells.
2. Thaw cells on ice
3. Transfer appropriate amount of cells to appropriately labelled Eppendorf tube (I would suggest using the same key as you used for the gibson assemblies).

Transform

4. Add 2 uL of plasmid DNA to each aliquot of cells.
5. Ice for 30 minutes. Prewarm heatblock to 42 degrees C.

   I would strongly recommend that you take this time to prelabel your plates and place them in the incubator to prewarm.

Heat Shock

6. Heat shock cells at 42C for appropriate amount of time. This varies based on which strain you are using.

   BL21 gets heat shocked for 10 seconds
   10Beta and 5alpha get heat shocked for 30 seconds
7. Ice for five minutes.
8. Pipette in SOC based on the amount of cells you used. 50 uL of cells get 950 uL of SOC, for reference.

Outgrow

9. Place in shaking incubator 250 rpm 37C for 1 hour (chlor, amp, or tet) or 2 hours (kan)

10. Remove bacteria from shaking incubator.

11. **INVERT EVERY TUBE 4-6 TIMES. IF YOU DONT DO THIS YOU WILL NOT HAVE A SUCCESSFUL TRANSFORMATION.**

Plate

12. Plate out 100 uL of bacteria.

    (We have been having lawn growth for a lot of constructs; you may want to do 50 uL if you have experience with this part overgrowing. Likewise, if you are doing a double transformation or a low copy number, do 150 uL).
    Use glass beads in a bunsen burner sterile field. Dispose of glass beads into ethanol.

13. Put plates in incubator upside down (agar side up, lid down). Let grow overnight.

    Do not be alarmed if you do not see colonies for up to 18 hours.

160916 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-15

THURSDAY, 9/15

Today's To-Do

General

- ☒ Autoclave dry materials-- Gravity 20

Miniprep

top priority in the morning, as long as you take the plates out of the incubator before they overgrow.

- ☒ UNS 55AS w/ sfGFP 1C3 MP1, 2, 3
- ☒ nanodrop (MP in fridge on white rack)
- ☒ glycerol stocks
- ☒ add to inventory

Colony PCRs and Inoculations

- ☒ Colony PCR all transformations with P008 and P009
  (see 160915 JPM *for lengths of constructs)*
  - ☒ image at 1:15PM
- ☒ Inoculate them in LB for miniprep. See gel pic below for which colonies to inoculate.
  A = UNS 55AS with NRII 1C3
  B = UNS 52S with NRII 1C3

Transform

- ☒ WM16_110, WM16_111 with and without addGene lacO array (pJD100) in 10 beta [4 transformations]
  (because maybe the addGene lacO didn't transform into BL21 because of strain incompatibility... it worked in 5 alpha.)

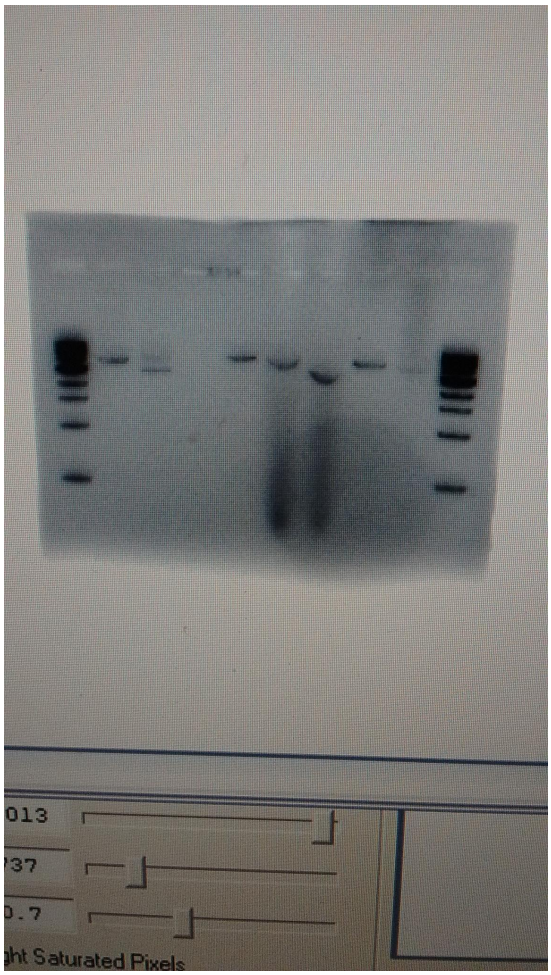
In @ 1:00 PM

Resuspending gBlocks to .1 pmol/uL.
These are constructions to build 25, 62, 77, 78, 81

Made glycerol stocks of the three minipreps that Kalen did of UNS 55AS w/ sfGFP 1C3.

Imaged gel of colony PCRs that Kalen set up (160916 KPC)

IMG_20160916_132338723.jpg



L: UNS 55AS with NRII 1C3 #1-4
R: UNS 52S with NRII 1C3 #1-4
Both should be slightly over 4kb; 55AS should be slightly longer than 52S.

It's weird that there is such discrepancy in the lengths of the inserts... however the plurarity of length options seems to be the correct length. Don't know what happened in lanes 6 and 7.

Inoculate: A1, 4, random-from-plate (55AS)
B1, 3, 4 (52S)

Out @ 1:45 PM

6:00 - 7:00 met with Joe about the Departmental Seminar on Friday

In @ 7:20 PM

Transforming into 10-beta:

- WM16_110 MP3 1C3 160903 Box 11 Slot 26 + pJD100 Amp 2 Box 11 Slot 2
- WM16_110 MP3 1C3
- WM16_111 MP3 1C3 160912 Box 11 Slot 41 + pJD100 Amp2 Box 11 Slot 2
- WM16_111 MP3 1C3

Made media for inoculations-- waiting until ~10:00 PM to inoculate so Minipreps can be later in the day tomorrow

Poured out caps and bleached tubes from today.

Outgrowth began at 8:25 PM

Out @ 8:40 PM
In @10:40 PM

Plated out Transformations

Inoculated colonies

Out @ 11:05 PM

iGEM Transformation

Introduction

This is how you insert your plasmid(s) into cells. Please be sure you know **which strain** you are using and you know the **appropriate amount of time** to heat shock your **specific strain**.

Materials

- Comp Cells - 10beta
- Plasmid DNA
- SOC
-

Procedure

Thaw Cells

1. Take out enough cells so that you can have at least 15 uL of cells per thing you are trying to transform. There is about 45-50 uL of competant cells per NEB tube of cells.

   Used one tube for 4 transformations (D, WM16_111 solo, got the least), having 12.5 uL cells each

2. Thaw cells on ice

3. Transfer appropriate amount of cells to appropriately labelled Eppendorf tube (I would suggest using the same key as you used for the gibson assemblies).

Transform

4. Add 2 uL of plasmid DNA to each aliquot of cells.

   Used 1:10 Miniprep:NFW dilution, 2uL of each MP for cotrasnformations.

5. Ice for 30 minutes. Prewarm heatblock to 42 degrees C.

   I would strongly recommend that you take this time to prelabel your plates and place them in the incubator to prewarm.

Heat Shock

6. Heat shock cells for appropriate amount of time. This varies based on which strain you are using.

   BL21 gets heat shocked for 10 seconds
   10Beta and 5alpha get heat shocked for 30 seconds

7. Ice for five minutes.

8. Pipette in SOC based on the amount of cells you used. 50 uL of cells get 950 uL of SOC, for reference.

Outgrow

9. Place in shaking incubator 250 rpm 37C for 1 hour (chlor, amp, or tet) or 2 hours (kan)
10. Remove bacteria from shaking incubator.
11. **INVERT EVERY TUBE 4-6 TIMES. IF YOU DONT DO THIS YOU WILL NOT HAVE A SUCCESSFUL TRANSFORMATION.**

Plate

12. Plate out 100 uL of bacteria.

    (We have been having lawn growth for a lot of constructs; you may want to do 50 uL if you have experience with this part overgrowing. Likewise, if you are doing a double transformation or a low copy number, do 150 uL).
    Use glass beads in a bunsen burner sterile field. Dispose of glass beads into ethanol.
13. Put plates in incubator upside down (agar side up, lid down). Let grow overnight.

    Do not be alarmed if you do not see colonies for up to 18 hours.

106917 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-16

FRIDAY, 9/16

Today's To-Do

Minipreps

- ☒ UNS 55AS and NRII 1C3
- ☒ UNS 52S and NRII 1C3

Colony PCR and Inoculation

- ☒ Colony PCR with P008 and P009 all colonies
- ☒ Inoculate them into M9 with Leucine for Induction and FACS tomorrow.

In @ 10:30 PM

Need to make more Leucine because Ethan found out that it is light-sensitive and we have been storing it in a Falcon tube.
Also we should make more Thiamine since it is light-sensitive and we have been storing it in a Falcon tube.

The fact that the last time we tried to grow 5 alpha in M9 it took like 40 hours suggests that our Thiamine has gone bad.

Made 50mL of 100x Leucine in M9 Glycerol (Filter Sterilized) (1 mg / mL) and stored it in a foil-wrapped falcon tube in the hood.
Did not make Thiamine stock because we couldn't find it-- need to ask Dr Saha.

Ethan inoculated each colony with 4 mL M9 Glycerol + Leucine.

Out @ 12:00 AM

160918 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-17

SATURDAY, 9/17

Today's To-Do

General

- ☐ Make more M9 Glycerol

Inductions and FACS

The bullet points below are the inoculants (all in 10 beta):

- *1: WM16_110 MP3 1C3 160903 Box 11 Slot 26 + pJD100 Amp 2 Box 11 Slot 2*
- *2: WM16_110 MP3 1C3*
- *3: WM16_111 MP3 1C3 160912 Box 11 Slot 41 + pJD100 Amp2 Box 11 Slot 2*
- *4: WM16_111 MP3 1C3*

☒ Dilute *(only if they aren't at midlog in the morning)* the inoculants so they can reach midlog. Into 4 mL M9 + Leucine.

☒ Make Glyerol Stocks of the inoculants which have grown!

☒ Induce with a 12-step aTC induction

** ~ aTC dilution protocol: ~* (these are in an aluminum-foiled rack on the side tray of the fridge)

- ☒ Make 1.45 mL 20,000 ng/mL aTc (1:100 dilution)
  - ☒ 14.5 uL of 2mg/mL aTc
    - ☒ (again for Chlor + Amp)
  - ☒ 1435.5 uL of M9 Glycerol w/ low-dosage Chlor
    - ☒ (again with low-dosage Chlor and Amp)
- ☒ Make 1.45 mL 200 ng/mL aTc (1:10,000 dilution)
  - ☒ 14.5 uL of 20,000 ng/mL aTc
    - ☒ again for (Chlor + Amp)
  - ☒ 1435.5 uL of M9 Glycerol with low-dosage Chlor
    - ☒ (again with low-dosage Chlor and Amp)
- ☒ Follow the table below:

Table2

	A	B
1	12	Add 125 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 5000 ng/mL aTc
2	11	Add 50 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 2000 ng/mL aTc
3	10	Add 25 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 1000 ng/mL aTc
4	9	Add 12.5 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 500 ng/mL aTc
5	8	Add 5 uL of 200000 ng/mL aTc to 250 ul diluted culture to make 200 ng/mL aTc
6	7	Add 250 uL of 200 ng/mL aTc to 250 ul diluted culture to make 100 ng/mL aTc
7	6	Add 125 uL of 200 ng/mL aTc to 250 ul diluted culture to make 50 ng/mL aTc
8	5	Add 50 uL of 200 ng/mL aTc to 250 ul diluted culture to make 20 ng/mL aTc
9	4	Add 25 uL of 200 ng/mL aTc to 250 ul diluted culture to make 10 ng/mL aTc
10	3	Add 12.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 5 ng/mL aTc
11	2	Add 5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 2 ng/mL aTc
12	1	Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc

- [ ] FACS

Prep for Macrogen Sending Tomorrow

- [x] All recent minipreps that have not been sequenced need to be sent to Macrogen tomorrow. Prep them now so we don't stress!
  - [x] make tube traveler
  - [x] dilute minipreps
  - [x] aliquot/dilute primers
  - [x] fill out spreadsheet
  - [x] submit online form
  - [x] obtain fedex envelope

In @ 12:10 PM

Inoculants were not at midlog-- in fact, it was hard to convince myself that they had growth at all! Remaining optimistic, making aTC solutions.

Kalen indeed used low-dosage Amp and Chlor for the inoculations last night.

Out @ 12:55 PM

Working on Presentation for Departmental Seminar on Friday

In @ 4:50 PM

Some inoculants have started to grow.

WM16_111 solo never grew :(

Induced the rest of the samples (in at 5:50 PM).

The WM16_110 and WM16_110 + pJD100 have no #6 induction because I messed that one up and ran out of culture.

Prepared glycerol stocks for inoculants-to-be-FACS'd... I'll add culture before FACSing in a few hours, to give them more time to grow before I glycerol them.

Out @ 6:20 PM

Working on Presentation for Departmental Seminar on Friday

In @ 11:10 PM

Set up FACS -- as usual technical difficulties are occuring.

Got it to work after a while. Sample quality seems to be better having put 10 uL culture into 500 uL PBS. Less noise in the SSC channel.

Ethan measured GFP concentrations in the plate reader. Concentration is linear with Plate reader fluorescence (au) on a log-log scale. Ethan measured 2.1 aTC inductions on the plate so we can get a conversion from MEFL -> Plate Reader au -> [GFP].

Unfortunately it looks like all of the #2 replicates (WM16_110 1C3 in 10beta) are not inducing well :(It looks like conditions 1-10 are autofluorescence and 11, 12 start to induce slightly. Why???

Settings: #2: FSC 600 SSC 550 FL1 800

The #1 replicates are similarly looking like they're mostly autofluorescence except for the last two [aTc] condition :(

Settings: #1: FSC 600 SSC 550 FL1 800

The #3 replicates also have the same issue!! WTF??

Does 10-beta not uptake atC as efficiently as other strains or something??

Settings: #3: FSC 600 SSC 550 FL1 800

This sucks.

How to Finish out iGEM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-09-20

TUESDAY, 9/20

There are two things that will impede the success of iGEM as a team during these final four weeks:

1. Resentment of your teammate
    - Everybody is going to work very hard. We will most likely distribute work amongst ourselves in a way that means not everybody is physically present and working at the same time. This will make it easy to feel like you are doing more work than anyone else. There is no way to prevent yourself from feeling these feelings, but you cannot let yourself start to believe that this is true. This will breed resentment and you will begin to resent the team and the project itself. We cannot let this happen.
    - The team exists so that we can help each other out when things are especially bad for someone. Furthermore, many people either didn't vote 'Yes' or expressed nervousness about the workload despite wanting to go forward. Be understanding.
        - If you feel like you can't continue at the pace that the team is expecting you to, whether it's temporary or long-term, you *must* communicate with the team and the team *must* be understanding of your situation. It is still valuable to come in and do wetlab protocols each day even if you aren't taking ownership of the project organization. If you say nothing then resentment will fester, and people will start avoiding the lab and the project. We cannot let this happen.
    - It will be particularly easy to resent me and Joe because we will be working predominantly on out-of-lab things. We will keep records on Benchling of the time we spend working, as if we were doing wetlab, so that we can have an objective metric for addressing this resentment when it arises.
2. Diffusion of Responsibility
    - You, specifically, have to be personally responsible for the project. There is no other way to complete the project in this time.
    - As a team you will have to determine the best way to organize yourselves to keep the project moving forward. My opinion is that there is no way to do this other than having short daily meetings where we review what happened today and determine what's happening tomorrow-- any other method is going to lead directly towards diffusion of responsibility. It must be clear not only who is *doing* what, but who is *responsible* for what.
    - I think the best way to induce this transition is to 'jump into the deep end'. I'll give you a briefing of where each subproject is, progress-wise, and how I learned how to do things like FlowCal Data Analysis or FACS, but I won't be there for the first few meetings, forcing the transition to occur. I'll come back after a system has developed.
        - The path-of-least resistance will be to have Ethan take over the role I've been playing. We cannot let this happen, as the project can only succeed if we have *many* people taking personal ownership of the project.
        - We will lose a few days' worth of work and progress time because of this transition. This is inevitable and the only way to ensure we complete the project within the four weeks.

Finally, always remember that you, personally, are going to make sacrifices.

160922 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-22

THURSDAY, 9/22

At this point I am not doing wetlab but rather focusing on making the Calculator. Unfortunately, today I'll be working on LearnSynBio instead...

In @ 11:30 PM

> Putting together LearnSynBio Math Module outlines for LK, CEM, and KPC to follow in high-resolution detail. The hope is that there will not be a need for interpretation regarding things like equations-to-draw.
>
> I wrote out a detailed outline of Math Video 1: Introduction to Biological Circuits I and sent it to LK, CEM, KPC, and JLM to see if there is sufficient information from a drawer's or a narrator's point-of-view.

Out @ 12:40 AM

> I'm going to work on tomorrow's departmental seminar talk now

160923 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-23

FRIDAY, 9/23

In @ 1:00 PM

Decided the outline for the 2016 Jamboree Presentation:

- I will introduce the motivation and concept of the Toolbox
- Joe will explain each part of the Toolbox and RiboJ and present appropriate Data
- Kalen will motivate the Calculator, describe the underlying model, and describe how to use the Calculator
- Kalen will present our pBAD circuit simulation system and the resulting Data
- I will re-summarize the impact of the Toolbox and Calculator
- I will describe Outreach
- I will Conclude and Acknowledge

See

Dropbox/iGEM 2016/Presentations/2016 WM Jamboree Presentation Outline.docx

for a more detailed treatment.

Out @ 1:30 PM

160924 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-24

SATURDAY, 9/24

In @ 11:00 PM

Setting up the FACS and teaching KPC, EMJ, and LK how to run FACS samples.

Running FACS with Kalen on the following samples:

- 1. UNS NRII 52S (1C3) + TetR (3K3) x 3 replicates - aTc [BL 21]
- 2. PDJ100 (Amp) + WM16_111 (1C3) x 3 replicates - IPTG [10 Beta]
- 3. PJD100 (Amp) + WM16_110 (1C3) x 3 replicates - IPTG [10 Beta]

And I'm assuming they are 10 beta but I can't tell.

Settings for all three samples are:

FSC 600 SSC 550 FL1 700 (or FL3 900 for Synth. Enhancer)

All FCS files are saved in Dropbox/iGEM 2016 Storage/160925. This folder is accessible from the Lab Mac facing the windowed wall.

Out @ 4:00 AM

160926 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-09-26

MONDAY, 9/26

In @ 10:15 AM

Working on reviewing the progress-so-far that we have on the Calculator. Looking into the feasibility of modularly tuning portions of the underlying ODE model to data we have already (ie. on the DBA or on the Syn. Enhancer specifically) before we run a predictive calculator attempt on a pBAD circuit.

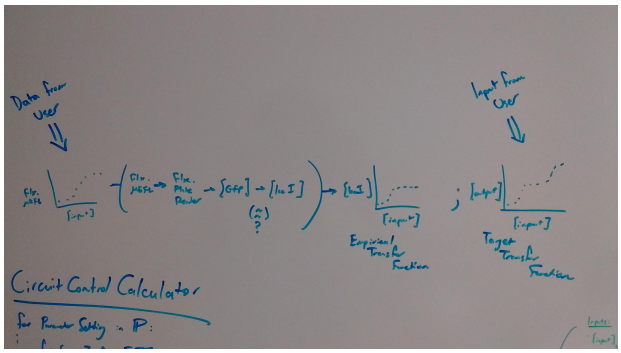
Thinking about project components that may have gotten left behind:

- **RIBOJ:** Did we ever re-measure the WM16_025 that we re-did? How did it looks? Sequence of the most recent one (160814 MP2 Box 10 Slot 63) says "Debatable" due to a few gaps at the beginning of the sequence... this is one of the geneBlocks that we ordered to re-do the beginning part.
  - We should:
    - Gibson Assemble the geneBlock with the requisite components, OR/AND
    - Transform the 160814 MP2 of WM16_025 with WM16_016 3K3 into BL21 to get a FACS curve *(if this hasn't been done already)*
      - *If it has*, we should analyze the new RiboJ data to see if we get curve collapse like in the Voigt paper.
- **BINDING ARRAY:** Did we ever re-try cotransforming the pTet+GFP combo with a control plasmid to compare data against the pTet+GFP combo cotransformed with UNS 85x tetO? *(turns out yes on 160913)*
  - If so, we need to analyze the data and see if it looked like tetO array worked.
  - If not, we should do this because a functional tetO array is easier to combine with Synth. Enhancer in a whole-Toolbox test.
- **COMBO CIRCUIT:** Right now it looks like the situation is that
  - lacO array (pJD100) only works in 10 beta for some reason but it has never been tested in LG3.300.
  - Synth. Enhancer has never been tested in 10 beta

  So to remedy this we should try testing the lacO array (also the tetO array) in LG3.300, and also the UNS Synth. Enhancer parts in 10 beta.
- **CALCULATOR COMPATIBILITY:** Do we need to do lacI vs. GFP degradation rate tests? How would that even work? Think more on this.

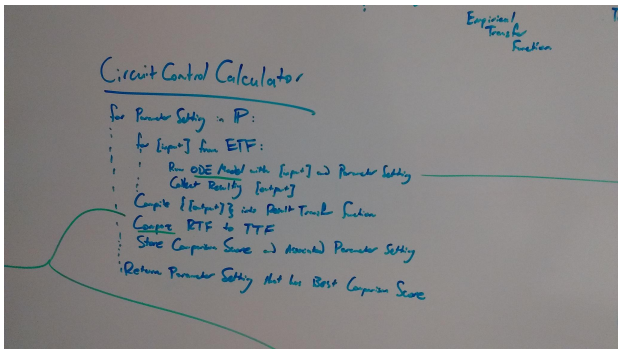
Here's a summary of the Calculator workflow as we currently envision it:

calc_inputs.jpg



The User-specified inputs to the Calculator

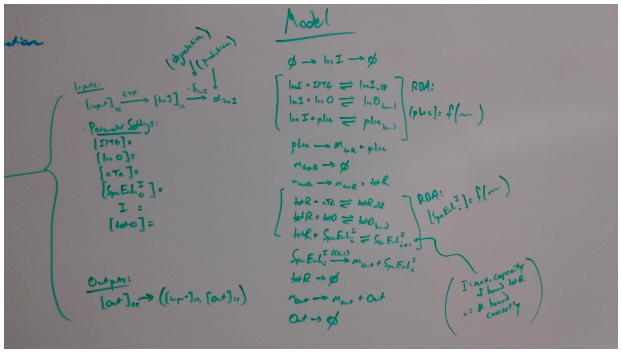
calc_algorithm.jpg



The pseudocode algorithm that is the Calculator. Parameter Settings correspond to Toolbox configurations... currently the Calculator brute-forces its way through every allowed one.

(Can we determine that only a subset of the possible Toolbox configuration will be necessary given the relationship between the Input Transfer Function and the Target Transfer Function?)

calc_model.jpg



The ODE Model which is iteratively run by the Calculator. Rapid Buffer Approximation makes it computationally more feasible (but does this imply it is now not infeasible?) to iteratively run this model many many times.

The Model does not currently account for fluctuations in plasmid copy number (for either the genes or the binding arrays). *The easy way to implement this is to Monte Carlo the results of steady-state copy numbers for each of these species. But this (a) introduces even more iterations to the Calculator and (b) still results in the* dynamics *arising from copy number fluctuations being ignored by the model, which might be the more important thing.*

(Can past iterations of [input] for a given Parameter Setting inform future iterations of [input] for the same Parameter Setting?)

And there needs to be another picture explaining how the Comparison between Result Transfer Function and Target Transfer Function will occur... need to go back and look at Kalen's old work on this.

Out @ 12:00 PM
In @ 1:50 PM

The nature of the ODE Model is such that degradation timescales of repressor proteins (and final output) will likely be on similar timescales-- hence although an individual RBA allows one to write

[free downstream promoter] = f([relevant upstream repressor]),

the successive RBAs in the model may actually be composable to obtain

[output] = f(g(h([lacI]))),

for example. This would indeed speed up model iteration time significantly (all integration would be removed from the process), but I worry that it crushes the timescale dynamics on the approach to steady state. Aren't these going to be important?

I feel like there should be interactions between these timecourses which create interesting phenomena that impact downstream processes... but then again an analytical steady-state solution would have to accomodate the eventual equilibrated results of said interactions, so simply writing it down may be OK?

Especially since we've basically thrown out the idea of taking kinetic measurements to validate or support anything *(but maybe a model tuning along these lines would yield a better chance of eventual predictive success?)*, it seems that maybe the composite collapse would be the way to go.

I need to determine if pre-steady-state dynamics get incorrectly ignored if I just write down a steady-state equation for [output] as a composite function of [input] only.

I also need to determine if kinetic rate constants can be more accurately determined through fitting to measurements-to-steady-state as opposed to simply the steady state value itself. If so, how would I do such a fitting? The relaxation of the ODE to its steady state does not involve additional parameters that aren't in the steady-state expression... perhaps the process is:

- (1) Fit parameters to the steady-state
- (2) Solve ODE model numerically and observe the fit between the solution and the empirical relaxation
- (3) Conclude that there exist additional underlying dynamics which were not accounted for, structurally, in the model
- (4) Include a generic term for the unknown mechanism and fit it accordingly
- (5) Extrapolate the generic term, with its fit parameters, to new kinetic data.
- (6) If the fit persists, done

    Else continue to overfit your model to your data

Out @ 3:05 PM

160927 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-09-27

TUESDAY, 9/27

In @ 9:30 AM

Presented the Calculator to Smith Lab Meeting. Some good suggestions:

- **Algebraic Model of the Toolbox:** One option of thinking about the Calculator might be to determine the functional form of each Toolbox Setting as a Hill Function t(x). Then for an empirical function e(x) and a target funtion g(x) we have
  t(e(x)) = g(x), which could be evaluated using either deconvolution or something much simpler and pointwise *(need to determine which it is)* to determine which t(x) is appropriate.
    This relies on modularity assumptions which I think we might already be making all over the place... the ODE model feels safer on that front but is it really? Interactions don't necesarily happen between things and competitive binding effects of RNAP vs. Repressor on a free promoter, for example, don't show up in the Model *(I think)* unless we make it a stochastic simulation
- **ODE Model of the Toolbox:** One thing to realize about the ODE model is that if we end up just running the whole thing to steady-state and writing down the answer (as I was thinking about in yesterday's Notes), then the Rapid Buffer Approximation ends up being just a regular part of the eventual solution (after all, the use of the Approximation is to solve a subset of the system in order to make more feasible the implementation of the ODE system as a whole... if we just write down the answer then we just carry through the idea to the whole system). Since we now seem to be moving away from the whole kinetic aspect of the time-evolution of the system, then it might be more feasible to simply (as I was thinking about before but not in the correct mindset) write down the steady-state solution to the ODE. This allows the Calculator to iteratively run just function evaluation instead of any integration.
- **Comparison of Result vs. Target Transfer Functions:** A good starting-point for determining a good comparison technique, taking advantage of the pointwise nature of the functions being compared, would be to make quadrileterals (with vertices t1 t2, r1 r2) and compute properties like area... vertical distance vs. horizontal shift vs. slope differences can be independently tuned and weighted, then all of these quadrilaterals can be summed/averaged over. A thought.
    - Another good thought is to, after a preliminary (or final) scoring of the fits of the model-resulting transfer functions, to output the top 10 functions to the user and let them choose by-eye their ideal choice (or perhaps the top 5 along each weighted fit dimension), and then output the parameter settings associated with the chosen function.

Out @ 11:15 AM
In @ 5:20 PM

Kalen had made a typo on the Macrogen spreadsheet so 6 / 70 reactions did not process. I called Macrogen and corrected the typo.

I set up the Gibson spreadsheet (Bulk tab) with today's gibson of 14 backbone + 25 gBlock.

Out @ 5:45 PM
In @ 11:30 PM

Set up FACS of WM16_110 + pJD100 IPTG induction (just one replicate) *[which strain??]*

FACS'd with Callan

Out @ 12:40 AM

160928 JPM

Made with Benchling

Project: iGEM 2016

Authors: Ethan Jones

Date: 2016-09-28

WEDNESDAY, 9/28

In @ 8:30 AM

Set up FACS of aTC induction from last night (they went overnight). These are:

Table1

	A	B
1	Key	Part
2	2	pACT-Tet (OA) + UNS 52S+DT BL21
3	3	pACT-Tet (OA) + UNS 55AS+DT BL21
4	4	pACT-Tet (OA) + UNS 55AS sfGFP BL21

2: FSC 600 SSC 550 FL3 600. Collecting 700,000 events to get approx. 15,000 relevant events.
3: FSC 600 SSC 550 FL3 600. Collection 500,000 events to get approx. 15,000 relevant events.
4: FSC 600 SSC 550 FL1 500. Collected 500,000 events to get approx. 15,000 relevant events.

If induction is happening at all in #2, it's happening at the "% of population that is fluorescent is increasing" level rather than "the value of the fluorescence that represents the 'active' population is increasing" level. ie. the value of the mode of the fluorescence peak does not increase, but the population-level mean may increase with induction.

I don't know if this is a property of the extreme discrepancies in copy number of the two plasmids (in fact, we had decided not to induce and FACS these samples until we got sequence information about UNS synthetic enhancer constructs on 3K3 so I don't know why these are being FACS'd right now), or if it's just how the synthetic enhancer is going to have to end up working.

The same phenomenon happened for #3 and #4.

Out @ 10:45 AM
In @ 12:00 PM

Kalen is finishing up the FACS.

Out @ 12:20 PM
In @ 1:20 PM

Analyzing FACS data from 160925 (110 and 111 with pJD100, and UNS 52S with NRII + TetR 3K3). Ran them through FlowCal.

110 and 111 look like good inuction is happening. Making summary plots of geometric mean.

Excel crashed and I couldn't recover my summary graphs (?!)... will have to re-make. 111 + pJD100 didn't look like it was inducing properly

Out @ 1:50 PM
In @ 8:30 PM

Analyzed FACS data from 160913, 160918, 160925. Turns out that when we express the 53 1A3 vs 53 1A3 + 85x tetO 1C3 in BL21 from 0824 as % of max. MEFL instead of just MEFL, we see that the 85x tetO array shifts the induction curve to the left as expected!!

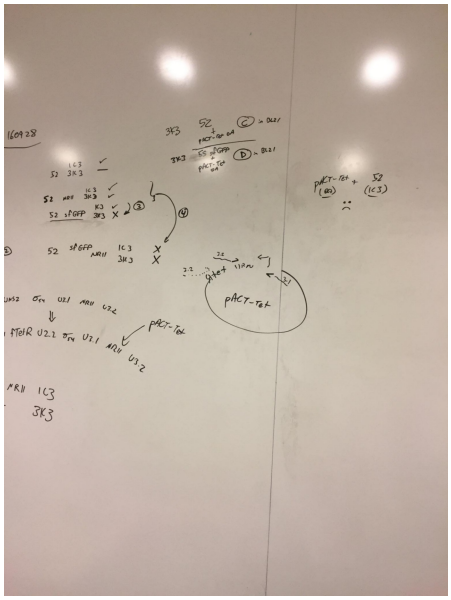
110 + pJD100 10 beta induced well, but the 110 10 beta construct did not grow on 0925. Need to try and obtain 110 tomorrow.

Other constructs didn't really induce. All information is on Dropbox/ iGEM 2016 / FACS Data.

iGEM MEETING:

- **Packing Slips:** From now on, don't remove any packages from the Office until you sign the Packing Slip and leave it with Charnel!!
- **Equipment:** Need to make sure you take really good care of this... wipe down balances etc.
- **Money:** The status is actually pretty good right now.

IMG_8350.JPG



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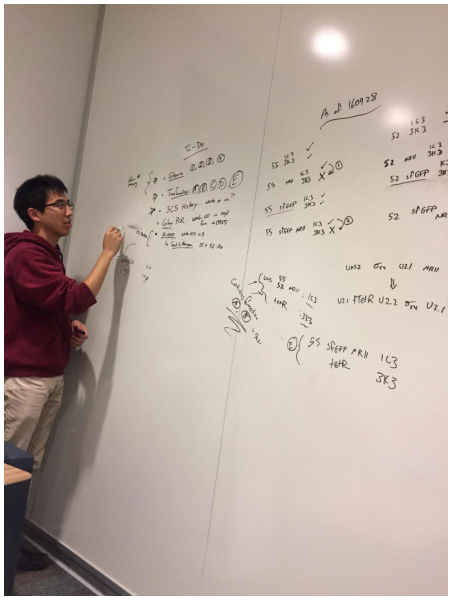

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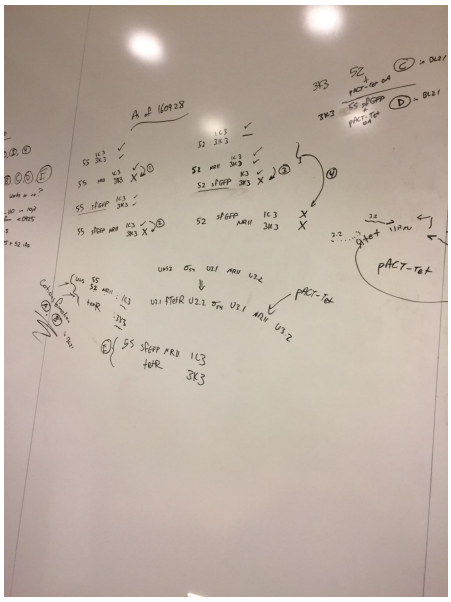

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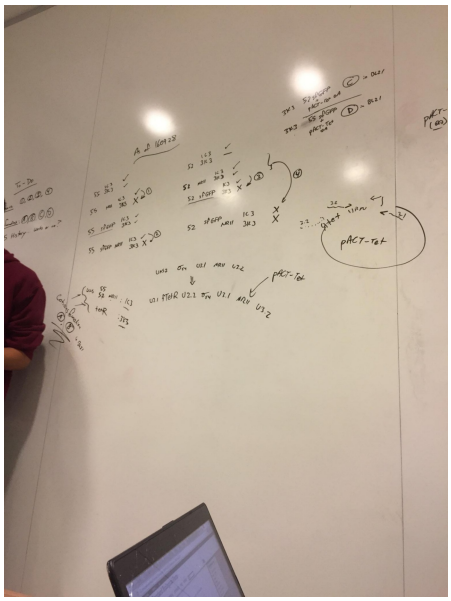


160930 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-09-30

FRIDAY, 9/30

In @ 5:10 AM (I know. Don't worry about it.)

Reviewing the old MATLAB code that currently contains our lacI submodel. I don't think we ever got the model to the point of incorporating protein expression, or if so it was all numeric and not analytical. Need to confirm where we're at with this--perhaps the [pLac] -> 0 problem isn't an issue?

An old MATLAB file I'd been relying on durin the two weeks 'PIAodeSolver' no longer seems to be working... no errors are thrown but the runtime is prohibitively long. I don't remember it acting this way... but given the new focus of the Model (Steady-State solving vs. RBA), it might be preferable to write this up from scratch anyhow.

Immediate Progress Goals for Today:

- Numerically evaluate steady-state values for [Protein]_ss that is produced from the pLac in an open pLac model, using BioNumbers parameter values and confirmed analytically, to see if the [pLac] -> 0 is a problem at the protein level
  - If so:
    - Start determining the best way to fit subcomponents of the model to empirically measured data
  - If not:
    - Proceeed to creating the synthetic enhancer submodel in the same regard

Out @ 5:40 AM
In @ 3:40 PM

Organizing short-, medium-, and long-term priorities for finishing the Calculator on-time. The **#1 priority** right now is determining the **feasibility of fitting empirical data to compartments of the ODE Model.** If this is not possible the Calculator likely has to be Algebraic (see Overleaf document for more details).

Re-thinking the nature of the combined-Toolbox circuit modification and how it would actually work mechanistically.

The biggest concern is that a lot of our measurements (especially given that we're now confirming Decoy Binding Array as a %-of-max measure, but this issue was present in horizontal-axis areas far earlier and I don't think I gave it appropriate treatement) are *relative* measures... how does this get captured in the Calculator? Will it turn out the algebraic method would be more appropriate? Relative measures don't really recognize amplitude modifications like RBS tuning, which would be necessary *in vivo* to get a circuit to function physically.

There are **two fundamental assumptions** we are making **to bridge the discrepancies between 'The way that we measure Toolbox parts to confirm their function' and 'The way to characterize these parts as they work *in vivo* as a circuit modification'**. They are:

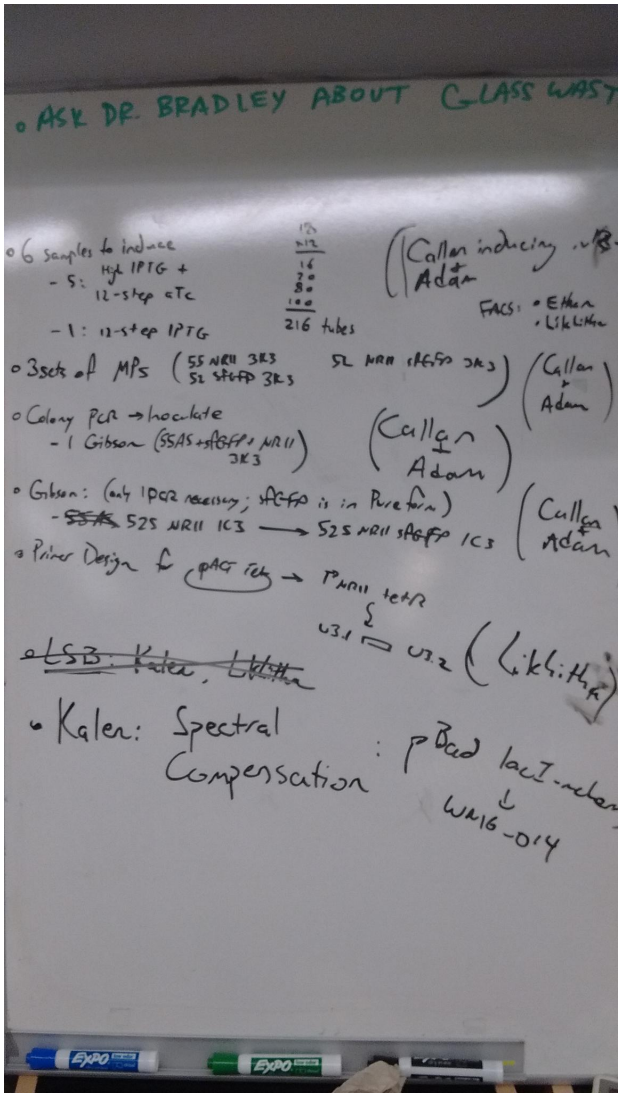
- We characterize parts as [out]/[out]_max when they really function as [out]
  - Transitioning from the proof-of-concept data to calculator characterization here requires the assumption that the concentration ranges of output protein are in some physical range where thinking of things in relative concentrations does not incorporate the issues of edge effects (too little protein -> lots of noise, or too much protein -> metabolic drain / saturation).
- We characterize the DBA and Synth. Enhancer as [inducer] when it should be [repressor].
  - Transitioning from proof-of-concept empirical data to calculator characterization requires the assumption that [repressor]_ss is influenced dynamically only, in a linear way, by [inducer] in such a way that making-relative the horizontal axis would preserve the shape of the Transfer Function.
    - This is a sketchy assumption to make, but doing so allows us to just reflect the empirical transfer functions across a vertical line.
    - The use of the fluorescent fusion repressors might alleviate this problem with the Synthetic Enhancers, but that requires good spectral compensation.

- *Need to run the spectral compensation pipeline on the pBad lacI-mCherry data that we have right now in order to make sure it can actually work!!*

Out @ 4:55 PM
In @ 5:50 PM

Team meeting happened. Tomorrow's schedule:

161001.jpg



Out @ 6:20 PM

Looked back at the two prior instances of Synthetic Enhancer FACS confirmation:

- OA two-binding site (52) + OA pACT-Tet in LG3.300 (0717)
- UNS 52S + UNS pACT-Tet; UNS 55AS + UNS pACT-Tet in LG3.300 (0817)

Note that both instances of successful confirmation are in LG3.300-- previously we had mistakenly believed that the 0817 FACS was in BL21.

This means we should do two things tonight:

- Re-transform the five Synth. Enhancer combos we have in BL21 currently (for FACS tomorrow) into LG3.300
- Transform the OA Synth. Enhancer parts into BL21 to see if we can get original functional replication of results

Additionally we should eventually:

- Try and get 85x tetO binding site shift in LG3.300, in case it turns out Synth. Enhancer only works in Lg3.300.

161001 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-10-01

SATURDAY, 10/1

In @ 12:10 PM

Determining protein-level production concentration in open pLac subsystem. Does [output] -> 0?

Out @ 1:00 PM
In @ 1:20 PM

Thought: Solving for [lacI]_ss involves solving for roots of a quartic function in the open full lacI subsystem. But adding addtional binders would increase the order of this polynomial correspondingly, but we know that starting with a quintic polynomial there is no general formula for its solution. So what's up with that? It seems that as-is we are conveniently within a simple-solution regime, but only conveniently so.

I solved out the quartic polynomial for [LacI]_ss in the form which MATLAB can numerically solve

It seems that if you finagle the transcriptin/translation rates from pLac in relatively reasonable (?) ways, then the fact that [pLac]_ss is extremely low won't cause a disappearance of [output protein]_ss.
Code:

```
% lacI Subsystem Final
%
% Last Edited on 161001 by JPM
%
% This script will solve for the steady-state values of [Out] in the
% subsystem where
%                     0 -> lacI -| pLac-Output
% and IPTG induction and lacO titration exist.
%
% The function requires one numerical root-finding of a quartic equation.
%
% Inputs:
%   - PT = [pLac]_tot
%   - IT = [IPTG]_tot
%   - OT = [lacO]_tot
%
% Outputs:
%   - TETRss = [Output]_ss
%
% The ODE model being solved is
%       NULL -> lacI -> NULL
%       lacI + pLac <-> pLacBound
%       lacI + IPTG <-> lacIBound
%       lacI + lacO <-> lacOBound
%       pLac -> pLac + mTetR
%       mTetR -> NULL
%       mTetR -> mTetR + TetR
```

```
%       TetR -> NULL
% with conserved quantities
%       pLacTOT = pLac + pLacBound
%       IPTGTOT = IPTG + lacIBound
%       lacOTOT = lacO + lacOBound

% Parameter settings (Rate Constants in nM and sec)
aL = 620; %Highly skeptical % lacI production (compressed) (comes from empirical function)
dL = .620728818; % lacI degradation
kpP = 6e5; % forward rate constant for lacI+pLac binding
kmP = 6e4; % reverse rate constant for lacI+pLac binding
kpI = 6e5; % forward rate constant for lacI+IPTG binding
kmI = 6e3; % reverse rate constant for lacI+IPTG binding
kpO = 6e5; % forward rate constant for lacI+lacO binding
kmO = 6e4; % reverse rate constant for lacI+lacO binding
aMT = 40*(1/120); % transcription rate of TetR from pLac
dMT = 1/120; % degradation rate of TetR mRNA
aT = 620; % translation rate of TetR from mRNA
dT = .620728818; % degradation rate of TetR protein

JL = aL/dL;
KP = kmP/kpP;
KI = kmI/kpI;
KO = kmO/kpO;
JMT = aMT/dMT;
JT = aT/dT;

% Input parameter settings (nM)
% 1 molecule ~ 1 nM
PT = 200; % Total pLac in cell
IT = 1e4; % Total IPTG in cell
OT = 48*200; % Total lacO in cell

% Solutions:
coeffs_for_LACIss = [1, ...
    KP + KI + KO - JL, ...
    KP*KI + KP*KO + KI*KO - KP*JL - KI*JL - KO*JL + KP*PT + KI*IT + KO*OT, ...
    KP*KI*KO + (PT+IT-JL)*KP*KI + (PT+OT-JL)*KP*KO + (IT+OT-JL)*KI*KO, ...
    KP*KI*KO*(PT+IT+OT-JL)]
LACIss = roots(coeffs_for_LACIss)
LACIss = LACIss(LACIss >= 0)
PLACss = KP.*PT./(KP + LACIss)
MTETss = JMT.*PLACss
TETRss = JT.*MTETss
```

with output:

```
>> lacI_subsystem_final

coeffs_for_LACIss =

    1.0000 -998.6159  870.2586  115.8142    1.8801


LACIss =

  997.7435
    0.9914
   -0.1000
   -0.0190


LACIss =

  997.7435
    0.9914


PLACss =

    0.0200
   18.3259


MTETss =

    0.8017
  733.0363


TETRss =

   1.0e+05 *

    0.0080
    7.3218
```

So production rates that have [lacI]_ss at 1000 yielded [TetR]_ss to be around 800. This uses BioNumbers sources for Rate Constants, as follows:

Table1

	A	B	C	D
1	**Parameter**	**Value**	**Rationale**	**Source**
2	aL	620 nM/sec	To set [lacI]_ss to be approx. 1000	Arbitrary
3	dL	.620728818 sec^(-1)	Assume concentraiton halves at doubling time, which is 67 min in M9	
4	kpP	6e+5		
5	kmP	6e+4		
6	kpI	6e+5		
7	kmI	6e+3		
8	kpO	6e+5		
9	kmO	6e+4		
10	aMT	40*(1/120)	Steady-state transcript count ~40	http://kirschner.med.harvard.edu/files/bionumbers/mRNA%20lifetimes%20of%20transcripts.pdf
11	dMT	1/120	mRNA lifetime ~2 min.	http://kirschner.med.harvard.edu/files/bionumbers/mRNA%20lifetimes%20of%20transcripts.pdf
12	aT	620 nM/sec	To set tetR translation to be similar to lacI translation	
13	dT	.620728818 sec^(-1)	Assume concentraiton halves at doubling time, which is 67 min in M9	
14	PT	200 nM	pSB1X3	
15	IT	10,000 nM	10 uM	
16	OT	48*200 nM	48x lacO array on 1X3	

Out @ 3:10 PM
In @ 10:00 PM

FACSing with Likhitha #1.4-1.6 and #2.4-2.6.

#1 (55AS 3K3 + pACT-Tet OA in BL21) did not have inductions until the final two aTC conditions... so basically it didn't seem to work.

Had a thought: Why are we doing 12- or 14- step ATC inductions on Synthetic Enhancer constructs? We really should be doing 20-step inductions to get a clear staircase!!

Currently we're kind of in a 'diagnostic' state to see if BL21 synthetic enhancer constructs can function (or if we can get our new synthetic enhancer constructs into LG3.300), but as soon as we move out of that stage we need to fix this.

Out @ 2:30 AM

161002 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-02

SUNDAY, 10/2

In @ 11:20 AM

Overnight aTC inoculations of 3.1, 4.3, 5.1-5.3 look well-grown... Setting these up for FACS

6.1-6.3 IPTG induction went in 7.5 hours ago but they look like they have no growth (they were induced at a stage where there were definitely cells in the solution, but that it was probably not midlog yet.) These will need more time so am waiting on these.

FACSing:

settings:

- #3 (55AS NRII 1C3 + tetR 3K3) FSC 600 SSC 550 FL3 700
- #4 (52S NRII 1C3 + tetR 3K3) FSC 600 SSC 550 FL3 700
- #5 (55AS NRII GFP 1C3 + tetR 3K3) FSC 600 SSC 550 FL1 550

FACS is not operational.... calling Tech Support tomorrow. Was unable to get measurements for Spherotech beads or 5.3. fcs files for 3.1, 4.3, and 5.1-5.2 are saved.

Out @ 5:00 PM

161003 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-03

MONDAY, 10/3

In @ 11:00 AM

calling tech support about facs. Fixed the FACS!!!! Here's the writeup from the Cell Sorter log:

```
Oct. 3, 2016
JPM
Called tech support about yesterday’s issue.
Case number:
01076119
Service mode (use only with Tech Support’s guidance!)
Username: service
Password: service
Tech support thinks that the issue of the Waste Stream Not Found error may be due to a poor stored image of the sample stream (as opposed to the pinhole stream which is visible in the Tip Swap wizard and is fine). He suggested unchecking the Fundamental Alignment box in Startup/Tip Swap in order to bypass the process which uses this stored image after a manually-aligned tip swap, and doing so would hopefully overrwrite the poor stored image. However we were unable to access the Tip Swap wizard due to communication drops between the machine and the computer… he instructed me to:
 Turn off ProSort, turn off Computer, turn off FACS, unplug the white USB connection between the FACS and computer, wait a few minutes, turn on FACS, plug in the white USB connection, turn on the computer, turn on ProSort, try Tip Swap.
 ********(The order in which the turning on occurs, in which the machine comes before the computer, is important!! The machine needs to be on for the computer to establish the connection between them).**********
 I did the process and the communication error seems to have improved-- whereas previously I was unable to proceed past the ‘Open chamber door and look for water droplets’ page, I was now able to do so. Startup occurred automatically open proceeding past this page, in order to initialize the stream. The process went into the 20-minute countdown phase, but the initial alignment of the stream is hitting the sort tray (this happens with this tip… however I think I prefer this to the other tip which is angled too far towards the front of the machine, causing the Waste Trough Filling error). Startup proceeded past the 20-minute calibration and moved on to Searching for Waste Stream. Stream not Found error occurred.
 Went through manual Tip Swap wizard and manually realigned the stream successfully. After doing so the Wizard prompted me with a Startup window… Tech support said to close out of the Wizard at this point, and to instead run QC (excluding Fundamental Alignment). I did this and it worked first time-- watching the stream on the Droplet Monitor it looked like it was supposed to.
 Tech Support was confident that at this point the system would take a new stream picture on the correctly-aligned stream, allowing it to calibrate itself properly in the automatic protocols within Startup next time. *********If this does not occur, however, after the 20-minute initialization phase if I get the error again I can go back to Manual Tip Swap, realign the stream, and proceed to QC as I did this time.**********
 I ran a sample from yesterday and found that the sample acquisition has gone back to normal.
 Shut down with DI Water.
```

Checking LK's primers (P183-P186). They match the design specs, which were to:

- Take the functional tetR cassette we use (WM16_022, which came from BBa_I739001) and insert it into the UNS2 region of the Synthetic Enhancer + NRII plasmid, in case that triple transformations of Synth. Enhancer + tetR plasmid + tetO array serve to be a hurdle we can't overcome.
- These primers use I739001 as their basis (as they overhang to UNS2.1, UNS2.2) and also include 13bp of the beginning of Suffix downstream of the Double Terminator... this was necessary to compromise between (a) having enough primer annealing region to bind successfully to I739001 in the reverse direction and (b) negating the self-dimerization that results between the end of B0015 and the UNS 2.2 region.

Ordered them.

Out @ 1:00 PM

161004 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-10-04

TUESDAY, 10/4

In @ 11:40 AM

The 8 inoculants of synthetic enhancer constructs in LG3.300 (see 161003-EMJ) have grown!!

We ran out of aTC so we had to do 12-step inductions for the 8 samples (3 replicates each), chopping off the top two aTC conditions. Hopefully it will be okay because those tend to be toxic to the cell anyway...

Once we FACS these tonight and assess a preliminary functionality of these constructs in LG3.300, we will have to re-do the good-looking ones with a denser aTC spread over the induction range (once we get more aTc)

Out @ 12:30 PM
In @ 3:30 PM

Set up inductions for the eight synthetic enhancer samples in LG3.300, all having 12-step [aTC] induction and 1 mM [IPTG]. These all went in at 4:15 PM, so we can FACS at 10:15 PM.

Out @ 4:40 PM
In @ 9:30 PM

Setting up FACS... it's not working again.....

Fixed the FACS. Running samples. All are in LG3.300 in Phillips Media.

#1 (55AS 3K3 + pACT-Tet OA): FSC 500 SSC 500 FL3 750
#2 (55AS GFP 3K3 + pACT-Tet OA): FSC 500 SSC 500 FL1 650
#3 (55AS NRII 1C3 + tetR 3K3): FSC 550 SSC 550 FL3 518
#4 (52AS NRII 1C3 + tetR 3K3): FSC 550 SSC 550 FL3 518
#5 (52AS NRII sfGFP 1C3 + tetR 3K3): FSC 500 SSC 500 FL1 500

#1 looks like only autofluorescence
#2 looks like only autofluorescence
#3 has distinguishable fluorescence, but doesn't seem like it induced.
#4 has distinguishable fluorescence but doesn't seem like it induced.
#5

The FACS is broken again, at a level that I think a tech needs to come in and examine.

We're going to store the cultures in a shaker at room temp. and measure them with the Plate Reader tomorrow.

Out @ 6:00 AM

161005 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-05

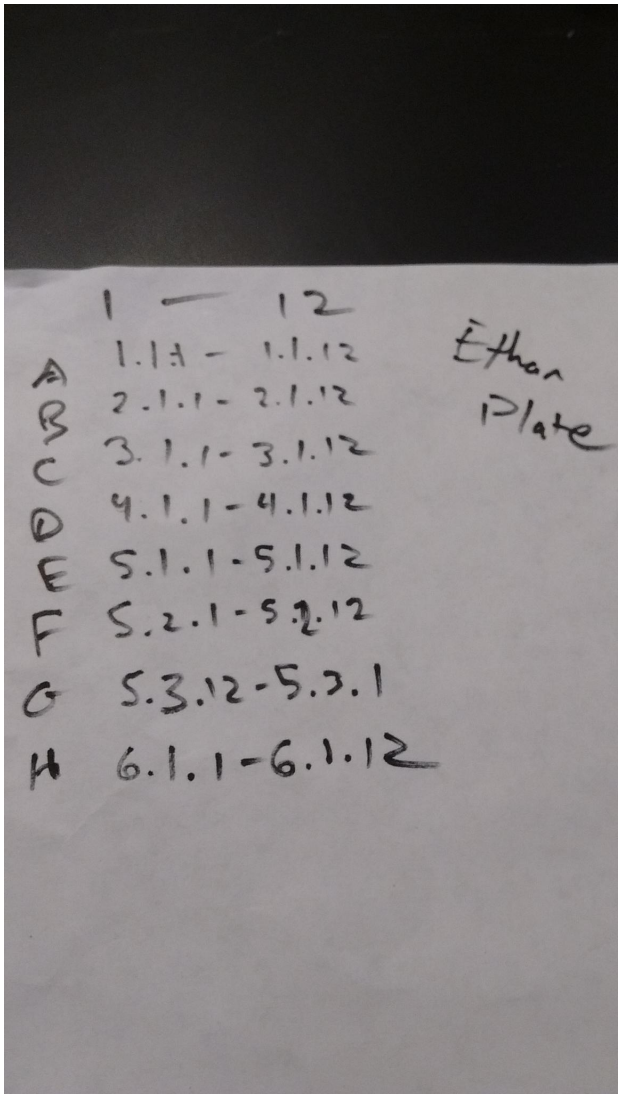
WEDNESDAY, 10/5

Talked to Dr. Bradley about the need to have a tech come look at the Fax. Sent Matt Goff an email about it.

In @ 5:40 PM

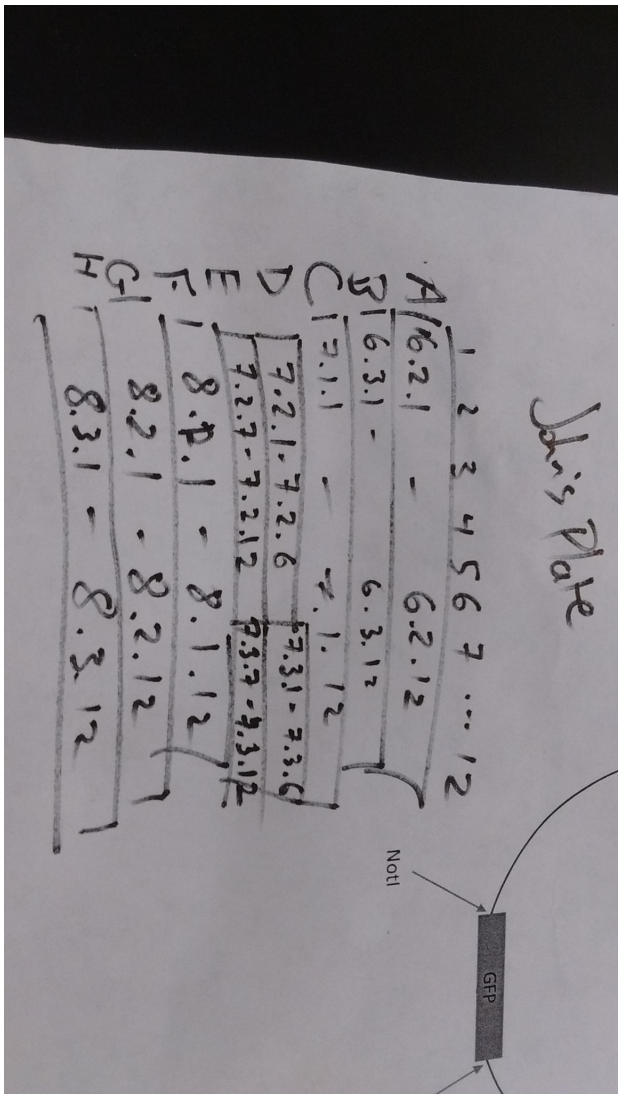
Set up inoculants in Plate Reader with Ethan, to measure.

IMG_20161005_185213306.jpg



Key is 161003-EMJ

IMG_20161005_184636045.jpg



Key is 161003-EMJ

The results: basically it looks like nothing induced.

161005-Platereader.xlsx

Out @7:35 PM

161006 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-06

THURSDAY, 10/6

I helped Christine set up Minipreps and Macrogen sequencing of 7 constructs according to 20161006 LK

Table1

	A	B	C
1	Key	Sample	Replicate
2	1	55AS TETR NRII SFGFP 1C3	MP1
3	2	52S tetR NRII sfGFP 3K3	MP1
4	3	52S tetR NRII sfGFP 3K3	MP2
5	4	52S tetR NRII sfGFP 3K3	MP3
6	5	52S NRII sfGFP 1C3	MP1
7	6	52S NRII sfGFP 1C3	MP2
8	7	52S NRII sfGFP 1C3	MP3

Matt responded to my email and thinks that the issue may be persistent upstream sample tube clogging... he asked for FCS files of data, which I sent.

I had Kalen transform the plate reader induction measurements by subtracting the fluorescence value of the uninduced condition and then converting to % of max... but it didn't elucidate anything.

In @ 9:30 PM

Ethan has transformed 52S (OA) + pACT-Tet (OA), with and without 85x tetO, in LG3.300, as well as 53 and 53 + 85x tetO in BL21, as well as plated out the 0817 52S + pact-Tet glycerol stock which worked. We inoculate these tomorrow and induce them on saturday in order to measure them on the plate reader to try and get synthetic enhancer measurements...

We will also use this opportunity to compare the results obtained from the following plate reader measurement methods:

- Grow up the induction in glass tubes as if we were FACSing, then trasnfer to plate reader only at the measurement stage
- Induce them in the plates and let them grow inside the plate, recording all the while.

Writing Math Outlines for Learn Syn Bio videos.

Out @ 12:30 AM

161007 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-07

FRIDAY, 10/7

In @ 7:30 PM

Kalen is setting up Colony PCRs of the transformations to inoculate into Phillips media or M9 tonight and induce tomorrow with aTC and measure on plate reader

Ethan and I did another known-concentration GFP plate-reader test to get across-day instrument variability with arbitrary units. Curves fit perfeclty over each other in the overlapping concentration region... higher concentration GFP region looked not only nonlinear but nonmonotonic... seems like I didn't sufficiently homogenize the GFP solution before I added it at the higher concentrations or something.

Results in Dropbox/iGEM 2016/FACS Data/161007 GFP

Cleaning up the lab

The gels did not look good: Gel images from 20161007 LK :

GEL images:

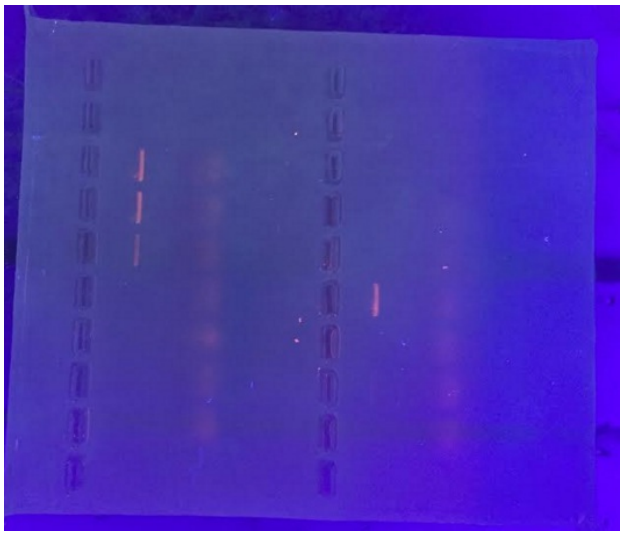
Table1

	A	B	C	D
1			size	primers
2	A	52S Kan (OA) + pACT tet (amp from OA)	3kb/3kb	44/45
3	A'	52S Kan (OA) + pACT tet (amp from OA)	3kb/3kb	42/43
4	B	52S Kan + pACT tet (amp from OA) + 85x TetO 1C3	3kb	44/45
5	B'	52S Kan + pACT tet (amp from OA) + 85x TetO 1C3	3kb	42/43
6	B''	52S Kan + pACT tet (amp from OA) + 85x TetO 1C3	3kb	8/9
7	C	pTet GFP + TetR (UNS) 1C3	2kb	8/9
8	D	pTet GFP + TetR (UNS) 1C3 + 85x TetO addgene (amp)	3kb	8/9
9	E	52S pACT tet 2 (OA)	3kb	44/45
10	E'	52S pACT tet 2 (OA)	3kb	42/43

GEL 1:
A1 2 3 4 A'1 2 3 4 (1st row)

B1 B2 B'1 B'2 B''1 B''2 (2nd row)

Screen Shot 2016-10-07 at 11.09.52 PM.png

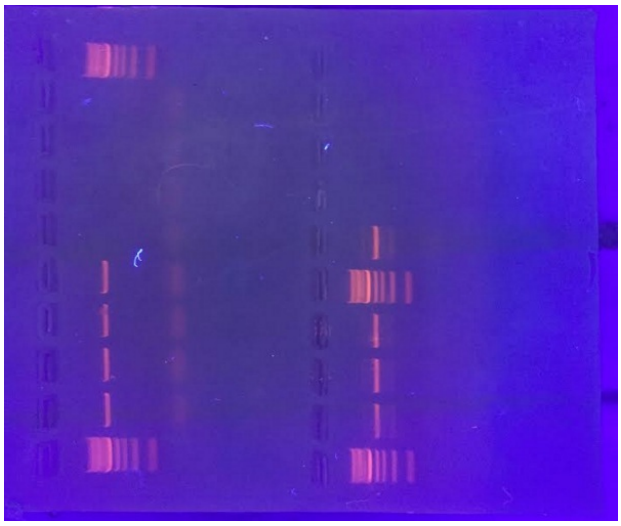


pACT tet is not there for A, but 2-4A have the 52s synthetic enhancer part.....pACT tet not there for B, but 52s is there and wrong primers were used to amplify the decoy binding array so can't tell if it is in there or not (only B2 had the 52s)

GEL 2:
E1 2 3 4 E'1 2 3 4

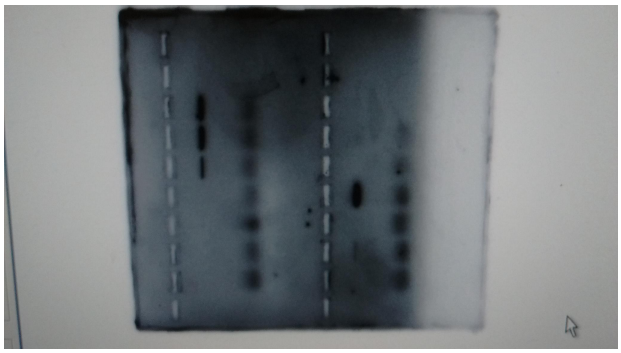
C1 2 3 4 D1

Screen Shot 2016-10-07 at 11.09.59 PM.png



pact tet is there for E but no 52S .....for C, all three colonies had the pTET gfp band, but there is slight double banding occuring, with the less prominent band around 500bp, which is not the size of pTET gfp or tetR alone..... and looksl ike pTET gfp is there for D, but the correct primers were not used to amplify the 85x tetO array so can't tell if array is in there or not

IMG_20161007_234512165.jpg



I re-took the first gel image on the upstairs gel imager... you can see that B2 has bands for both pACT-Tet and 52S.

- We are proceeding to inoculate *(in around 12:00 AM)*:
  - 53 solo in BL21 in M9
  - 53 + 85x tetO in BL21 in M9
  - B2 (52S OA + pACT-Tet OA + 85x teTO) in Phillips Broth

  For aTC induction tomorrow, both in-tube and in-plate
- Tomorrow we will also Colony PCR the A and E colonies gain (fresh transformations and 0817 FACS-confirmed glycerols of 52S OA + pACT-Tet OA) again.
- Tomorrow we will also re-try assembly of WM16_025.
- Tomorrow we will also try to successfully get a WM16_110 solo measurement again.
  - We may have to also re-attempt a WM16_110 + 48x lacO array measurement, because of the fact that our current situation is:
    - We have 110 + lacO 10 beta on FACS
    - We do not have 110 solo 10 beta induction
- Tomorrow we will also have to organize a structure to make it easy to make part pages.

Out @ 12:30 AM

161008 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-08

SATURDAY, 10/8

In @ 11:40 AM

The one replicate of 52S OA + pACT-Tet OA + 85x tetO 1C3 was at midlog when I came in. Inducing with aTC and IPTG:

- ☒ *Make 20,000 ng/mL aTC:*
  - ☒ 5 uL aTC to 495 uL Phillips Broth (Kan Amp Chlor, all low)
- ☒ *Make 200 ng/mL aTC:*
  - ☒ 5 uL 20,000 ng/mL aTC to 495 uL Phillips Broth (Kan Amp Chlor, all low)
- ☒ *aTC concentrations:*

Table1

	A	B
1	14	Add 125 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 10000 ng/mL aTc
2	13	Add 62.5 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 5000 ng/mL aTc
3	12	Add 25 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 2000 ng/mL aTc
4	11	Add 12.5 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 1000 ng/mL aTc
5	10	Add 6.25 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 500 ng/mL aTc
6	9	Add 2.5 uL of 200000 ng/mL aTc to 125 uL diluted culture to make 200 ng/mL aTc
7	8	Add 125 uL of 200 ng/mL aTc to 125 uL diluted culture to make 100 ng/mL aTc
8	7	Add 62.5 uL of 200 ng/mL aTc to 125 uL diluted culture to make 50 ng/mL aTc
9	6	Add 25 uL of 200 ng/mL aTc to 125 uL diluted culture to make 20 ng/mL aTc
10	5	Add 12.5 uL of 200 ng/mL aTc to 125 uL diluted culture to make 10 ng/mL aTc
11	4	Add 6.25 uL of 200 ng/mL aTc to 125 uL diluted culture to make 5 ng/mL aTc
12	3	Add 2.5 uL of 200 ng/mL aTc to 125 uL diluted culture to make 2 ng/mL aTc
13	2	Add 1.25uL of 200 ng/mL aTc to 125 uL diluted culture to make 1 ng/mL aTc
14	1	Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc

125 uL because there was insufficient volume for 250 uL

- ☒ *add 1mM IPTG:*
  - ☒ Add 1.25 uL 100 mM stock IPTG to 125 uL culture
    I should've done this first to save tips!! :(:(:(

Inductions went in at 12:30 PM, labeled 1.1-1.14.
Didn't do plate reader induction due to lack of volume, and also because induction on this part is inconsistent. Will do with 53.

Autoclaving more glass culture tubes. In at 1:20 PM

We are running low on P10 tips but we have receipt of order confirmation from Genessee that we ordered 5 boxes on Sep. 28... furthermore we also ordered 2 boxes of P1000 and 1 box of P200 which did arrive... so if these new P10 tips don't show up on Tuesday (Monday is a federal holidy :(:(:( ) it's gonna be a problem.....

Looking into WM16_110's performance history. Which MPs worked when? etc.

It turns out that **the successful WM16_110 1C3 + pJD100 Amp 10beta induction on 160925 was done with 110 MP3 and pJD100 MP1**. There have been two successive times to try growing WM16_110 1C3 solo 10beta, once with MP2 and once with MP3. Both were unsuccessful at the growth stage (plate or inoculation). **The successful transformation (but unsuccessful induction) of 110 1C3 +/- pJD100 10beta on 160918 was done with MP3.** This seems to suggest it might be worth re-trying MP3 transformation in 10beta...

Looked into the previous assembly attempts at WM16_025 using the new gBlock. These previous attempts were incorrectly done using [WM16_P040 (sfGFP Fwd) + WM16_013 (UNS2Rev)] on WM16_014, when in fact we needed to do [WM16_P014 (Scar RBS2 cI Fwd) + WM16_019 (UNS2 Rev)] on WM16_015.

Ethan set up Gibsons of (161008-EMJ):

- Move WM16_029 MP3 1C3 onto 1A3
  In order to co-transform that with a 23 1C3 from Kit to measure repressor functionality
- WM16_025 1C3 (using the above correct procedure)

Ethan and I set up 10 uL Colony PCRs that repeat the A and E transformations' Colony PCRs from yesterday. Both are 52S OA + pACT-Tet OA so we did:

- to get 52S: P042 P043 1:30 64C [labeled A1-8 E1-8, P1]
- to get pACT-tet: P044 P045 1:30 59C [Labeled A1-8 E1-8, P2]

Ethan is making new IPTG stock from the solid IPTG which came in. Made 10 mL 100 mM IPTG (same concentration as the Ready-to-Use stock) and 4 mL 1 M IPTG in filter-sterilized water.

Out @ 4:30 PM
In @ 5:00 PM

Ran the Gel of the Gibson PCRs. Only the 14 backbone PCR worked, the 29 insert PCR did not and the 15 backbone PCR did not.

- Turns out the 15 backbone PCR was done incorrectly (P19 was used instead of P13). Ethan is re-doing it with the parameters
  P014 P013 66C 2:00 on WM16_015 1C3 MP3 160605
- Don't know why the WM16_028 MP3 P008 P009 PCR didn't work... however we are still waiting on functional confirmation of pBad induction capability on this part so we will hold off on trying to re-do this PCR until that can be demosntrated on a particular miniprep.

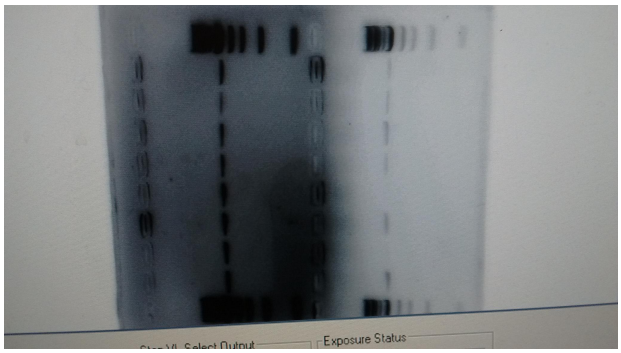
Out @ 5:20 PM
In @ 6:50 PM

Set up DpnI of the 15 backbone PCR.

- 2.7 uL CutSmart
- 0.5 uL DpnI
- 24 uL PCR Product

Ran Gels of Colony PCRs, which were the same plates that we ran colony PCRs on yesterday. The colony PCRs for A looked great... why were they so bad yesterday? It seems like we've been messing up a lot of PCRs lately, need to be more careful:

161008_A.jpg

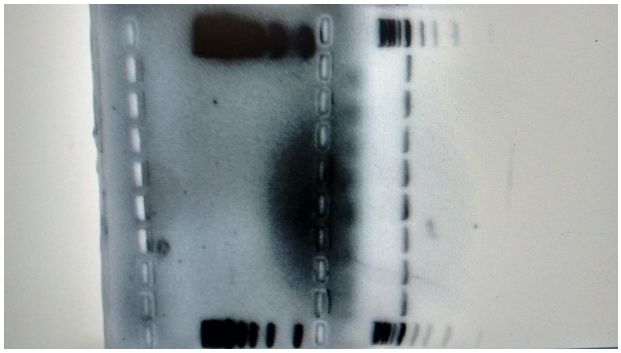


52S OA + pACT-Tet OA fresh transformation LG3.300

Left: P42/P43 to amplify 52S OA
Right: P44/P45 to amplify pACT-Tet OA

Looks like A1, A3, A5, A6, A7, A8 are good.
Prioritize A1, A3, A6 for inoculation and induction.

161008_E.jpg



52S OA + pACT-Tet OA LG3.300 Glycerol Stock

Left: P42/P43 to amplify 52S OA
Right: P44/P45 to amplify pACT-Tet OA

As before, looks like most colonies did not have both plasmids present.
E2 is the only maybe-having-52S colony.

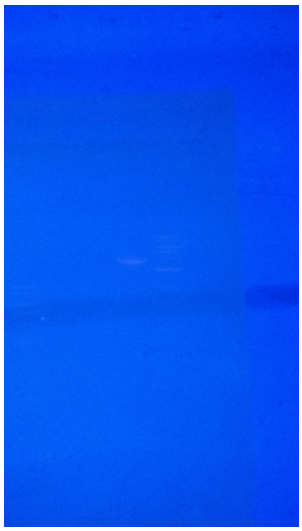
Inoculations of 53 1C3 solo BL21 and 53 1C3 + 85x tetO addGene Amp BL21 have not grown yet after 19.5 hours in the shaker. (M9 Glycerol Low-Antibiotic culture is what they are supposed to be).

Macrogen Results came back yesterday (55AS TETR NRII SFGFP 1C3, 52S tetR NRII sfGFP 3K3, and 52S NRII sfGFP 1C3) and LK analyzed them-- all but one replicate were confirmed.

Out @ 7:40 PM
In @ 8:30 PM

Ethan is setting up PCR purification of the 15 backbone PCR to be Gibson'd with the 25 insert gBlock.

161008_Gibson.jpg



Gel looks good-- band is slightly under 4kb (should be 3,737 bp).

Also this is functional confirmation that you can indeed re-use the empty wells of an old gel that's been sitting around in the waste bag for a week. The picture isn't high quality but in-person it looks perfect.

Gibson will be:

Table2

	A	B	C	D	E	F	G
1	**Backbone**	**WM16_015 with P13 / P14**	**0.06**	**3737**	**185.2**	**0.7990561555**	
2	Insert	WM16_025 gBlock	0.18	NA	NA	1.8	2.4009

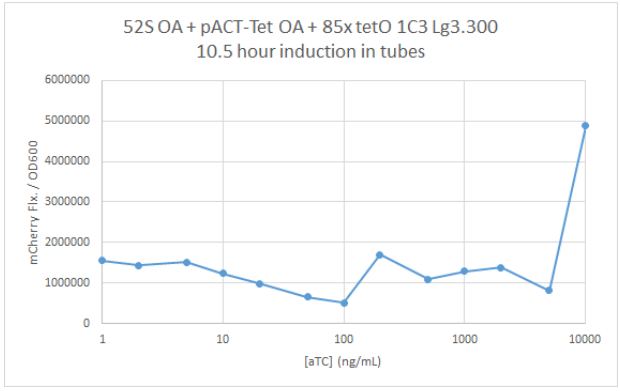
Recall that all gBlocks are resuspended to be 0.1 pmol/uL

Made part pages for all 8 RBS characterization parts

Measuring the 52S OA + pACT-Tet OA + 85x tetO 1C3 LG3.300 inductions from this morning at 10:00 PM (9.5 hour aTC induction) in the Plate Reader. Put 125 uL culture into each well. OD600 and mCherry Flx (587/610). However the results do not convincingly show induction.

See Dropbox / iGEM 2016 / FACS Data / 161008

clipboard_2016-10-09_13:50:54.png



Ugh. Also what's going on with the 10,000 point?? It's not entirely a function of dividing by a small OD600 value, as its raw Fluorescence is still the highest in the series without normalizing by OD600.

You also don't really see the toxicity condition we saw earlier in the summer at high [aTC].

At 10:15 PM (22.5 hrs) the 53 1C3 + 85x tetO 1A3 culture looked like it was around midlog. I induced them as:

- ☒ *Make 20,000 ng/mL aTC: (EMJ)*
  - ☒ 5 uL aTC to 495 uL M9 Glycerol (Amp Chlor, all low)
- ☒ *Make 200 ng/mL aTC: (EMJ)*

- ☒ 5 uL 20,000 ng/mL aTC to 495 uL M9 Glycerol (Amp Chlor, all low)

☒ *aTC concentrations: (JPM)*

Table3

	A	B
1	13	Add 250 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 10000 ng/mL aTc
2	12	Add 125 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 5000 ng/mL aTc
3	11	Add 50 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 2000 ng/mL aTc
4	10	Add 25 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 1000 ng/mL aTc
5	9	Add 12.5 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 500 ng/mL aTc
6	8	Add 5 uL of 200000 ng/mL aTc to 250 ul diluted culture to make 200 ng/mL aTc
7	7	Add 250 uL of 200 ng/mL aTc to 250 ul diluted culture to make 100 ng/mL aTc
8	6	Add 125 uL of 200 ng/mL aTc to 250 ul diluted culture to make 50 ng/mL aTc
9	5	Add 50 uL of 200 ng/mL aTc to 250 ul diluted culture to make 20 ng/mL aTc
10	4	Add 25 uL of 200 ng/mL aTc to 250 ul diluted culture to make 10 ng/mL aTc
11	3	Add 12.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 5 ng/mL aTc
12	2	Add 5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 2 ng/mL aTc
13	1	Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc

These went in at 10:30 PM.

Ethan is transforming / plating the following:

Plating

	A	B	C	D	E	F
1	**Key**	**Name**	**Strain**	**Purpose**	**Abx**	**Notes**
2	A	WM_025 1C3	5-alpha	Miniprep pipeline	1C3	
3	B	WM_110 mp3	BL21	Induction	1C3	
4	C	WM_110 mp3 +pjd mp1	BL21	Induction	1C3/Amp	
5	D	WM_029 1C3 mp1	BL21	Induction	1C3	
6	E	WM_029 1C3 mp2	BL21	Induction	1C3	
7	F	WM_029 1C3 mp3	BL21	Induction	1C3	
8	G	WM_029 1C3 gs1	5-alpha	Induction	1C3	Restreak
9	H	WM_029 1C3 gs2	5-alpha	Induction	1C3	Restreak
10	I	WM_029 1C3 gs3	5-alpha	Induction	1C3	Restreak
11	J	WM16_014 1C3 + WM16_016 3K3 160713 GS1	BL21	Induction	1C3 / 3K3	Restreak
12	K	WM_110 mp3	10 beta	Induction	1C3	
13	L	WM_110 mp3 +pjd mp1	10 beta	Induction	1C3/Amp	

A: To measure WM16_025 induction in BL21 with WM16_016 3K3 to get RiboJ measurements
B and C: To characterize the pJD100 48x lacO array.. this was erroneously transformed into BL21 (We have historically had extremely poor transformation success of 48x lacO array into BL21)
D, E, and F: To determine if any of these replicates are able to be functionally induced (this is currently not known) (we are assuming BL21 will provide best-induction conditions for pBad)
G, H, I: To determine if any of these replicates are able to be functionally induced (this is currently not known) (we are using 5alpha to put 29 on the same conditions as the successful 28 induction we got earlier)
J: To use as a known good-induction construct to optimize our plate reader measurement protocol (ie. is it functionally equivalent to induce in-plate than in-tube? Can we get comprable 200x induction levels that we'd been seeing before on FACS?)
K and L: To characterize the pJD100 48x lacO array in 10 beta

I started transformations of K and L 30min behind the rest of the transformations

Out @ 11:45 PM

iGEM Transformation

Introduction

This is how you insert your plasmid(s) into cells. Please be sure you know **which strain** you are using and you know the **appropriate amount of time** to heat shock your **specific strain**.

Materials

- Comp Cells
- Plasmid DNA
- SOC
-

Procedure

Thaw Cells

1. Take out enough cells so that you can have at least 15 uL of cells per thing you are trying to transform. There is about 45-50 uL of competant cells per NEB tube of cells.
2. Thaw cells on ice
3. Transfer appropriate amount of cells to appropriately labelled Eppendorf tube (I would suggest using the same key as you used for the gibson assemblies).

Transform

4. Add 2 uL of plasmid DNA to each aliquot of cells.
5. Ice for 30 minutes. Prewarm heatblock to 42 degrees C.

   I would strongly recommend that you take this time to prelabel your plates and place them in the incubator to prewarm.

Heat Shock

6. Heat shock cells for appropriate amount of time. This varies based on which strain you are using.

   BL21 gets heat shocked for 10 seconds
   10Beta and 5alpha get heat shocked for 30 seconds
7. Ice for five minutes.
8. Pipette in SOC based on the amount of cells you used. 50 uL of cells get 950 uL of SOC, for reference.

Outgrow

9. Place in shaking incubator 250 rpm 37C for 1 hour (chlor, amp, or tet) or 2 hours (kan)

10. Remove bacteria from shaking incubator.

11. **INVERT EVERY TUBE 4-6 TIMES. IF YOU DONT DO THIS YOU WILL NOT HAVE A SUCCESSFUL TRANSFORMATION.**

Plate

12. Plate out 100 uL of bacteria.

    (We have been having lawn growth for a lot of constructs; you may want to do 50 uL if you have experience with this part overgrowing. Likewise, if you are doing a double transformation or a low copy number, do 150 uL).
    Use glass beads in a bunsen burner sterile field. Dispose of glass beads into ethanol.

13. Put plates in incubator upside down (agar side up, lid down). Let grow overnight.

    Do not be alarmed if you do not see colonies for up to 18 hours.

161009 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-09

SUNDAY, 10/9

In @ 11:00 AM

Some plates from last night had grown decently; others needed more time.

Synthetic Enahncer (52S OA + pACT-Tet OA LG3.300) cultures in phillips broth were at ~midlog.

- Tube-based induction:
  - *Make 20,000 ng/mL ATC:*
    - ☒ Add 15 uL 2 mg/mL aTC (Ethan stock)
    - ☒ Add 1485 uL Phillips Broth (Low Amp Low Kan)
  - *Make 200 ng/mL ATC:*
    - ☒ Add 14 uL 20,000 ng/mL ATC from above
    - ☒ Add 1485 uL Phillips Broth (Low Amp Low Kan)
  - *Add IPTG to 1 mM:*
    - ☒ 2.5 uL 100 mM Stock (ready-to-use) IPTG to each glass tube (14 per replicate)
  - *Induce with aTC:*

Table2

	A	B
1	14	Add 250 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 10000 ng/mL aTc
2	13	Add 125 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 5000 ng/mL aTc
3	12	Add 50 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 2000 ng/mL aTc
4	11	Add 25 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 1000 ng/mL aTc
5	10	Add 12.5 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 500 ng/mL aTc
6	9	Add 5 uL of 200000 ng/mL aTc to 250 ul diluted culture to make 200 ng/mL aTc
7	8	Add 250 uL of 200 ng/mL aTc to 250 ul diluted culture to make 100 ng/mL aTc
8	7	Add 125 uL of 200 ng/mL aTc to 250 ul diluted culture to make 50 ng/mL aTc
9	6	Add 50 uL of 200 ng/mL aTc to 250 ul diluted culture to make 20 ng/mL aTc
10	5	Add 25 uL of 200 ng/mL aTc to 250 ul diluted culture to make 10 ng/mL aTc
11	4	Add 12.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 5 ng/mL aTc
12	3	Add 5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 2 ng/mL aTc
13	2	Add 2.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 1 ng/mL aTc
14	1	Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc

These went in the shaker at 37c 250 RPM at 12:10 PM

- Plate-Based Induction:
  - *Make 20,000 ng/mL ATC:*
    - ☒ Add 15 uL 2 mg/mL aTC (Ethan stock)
    - ☒ Add 1485 uL Phillips Broth (Low Amp Low Kan)
  - *Make 200 ng/mL ATC:*

- ☒ Add 14 uL 20,000 ng/mL ATC from above
- ☒ Add 1485 uL Phillips Broth (Low Amp Low Kan)

- *Add IPTG to 1 mM:*
  - ☒ 1.25 uL 100 mM IPTG (ready-to-use) to each well (14 per replicate)
    (I added 2.5 uL IPTG to #7, #8, #13, #14 of each replicate to compensate for extra volume)
- *Add aTC:*

Table1

	A	B
1	14	Add 125 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 10000 ng/mL aTc
2	13	Add 62.5 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 5000 ng/mL aTc
3	12	Add 25 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 2000 ng/mL aTc
4	11	Add 12.5 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 1000 ng/mL aTc
5	10	Add 6.25 uL of 20000 ng/mL aTc to 125 uL diluted culture to make 500 ng/mL aTc
6	9	Add 2.5 uL of 200000 ng/mL aTc to 125 uL diluted culture to make 200 ng/mL aTc
7	8	Add 125 uL of 200 ng/mL aTc to 125 uL diluted culture to make 100 ng/mL aTc
8	7	Add 62.5 uL of 200 ng/mL aTc to 125 uL diluted culture to make 50 ng/mL aTc
9	6	Add 25 uL of 200 ng/mL aTc to 125 uL diluted culture to make 20 ng/mL aTc
10	5	Add 12.5 uL of 200 ng/mL aTc to 125 uL diluted culture to make 10 ng/mL aTc
11	4	Add 6.25 uL of 200 ng/mL aTc to 125 uL diluted culture to make 5 ng/mL aTc
12	3	Add 2.5 uL of 200 ng/mL aTc to 125 uL diluted culture to make 2 ng/mL aTc
13	2	Add 1.25uL of 200 ng/mL aTc to 125 uL diluted culture to make 1 ng/mL aTc
14	1	Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc

Note the half-volume to accomodate the wells

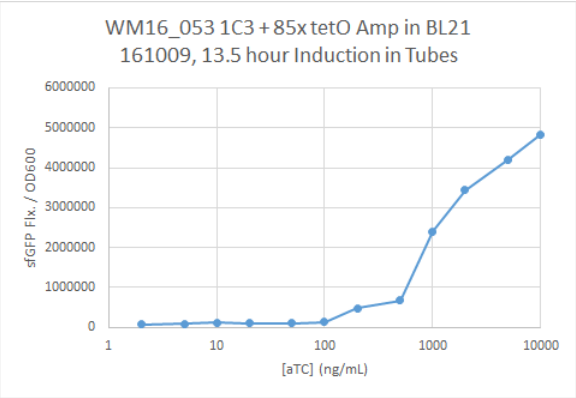
- *Program Plate Reader:*

```
Use Lid
Set Temperature 37C
Start Kinetic (12 hours, 10 minute interval)
    Shake: Double Orbital (Continuously)
    Read Absorbance 600 (OD600)
    Read Fluorescence 587/610 (mChery)
End Kinetic
```

This went in at 12:30 PM

I also measured last night's WM16_053 1C3 + 85x tetO 1A3 induction (one replicate, 14 induction conditions) in the plate reader at 12:00 PM (13.5 hour induction).

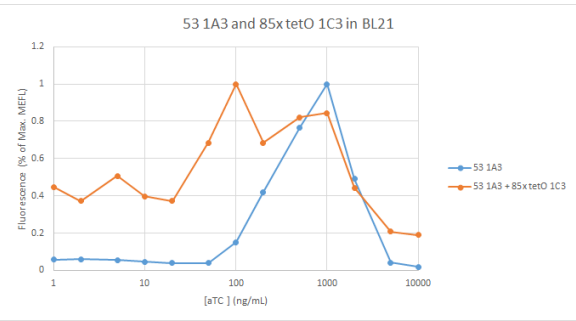
clipboard_2016-10-09_13:41:48.png



It doesn't look that bad, actually. But it seems to be inconsistent with our previous 53 +/- 85x tetO measurements (reproduced below)

This from 160824 measurements.

clipboard_2016-10-09_13:46:04.png



Note how the 161009 measurement does not exhibit the high-[aTC] drop-off (which we attributed to aTC toxicity) and also begins increasing later than either of these curves here.

Could it be that the concentrations are not being measured consistently??

Now that we have much more atC we might try 20,000 and 50,000 ng/mL conditions on some of these.

Recall that yesterday's synthetic enhancer measurement was also not toxic at high [aTC], despite the fact that yesterday I induced with the Clontech aTC and for today's induction Ethan used his home-made aTC solution. Mysterious. 161008 JPM

At 12:40 PM the glycerol streak of WM16_014 1C3 + WM16_016 3K3 had not yet grown at all despite the other glycerol stocks growing... I re-streaked the other glycerol stocks of this one just in case.

Made LB Agar plates (1L) for chlor plates:

- ☒ 15 g Agar
- ☒ 25g LB Broth Mix

In autoclave @ 1:30 PM

The WM16_053 1C3 solo BL21 in M9 glycerol Chlor (low) has still not grown. We are going to Colony PCR more colonies from the plate (it grew super well-- C from 161007 trasnformations) and inoculate them tonight to try and get a good solo 53 to work.

Reading into the synthetic enhancer paper again, I came across this paragraph which I hadn't noticed (or realized the significance of) before:

clipboard_2016-10-09_14:29:28.png

Repression Ratio Measurement Assay

Synthetic enhancer strains containing the pACT-Tet or pACT-Tra plasmid were initially grown in LB, resuspended in the low growth buffer, and dispensed in the 48-well plates. In this case, appropriate concentrations of aTc or 3OC8 (sigma) were dispensed in each well, spanning four to six orders of magnitude. For each strain, we used two plates to allow for 94 different readings of fluorescence as a function of aTc concentration (two wells were used as –IPTG controls). We carried out each measurement in duplicates, i.e., four plates per measurement.

To compute the repression ratio levels as a function of aTc or 3OC8 concentrations, each fluorescence ratio value was calculated using a running average algorithm. This entails averaging three to five raw fluorescence readings for every fluorescence value shown, whereby the averaging is carried over adjacent inducer concentrations. This algorithm is used to smooth out short-range fluctuations and highlights the large-scale features that span wide concentration ranges.

WTF Roee Amit.

Measurement graphs might look more like the paper if we performed a similar moving average transformation on the fluorescence data.

Out @ 2:40 PM
In @ 4:00 PM

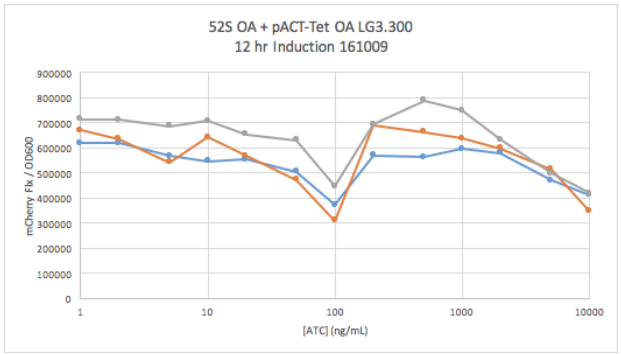
Called Sheraton Boston Hotel and placed John Mitchell, Kalen Clifton, and Likhitha Kolla's names explicitly on the reservation booking so that EXTREEMS and HHMI funding can go through without hitches.

Set up Colony PCRs for the transformations from last night. The only plate that didn't have eventual growth was C (the erroneous 110 1C3 + pJD100 Amp in BL21 transformation), so we replaced this with C from 161007 (WM16_053 1C3 solo in BL21) to try and re-do inoculations (the inoculants still have not grown after over 24 hours at this point).

Out @ 5:40 PM
In @ 12:40 AM

Kinetic measurement (12hrs) from Plate Reader finished-- exported data. Took a new single-timepoint measurement of the tube-based induction (13 hrs) as well and exported that. All data in Dropbox / iGEM 2016 / FACS Data / 161009

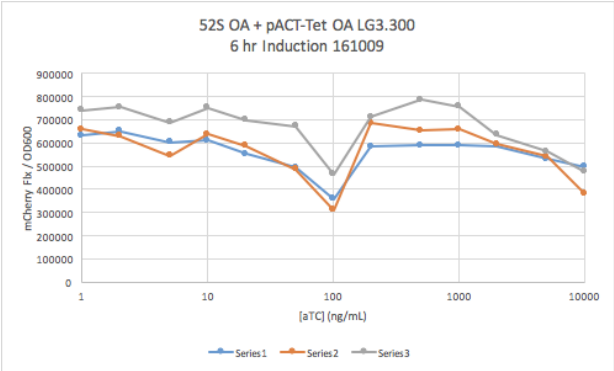
clipboard_2016-10-10_01:31:32.png



Kinetic in Plate:

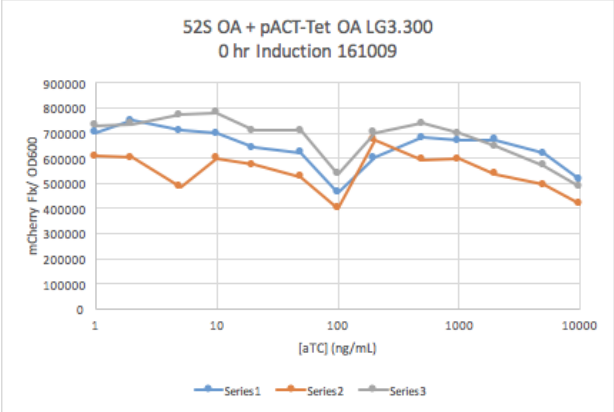
The 12 hour induction timepoint from the kinetic measurement takes a similar shape to what we tend to see with the synthetic enhancer inductions... a sloping decrease toward the middle, a rebound, then another sloping decrease. It seems like induciton isn't really happening and what's going on is really more a factor of the variable volume or something added by the dilution method, perhaps....?

clipboard_2016-10-10_01:52:36.png



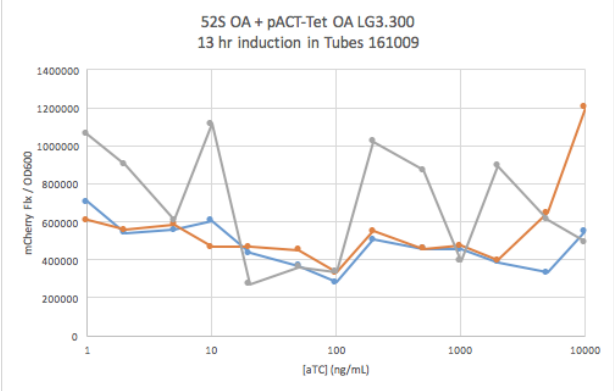
If it's any consolation we can see that the 6hr timepoint looks exactly the same as the 12hr timepoint... probably because induction didn't occur.

clipboard_2016-10-10_01:55:25.png



Yep, here's the 0 hour timepoint.

clipboard_2016-10-10_01:40:50.png



Inductions in Tubes:

This looks like it failed completely. Replicate 2 (the orange one)... maybe?? But why is it so inconsistent??

There are so many places where the induction failure could be occurring...
However, so far it seems that tube-based inductions at least get a high-concentration [aTC] level to be higher than a lower-concentration [aTC] level, so that distinction might end up being important. Tomorrow we'll induce 14+16 in both tubes and plates to provide more evidence of there being a functional difference here.

Out @ 2:15 AM

161011 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-10

MONDAY, 10/10

Explicit attempt to Replicate 52S measurements from Amit et al Synthetic Enhancer Paper:

1. Make correct-concentration Antibiotics:
    - Make **100 mg/mL** Amp (1000X)
        - ☒ Add 1.0 g Ampicillin (in 4C fridge) to
        - ☒ 10 mL Millipore Water
        - ☐ Label aliquots as explicilty 100 mg/ mL Amp
    - Make **20 mg/mL** Kan (1000X)
        - ☒ Add 0.20 g Kanamycin (in the cupboard over the 3D printers) to
        - ☒ 10 mL Millipore Water
        - ☐ Label aliquots as explicitly 20 mg / mL Kan *(this is different from our usual stock concentration!)*

2. Obtain three good colonies of LG3.300 52S OA + pACT-Tet OA cotransformation.
    - ☒ Inoculate them in 3 mL LB overnight with Kan and Amp from above.

3. Inoculate 5 uL of each overnight colony culture in 20 mL LB with Kan and Amp from above *(they are 1000X so you need 20 uL Kan and 20 uL Amp per flask)* in a 125 mL Flask at 37C, 250 rpm, until **midlog**

    You will have to either install the flask-holders in our shaker or go upstairs

    Midlog means OD600 = 0.6. Use the Nanodrop, Plate Reader, or some other spectrophotometer to determine OD600.

    *During this time you can **start setting up aTC** according to the table in step 6*

    - ☒ In at 11:00 AM *(it ended up taking about 3~4 hours to reach midlog)*

4. Use the big centrifuge upstairs to spin down the LB cultures at midlog.
    - ☒ Remove supernatant

    It took a while to figure out what settings are required to create a pellet from 20 mL volume at midlog growth... eventually I found that 13,000 rpm for 15 min will do the job.

5. Resuspend each pellet in 100 mL Sigma 54 Broth with Kan and Amp from above
    - ☒ Then set aside 2 mL in a glass culture tube, from each replicate *(I forgot to do this on #2 and #3 :( )*
    - ☒ After setting aside the 2mL, add 1 mL 100 mM IPTG to each replicate

6. Dispense each replicate in 2 mL increments amongst 47 glass tubes, which each contain [aTC] solution **already**.

Table1

	A	B	C
1	**Add _____ uL**	**of ____ ng/mL aTC**	**to 2 mL Sigma 54 Broth to get _____ ng/mL aTC**
2	0.5	200	0.05
3	0.642193132	200	0.064219313
4	0.824824037	200	0.082482404
5	1.059392663	200	0.105939266
6	1.360669384	200	0.136066938
7	1.747625066	200	0.174762507

8	2.244625629	200	0.224462563
9	2.882966325	200	0.288296632
10	3.702842346	200	0.370284235
11	4.755879846	200	0.475587985
12	6.108386745	200	0.610838674
13	7.845528028	200	0.784552803
14	10.07668843	200	1.007668843
15	12.9423602	200	1.29423602
16	16.62298966	200	1.662298966
17	21.35033958	200	2.135033958
18	27.42208288	200	2.742208288
19	35.22054657	200	3.522054657
20	45.23678621	200	4.523678621
21	0.581015068	20000	5.810150682
22	0.746247773	20000	7.462477725
23	0.958470388	20000	9.584703883
24	1.231046201	20000	12.31046201
25	1.58113883	20000	15.8113883
26	2.030792994	20000	20.30792994
27	2.608322626	20000	26.08322626
28	3.350093752	20000	33.50093752
29	4.302814396	20000	43.02814396
30	5.526475706	20000	55.26475706
31	7.098129482	20000	70.98129482
32	9.116740004	20000	91.16740004
33	11.70941563	20000	117.0941563
34	15.03941259	20000	150.3941259
35	19.31641494	20000	193.1641494
36	24.80973802	20000	248.0973802
37	31.86528671	20000	318.6528671
38	40.92733654	20000	409.2733654
39	0.525665089	2000000	525.6650885
40	0.675157019	2000000	675.1570189
41	0.867162401	2000000	867.1624009
42	1.113771476	2000000	1113.771476
43	1.430512785	2000000	1430.512785
44	1.83733097	2000000	1837.33097

44	[illegible]	[illegible]	[illegible]
45	2.35984266	2000000	2359.84266
46	3.030949497	2000000	3030.949497
47	3.892909899	2000000	3892.909899
48	5	2000000	5000

Note that 2,000,000 ng/mL is the stock 2mg/mL concentration.

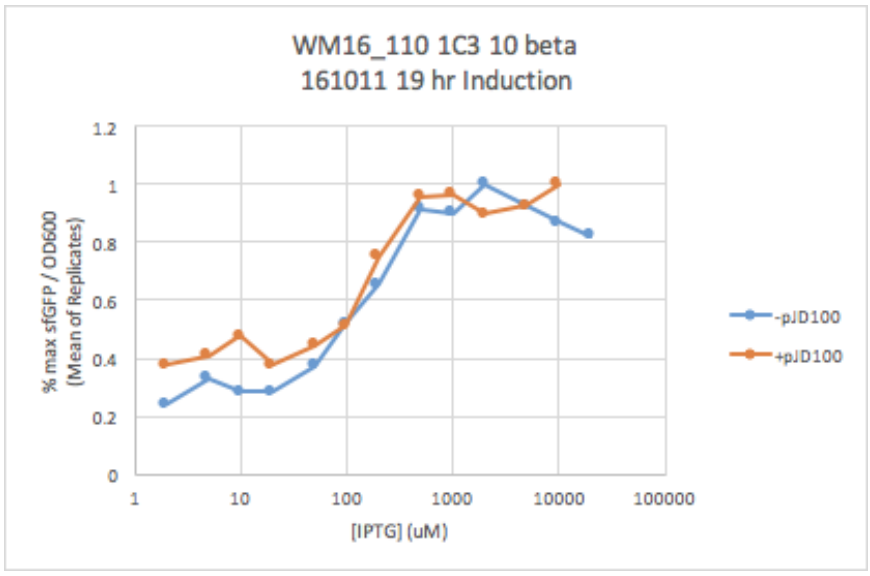
Also note that this is the table for one replicate, and it requires:
202.55 uL 200 ng/mL aTC solution
182.80 uL 20,000 ng/mL aTC solution
20.73 uL Stock (2 mg/mL) aTC Solution

7. Let the tubes incubate at 37C 250 rpm until "steady-state of growth"... presumably a long time.

8. Measure 200 uL aliquots in the plate reader, taking OD600 and mCherry Fluorescence (580/610). Each replicate should take up 48 wells (47 inductions + 1 no-IPTG control).

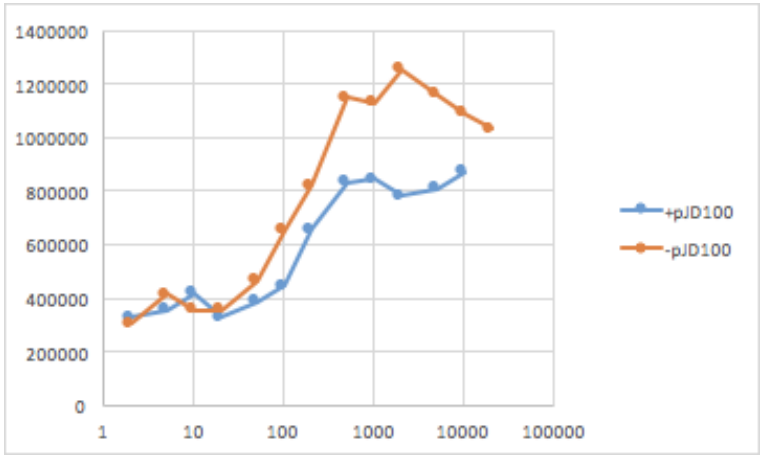
In @ 12:10 PM

clipboard_2016-10-11_13:54:01.png



Looks like pJD100 didn't have an effect on induction of WM16_110.

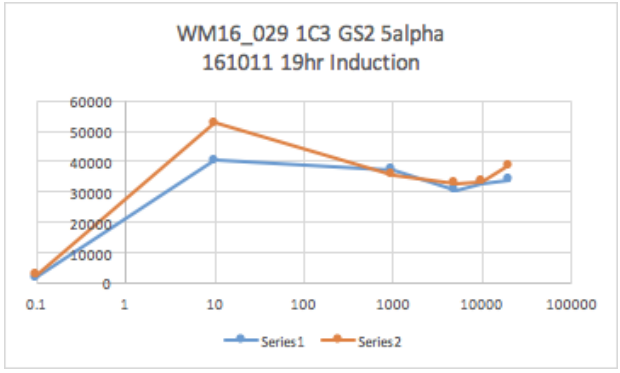
clipboard_2016-10-11_13:54:33.png



If you don't express the above graph as %max, you can see a reduction in max expression that is associated with the presence of pJD100 and Amp selection

Also the colors are swapped from the above graph, sorry.

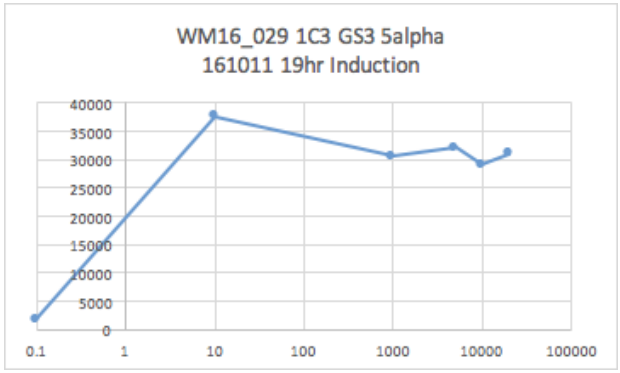
clipboard_2016-10-11_14:35:23.png



29 induced in 5 alpha too.

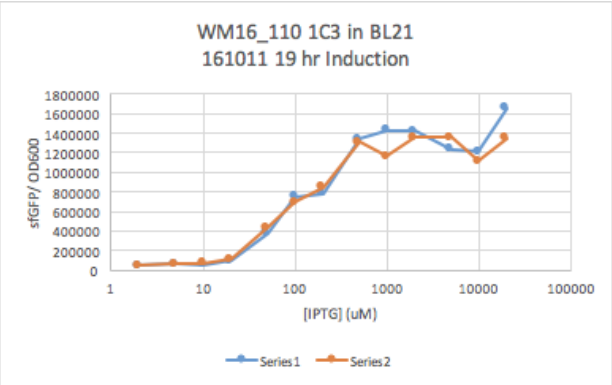
0 point became 0.1 to display on graph

clipboard_2016-10-11_14:35:27.png



as above.

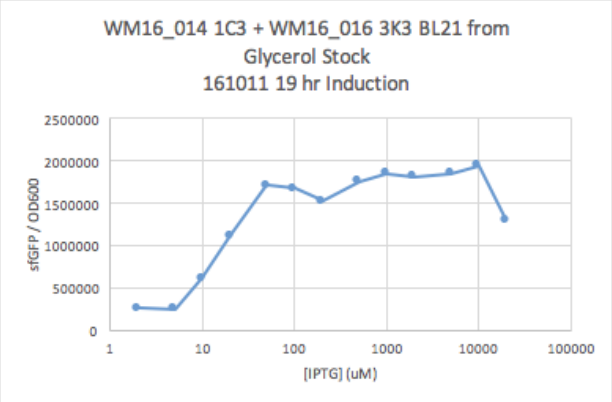
clipboard_2016-10-11_14:10:11.png



WM16_110 induced quite well in BL21, also. However the version with pJD100 never grew (as has happened several times now in BL21)

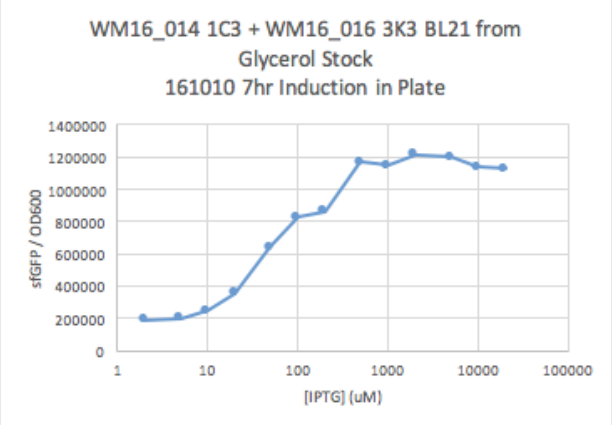
These are two replicates

clipboard_2016-10-11_14:12:12.png



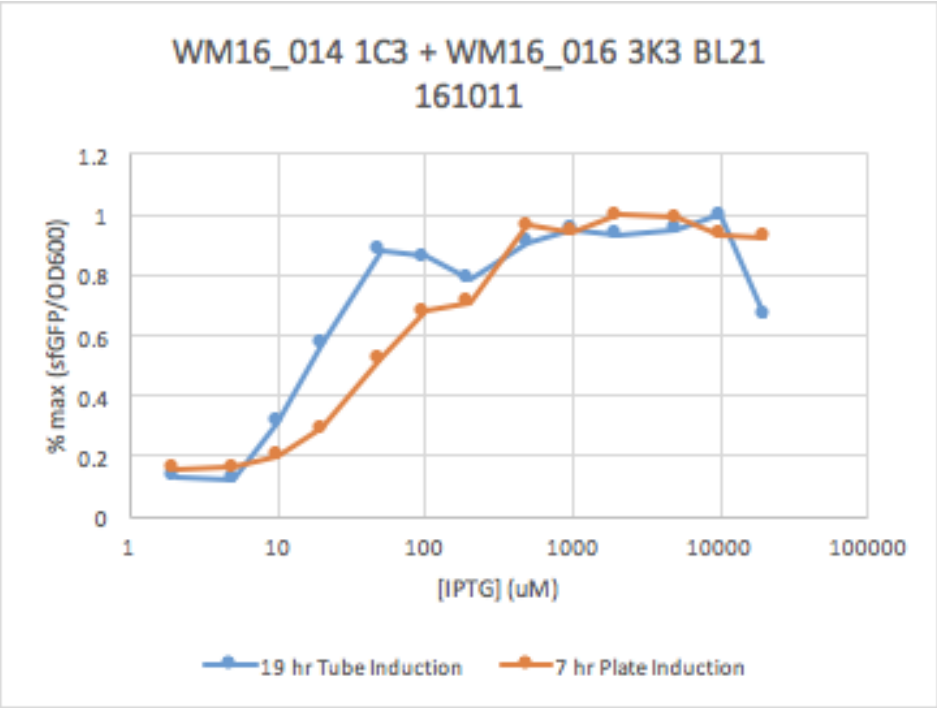
This is the 14/16 cotransformation induced in tubes

clipboard_2016-10-11_14:15:07.png



This is the same 14/16 cotransformation induced in plate
(I would have taken the 7hr timepoint of the tubes version, but it seemed like it wasn't grown it all at the time so I let it go overnight)

clipboard_2016-10-11_14:31:31.png



Interestingly, it seems to not matter whether we induced in tubes or in plates! (125 uL volume each)

That's good news.

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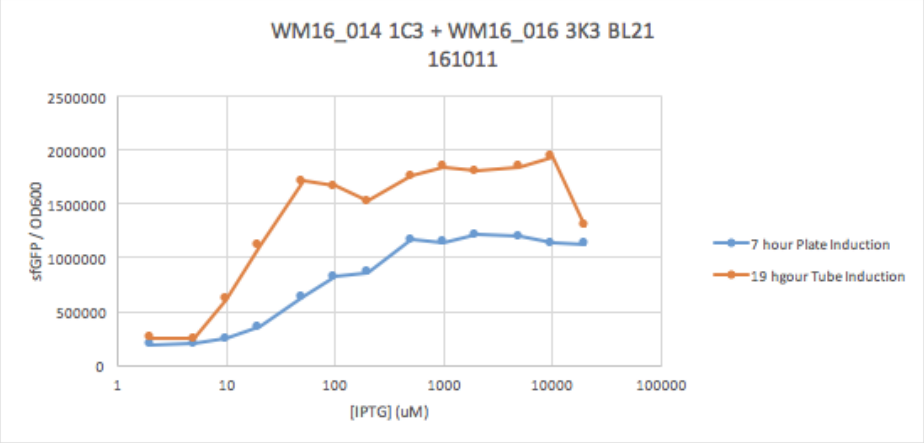
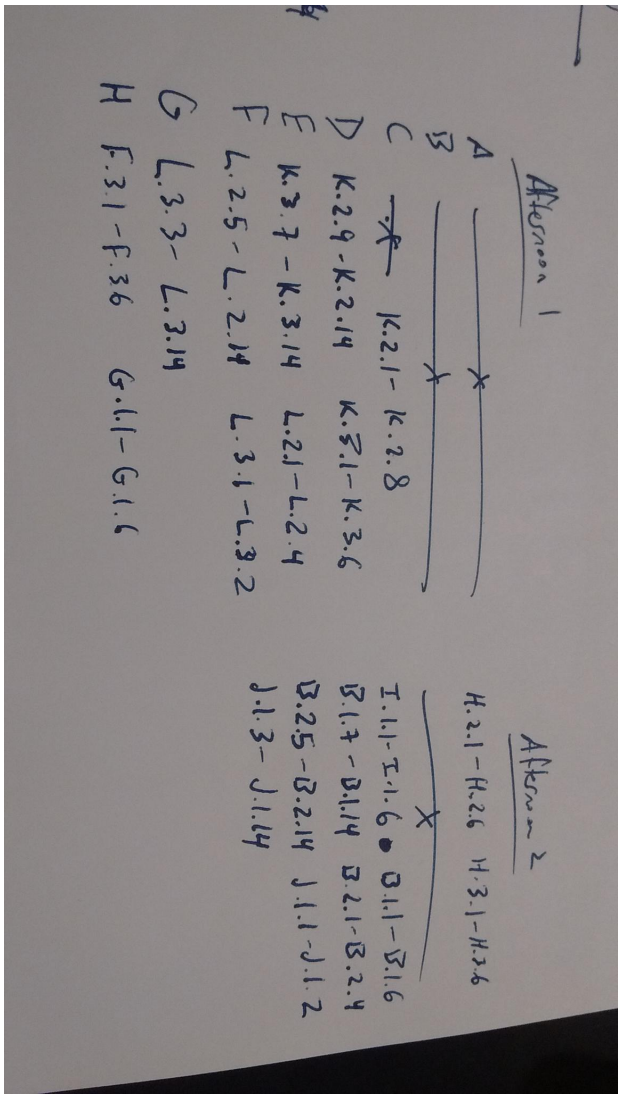


When it's not %max, you can see that the Tube induction reached a higher expression level, but also it had 12 more hours to grow/induce.

Note that the colors have flipped from the previous graph, sorry.

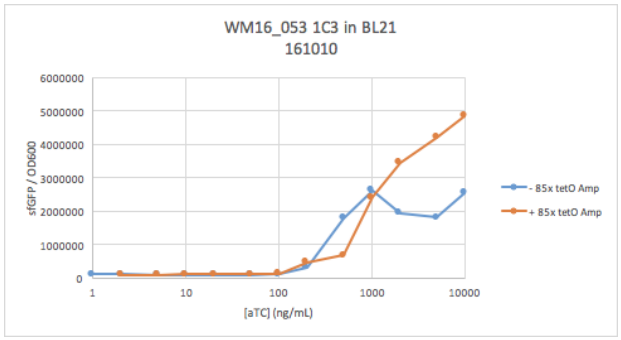
161011_key.jpg



Btw this is the plate layout

I then went back and overlayed the WM16_053 1C3 BL21 with and without 85x tetO Amp and got:

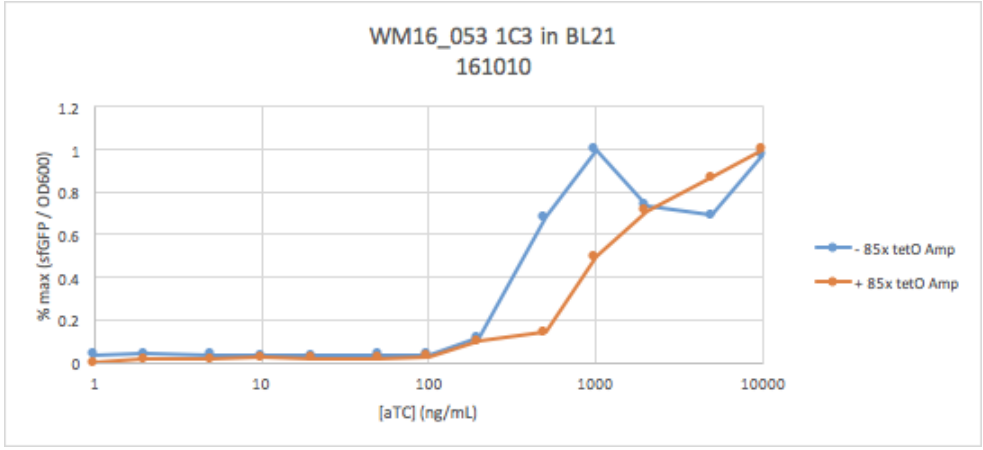
clipboard_2016-10-11_14:54:54.png



So it's weird that the no-array case has lower expression, but if we convert to % of max....

(or it might just be that high concentrations led to something deleterious in the -85x tetO condition but not the +)

clipboard_2016-10-11_14:55:19.png



... then it looks like the array induces a *rightward* shift??

However it is important to note that the + tetO array condition was induced for 1.5 hrs longer, and that they were done on different (though sequential) days...

+ array was 161009 JPM
- array was 161010 JPM

Ethan is setting up Gibsons to:

- Move WM16_029 1C3 MP3 onto 1A3 backbone
- Move WM16_023 (pTet GFP, K1493504) onto 1A3 backbone (no UNS)

Likhitha transformed the Gibsons as well as (20161011 LK)

- WM16_053 1A3 +/- 85x tetO 1C3 in BL21
- WM16_053 1C3 +/- 85x tetO 1K3 in BL21

Callan induced the Synthetic Enhancer samples and put them in the Incubator at around 5:00 PM

Out @ 5:45 PM

161010 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-10

MONDAY, 10/10

In @ 11:10 AM

When I came in today, most of the BL21 inoculation had grown to midlog. Recall that our key is

Table4

	A	B	C	D	E	F
1	**Key**	**Constructs**	**Strain**	**Abx**	**Inducer**	**Notes**
2	A	WM16_025 1C3	5 alpha	Chlor		Not for induction
3	B	WM16_110 1C3	BL21	Chlor	IPTG	
4	C	WM16_053 1C3	BL21	Chlor	aTC	
5	D	WM16_029 1C3 MP1	BL21	Chlor	Arabinose	
6	E	WM16_029 1C3 MP2	BL21	Chlor	Arabinose	
7	F	WM16_029 1C3 MP3	BL21	Chlor	Arabinose	
8	G	WM16_029 1C3 GS1	5 alpha	Chlor	Arabinose	
9	H	WM16_029 1C3 GS2	5 alpha	Chlor	Arabinose	
10	I	WM16_029 1C3 GS3	5 alpha	Chlor	Arabinose	
11	J	WM16_014 1C3 + WM16_016 3K3	BL21	Chlor Kan	IPTG	
12	K	WM16_110 1C3	10 beta	Chlor	IPTG	
13	L	WM16_110 1C3 + pJD100	10 beta	Chlor Amp	IPTG	

Specific minipreps can be found in 161008 JPM

The following were at midlog (each sample was inoculated with 3 replicates):

- B.3
- J.3
- D.1,2,3
- E.1,2,3
- F.1,2,3
- C.1,2,3

So Callan and I induced them (in Tubes):

Make 20,000 ng/mL ATC:

- ☒ Add 15 uL 2 mg/mL aTC (Ethan stock)
- ☒ Add 1485 uL M9 Glycerol (Low Chlor)

Make 200 ng/mL ATC:

- ☒ Add 14 uL 20,000 ng/mL ATC from above
- ☒ Add 1485 uL M9 Glycerol (Low Chlor)

14-step aTC:

Table2

	A	B
1	14	Add 250 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 10000 ng/mL aTc
2	13	Add 125 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 5000 ng/mL aTc
3	12	Add 50 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 2000 ng/mL aTc
4	11	Add 25 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 1000 ng/mL aTc
5	10	Add 12.5 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 500 ng/mL aTc
6	9	Add 5 uL of 200000 ng/mL aTc to 250 ul diluted culture to make 200 ng/mL aTc
7	8	Add 250 uL of 200 ng/mL aTc to 250 ul diluted culture to make 100 ng/mL aTc
8	7	Add 125 uL of 200 ng/mL aTc to 250 ul diluted culture to make 50 ng/mL aTc
9	6	Add 50 uL of 200 ng/mL aTc to 250 ul diluted culture to make 20 ng/mL aTc
10	5	Add 25 uL of 200 ng/mL aTc to 250 ul diluted culture to make 10 ng/mL aTc
11	4	Add 12.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 5 ng/mL aTc
12	3	Add 5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 2 ng/mL aTc
13	2	Add 2.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 1 ng/mL aTc
14	1	Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc

We used Ethan's filtered aTC

Make 100mM Arabinose

- ☒ 0.15g arabinose
- ☒ 1mL Low Chlor M9 Glycerol

Make 1mM Arabinose

- ☒ 10uL 100mM arabinose
- ☒ 990uL Low Chlor M9 Glycerol

- ☒ Add 250uL culture to each tube

Table1

	A	B
1	1	Add 0uL to 250uL culture to make 0uM arabinose
2	2	Add 2.5 uL 1mM arabinose to 250uL culture to make 10uM
3	3	Add 2.5 uL 100mM arabinose to 250uL culture to make 1mM
4	4	Add 12.5 uL 100mM arabinose to 250uL culture to make 5mM
5	5	Add 25uL 100mM arabinose to 250uL culture to make 10mM
6	6	Add 50uL 100mM arabinose to 250uL culture to make 20mM

We only did a 6-step induction because the number of samples makes higher-step inductions impractical, and we are only doing this to get a diagnostic of whether a given WM16_029 replicate is even able to induce at all.

Make 1 mM IPTG:

- ☒ 10uL stock IPTG (100mM)
- ☒ 990 uL apropriate media (Low Chlor M9 Glyceorl or Low Chlor Low Kan M9 Glycerol)

Table3

	A	B
1	1	Add 0uL to 250uL culture to make 0uM IPTG
2	2	Add 0.5uL 1mM IPTG to 250uL culture to make 2uM
3	3	Add 1.25uL 1mM IPTG to 250uL culture to make 5uM
4	4	Add 2.5uL 1mM IPTG to 250uL culture to make 10uM
5	5	Add 5uL 1mM IPTG to 250uL culture to make 20uM
6	6	Add 12.5uL 1mM IPTG to 250uL culture to make 50uM
7	7	Add 25uL 1mM IPTG to 250uL culture to make 100uM
8	8	Add 50uL 1mM IPTG to 250uL culture to make 200uM
9	9	Add 1.25uL 100mM IPTG to 250uL culture to make 500uM
10	10	Add 2.5uL 100mM IPTG to 250uL culture to make 1mM
11	11	Add 5uL 100mM IPTG to 250uL culture to make 2mM
12	12	Add 12.5uL 100mM IPTG to 250uL culture to make 5mM
13	13	Add 25uL 100mM IPTG to 250uL culture to make 10mM
14	14	Add 50 uL 100 mM IPTG to 250 uL culture to make 20 mM

We used the Ready-to-Use IPTG

This first batch of induction went into the shaker at 1:00 PM.

> *Note that I had intended for 14 + 16 to be induced both in tubes and in-plate to further support / refute the currently-held notion that tube induction works while plate induction doesn't (our only test has been on 52S OA + pACT-Tet OA LG3.300). However the inoculations were done with only enough for one 14-step induction and I did not correspondingly half the volumes of the induction condition to account for this... hence we will have to wait for replicates #2 and #3 to grow to midlog to do this.*

Callan is making Glycerol Stocks of the cultures which we induced this morning.

At 1:30 PM (15 hours after inoculation) the miniprep cultures have not grown well... one replicate has present but non-turbid growth and the other two replicates look quite clear. This is unfortunate because WM16_025 is a critical part... if they haven't grown yet in a few hours we will miniprep the good replicate, colony PCR more colonies from the plate, and inoculate them tonight for miniprep and sending to Macrogen tomorrow.

Out @ 1:50 PM
In @ 2:45 PM

Setting up miniprep of the one replicate of WM16_025 which grew.

Yield was very poor (purity was fine)... ~30ng/uL even after taking the elution and re-running it through the column a second time. Culprit factors include not warming the EB and the poor growth of the inoculant.

We are doing more Minipreps tomorrow so I am not *too* concerned.

I made a glycerol stock of this miniprep.

One replicate each of 110 10 beta and 110 + pJD100 10 beta grew, and the other two replicates of 14 + 16 seem to be growing as well. These are probably ready for induction within the hour.

Callan and I induced the following, using the same induction conditions as above (still using ready-to-use IPTG, for example):

- B.1,2
- F.3
- G.1
- H.2,3
- I.1
- J.1
- K.2,3
- L.2,3

but where J .1 (14 + 16) was done in both tubes and in Plate with 125 uL Volume in each.

Plate setup was: D1-12, E1-2 in induction order #1 - 14.

```
Use Lid
Set Temperature 37C
Start Kinetic (8 hours, 10 minute interval)
    Shake: Double Orbital (Continuously)
    Read Absorbance 600 (OD600)
    Read Fluorescence 485/510 (sfGFP)
End Kinetic
```

This covers induction of all of the samples we wanted to induce.

Inductions went in at 5:00 PM

We were only able tomake glycerol stocks of B.1 because the rest ran out of volume during the induction... we will have to make glycerols of the uninduced sample if we want them later.

I set up Colony PCRs of WM16_025 1C3 5 alpha colonies from 151008 transformation plate to try and get more inoculants to grow to successfully miniprep tomorrow. I did:

1 colony in 10 uL NFW
Master Mix: 50 uL Q5, 5 uL P008, 5 uL P009, 30 uL NFW
8 reactions from 8 colonies
66C annealing, 1:00 extension

Out @ 5:20 PM
In @ 7:45 PM

Talking to Marissa from Broad Run iGEM about some advice they requested about outreach and math modeling:

- They used some of the 2015 iGEM Outreach Booklet activities in their Outreach and wanted us to mention this fact on our wiki when we talk on the Collaboration page about our relationship with them. I said we would.

- They offered to talk to the teachers / admins in charge of their Independent Biology Research class that they have there to try and incorporate LearnSynBio into the class once it's available. I said we would appreciate it.
- They wanted to ask for help with implementing the equation they'd made for their model into MATLAB. I said I would help them.

Reading the Synthetic Enhancer paper again to try and find clues about WHY this isn't working

- **Orientation of binding sites** (ie. being alternating) is explicitly mentioned in the paper but I don't recall if we have confirmed orientation in the 52S / 55AS sequencing data... checking that out
  - I determined the original orientations of tetO1 and tetO2 from the source paper Hillen & Berens 1994: "Mechanisms Underlying Expression of TN10 Encoded Tetracycline Resistance". I determined that:

Table5

	A	B
1	Site	Sequence (5' -> 3')
2	tetO1	actctatcattgatagagt
3	tetO2	tccctatcagtgatagaga

Note that the labels are swapped from what Orna Atar labeled.
Also, these are near-symmetric.

and that this implies that 52S, according to our 160722 sequence information, contains the orientation:

[-WM16_P042->] <- tetO2 - <- tetO1 - <- tetO2 -

and that, according to the Orna Atar sequences, 62S and 82S both contain the orientation:

-tetO2-> -tetO1-> -tetO2-> -tetO2-> -tetO1-> -tetO2->

which, given that the Orna Atar sequences are 3' <- 5', fits the assumption that the six-repeat cassettes were created from two copies of the three-copy cassette, which *is* 52S.

Furthermore, though it's hard to be 100% confident, the paper seems to suggest that -tetO1-> and -tetO2-> yield binding on opposite 'sides' of the DNA *(Fig. 1 legend's claim that the genes have "Divergent Polarities")*, which implies that 52S does indeed meet Amit's claim that the orientation should overlap between adjacent operator sites for proper enhancer functionality.

  - In addition, according to 160722 sequence, the 55AS plasmid does not contain *either* tetO1 or tetO2 by low-alignment BLAST under the same parameters which I used on 52S...

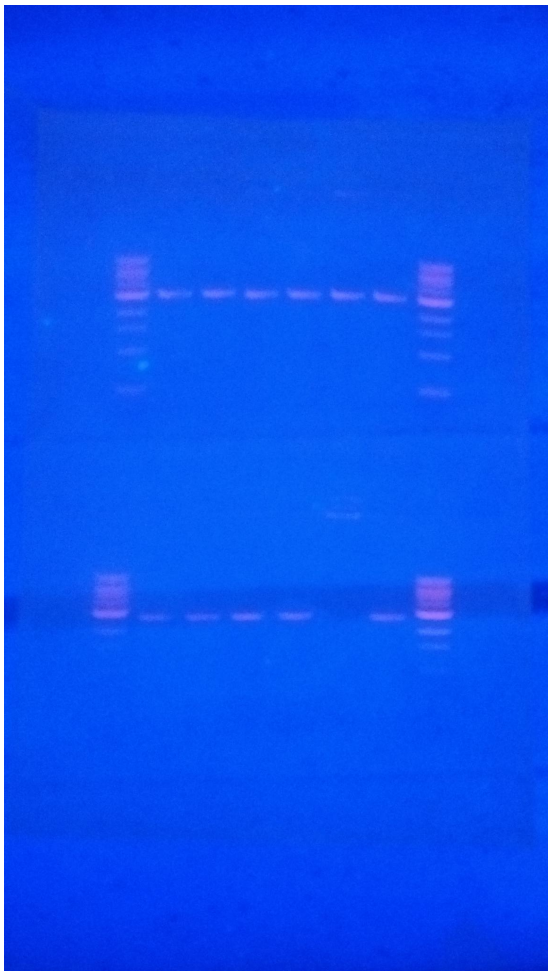
- Paper explicitly lists **binding affinities** of tetO2 and tetO1: 10 pM and 30-50 pM, respectively.
- Flx. was measured at **580/610** insted of 571/610 which we were using for mCherry
- **Abx. concentrations** used for pACT-Tet with Synthetic Enhancer were 20 ug/mL Kan and 100 ug/mL Amp
  - Our stock Kan is 10 mg/mL and stock Amp is 100 mg/mL.
    - *(is our stock Kan really 10 mg/mL? This is what is given by the iGEM 2015 Important Protocols file but I am not confident that people have been following this).*

So I have convinced myself that the part 52S, barring mutation, is not the error, and that the one staircase we observed in the past is probably legitimate. I think 55AS is broken.

I set up Colony PCRs of the 161007 transformations of pACT-Tet OA + 52S OA into LG3.300 in order to follow Amit's protocol to the letter tomorrow.

Master Mix was 40 uL Q5; 4 uL P042 (P044); 4 uL P043 (P045); 24 uL NFW
Six colonies into 10 uL NFW
1:30 extension time, 64C (59C) annealing temp.

161010.jpg



The gel looked great!
Top: P044/P045 to detect pACT-Tet
Bottom: P042/P043 to detect 52S

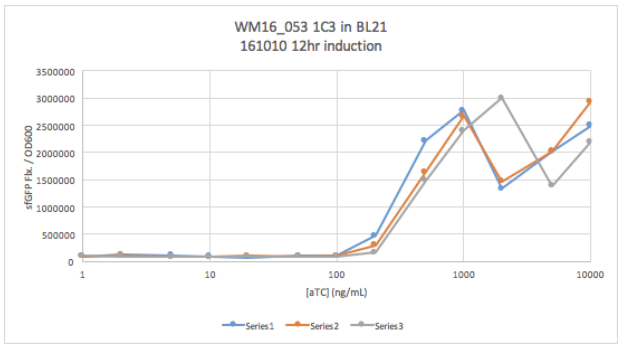
I inocualted colonies #1, 2, 3 into 4 mL LB with 20 ug/mL Kan and 100 ug/mL Amp as Amit specified in the paper.

Trivia: This is probably the shortest time that the colonies have sat in water before being inoculated, as I basically didn't wait at all between Colony PCR -> Gel -> Inoculation.

The kinetic run for the 14 1C3 + 16 3K3 BL21 induction finished. Got the data.

Loaded the morning inductions on a plate and measured those. Got some good induction-- this will make for good inputs into testing the Model!

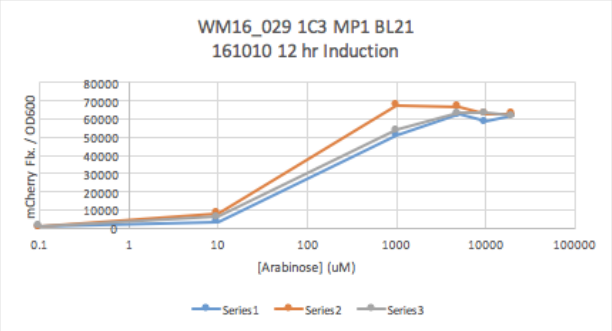
clipboard_2016-10-11_01:55:08.png



WM16_053 1C3 BL21 induced! Now we can compare this to the 85x tetO array...?

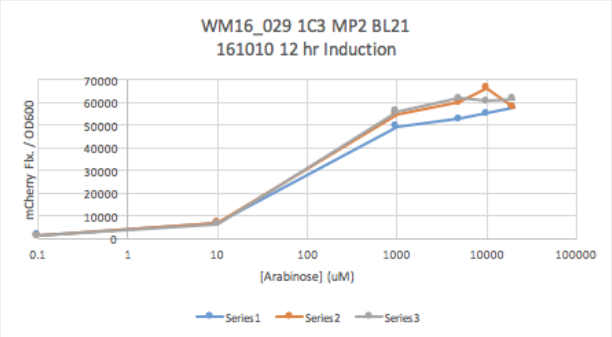
Also it looks like replicate 3 might have had an off-by-one error to ther replicates or something toward the end idk

clipboard_2016-10-11_02:00:06.png



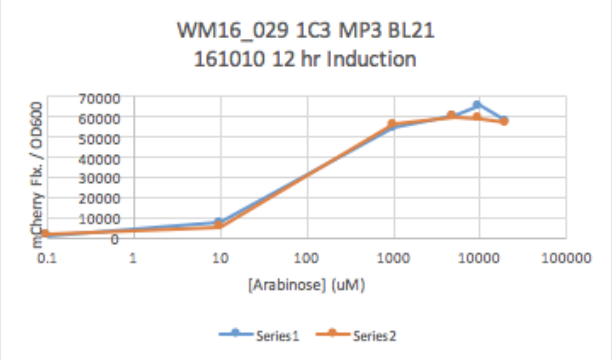
WM16_029 looks like it functions as well!
(The 0 point is set to 0.1 so it can be plotted)

clipboard_2016-10-11_02:01:31.png



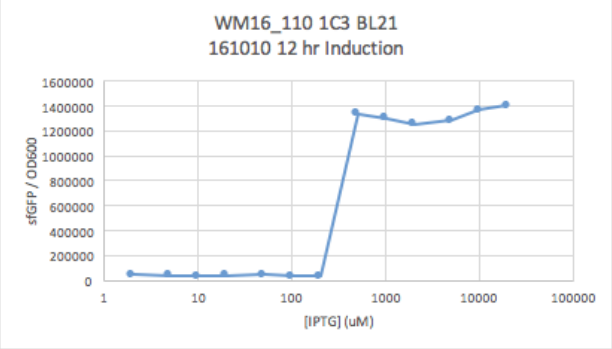
MP2 worked about the same

clipboard_2016-10-11_02:02:32.png



MP3 looks okay too, which is interesting considering that this is the miniprep which didn't look good in 5 alpha when we induced it earlier on

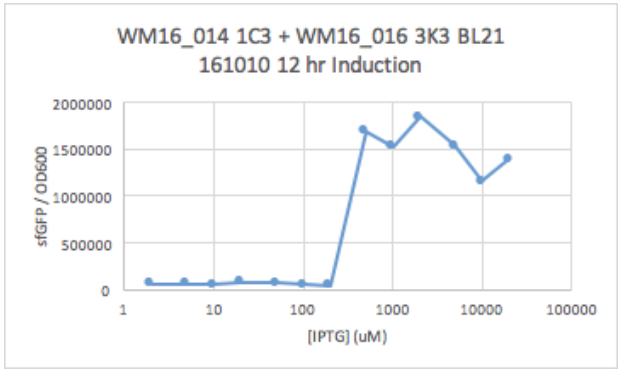
clipboard_2016-10-11_02:06:04.png



Wow I have not seen such a steep transition in a while!!

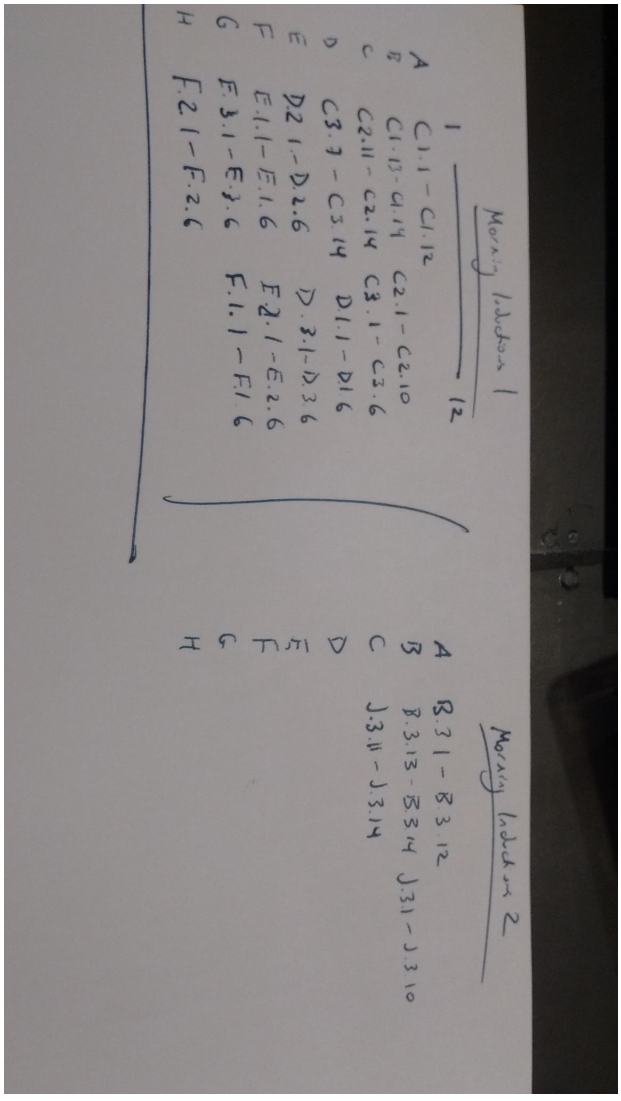
Too bad our 110 + pJD100 construct is in 10 beta... but that's coming up in the afternoon induction

clipboard_2016-10-11_02:09:05.png



Why is the high-concentration region so noisy?

161010_morning_key.jpg



This is the plate layout by the way.

I looked at the Afternoon Inductions (it has been 9 hours for them) but many looked like they still needed more time to grow to get to turbidity... will wait overnight for these ones.

Out @ 2:20 AM

161012 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-10-12

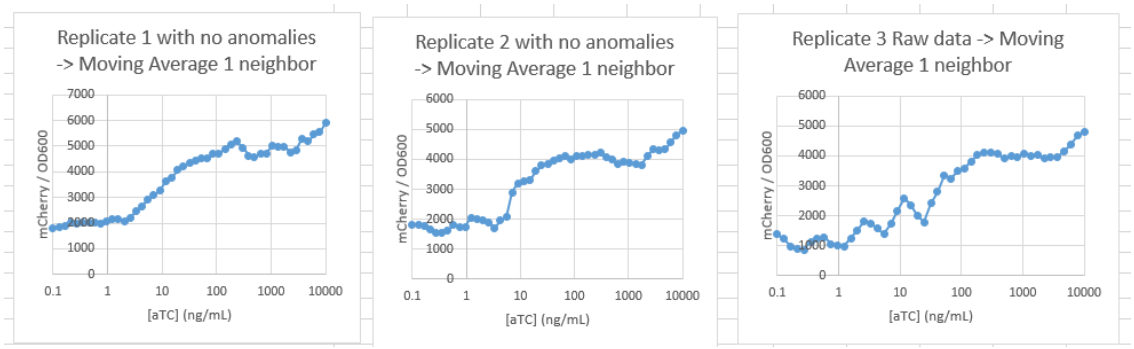
WEDNESDAY, 10/12

In @ 12:30 PM

Adam set up the 52S OA + pACT-Tet OA LG3.300 inductions in the plates. They had been inducing for 20 hours at this point. I measured them, and put the cultures back in the shaker (37C 250 rpm) just in case they stil hadn't reached "saturation of growth" yet... the cultures looked well-grown but not insanely turbid.

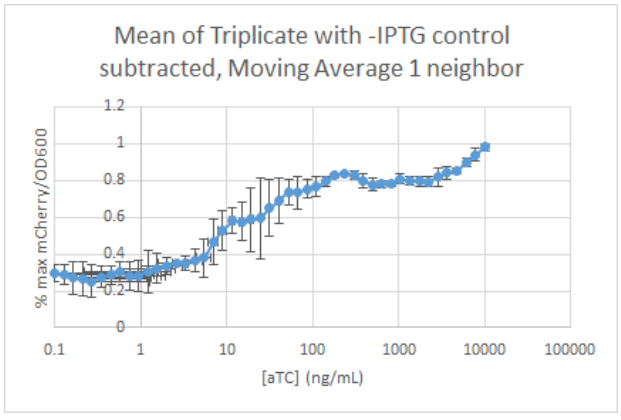
I measured at 580/610 as Amit did for mCherry.

clipboard_2016-10-12_14:53:33.png



Replicate 2 looks pretty good.
Dropbox / iGEM 2016 / FACS Data / 161012 / 52S OA pACT-Tet OA 580 610.xlsx

clipboard_2016-10-12_15:18:30.png



There are 4 steps here, Low, Mid1, Mid2, and High, corresponding to 3 bound, 2 bound, 1 bound, and 0 bound, as expected.

The 2-bound configuration is less probable than the 1-bound configuration, it seems. Can we justify this with a mechanistic hand-wave?

Out @ 1:50 PM
In @ 6:30 PM

Planning out the rest of this week experimentally. Want to:

- Synthetic Enhancer... get an array-shifted staircase and a staircase from a modified version of 52S:
  - Transform into Lg3.300 *(today)*:
    - 52S + NRII 1K3 AND tetR 1A3
    - 52S sfGFP + NRII 3K3 AND tetR 1A3

                - 52S tetR sfGFP + NRII 3K3
                - 52S tetR sfGFP + NRII 3K3 AND 85x tetO 1C3
                - 52S OA Kan AND pACT-Tet OA Amp AND 85x tetO 1C3
            - Induce using the 161011 protocol *(Start overnight on Friday to induce on Saturday)*:
                - The above five transformations
                - 52S OA Kan AND pACT-Tet OA Amp LG3.300 from 161007 plate
    - 85x tetO array... get a better graph
        - Induce (16-step) *(tomorrow)*:
            - 53 1C3 + 85x tetO 1K3 BL21
            - 53 1A3 + 85x tetO 1C3 BL21
    - pBad TetR-mCherry: Get Functional Induction Characterization
        - After MP tomorrow, cotransform with pTet GFP (two different Abx combos possible)
    - RiboJ:
        - After sequence confirmation tomorrow night, cotransform 25 1C3 with 16 3K3 in BL21 to get induction curve
        - ALSO cotransform all other RiboJ parts with 16 (or grow up glycerol stocks of them) to get induction curves on plate reader to match with the 25 induction curve

Out @ 8:00 PM

161013 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-12

WEDNESDAY, 10/12

aTC Inductions for WM16_053 +/- 85x tetO in BL31.

- Do inductions for WM16_053 1A3 +/- 85x tetO 1C3 in a 96-well black plate reader plate. All three replicates for each should fit exactly.
- Do inductions for WM16_053 1C3 +/- 85x tetO 1K3 in tubes.

Make 20,000 ng/mL ATC:

- ☒ Add 10 uL 2 mg/mL aTC (Ethan stock)
- ☒ Add 990 uL M9 Glycerol (Low Amp Low Chlor // Low Chlor Low Kan)

Make 200 ng/mL ATC:

- ☒ Add 10 uL 20,000 ng/mL ATC from above
- ☒ Add 990 uL M9 Glycerol (Low Amp Low Chlor // Low Chlor Low Kan)

16-step aTC.xlsx

Last night LK did the transformations 20161012 LK

Popped in in the morning and excised a fungal growth from plate A.

In @ 3:30 PM

Inoculations of WM16_029 1A3 and WM16_023 1A3 (no UNS) were well-grown. LK is miniprepping.

Inoculations of WM16_053 1A3 +/- 85x tetO 1C3 were at near-midlog. Setting up inductions in plate... the WM16_053 1C3 +/- 85x tetO 1K3 inductions still had not grown yet.

Plate induction went in at 5:00 PM for a 7 hr kinetic measuring sfGFP at 485/510.

Plate setup was:

WM16_053 1A3 BL21 #1.1 - 16 WM16_053 1A3 BL21 #2.1 - 16 WM16_053 1A3 BL21 #3.1 -16
WM16_053 1A3 + 85x tetO 1C3 BL21 #1.1 - 16 WM16_053 1A3 + 85x tetO 1C3 BL21 #2.1-16 WM16_053 1A3 + 85x tetO 1C3 BL21 #3.1 - 16

Out @ 5:15 PM

At 10:00 PM the other cultures have some cells, it looks like, but definitely aren't at midlog yet... will give them more time since they'll need overnight aTC induction anyways.

Did some sleuthing back at old records because I was worried about how the only two transformation plates with no growth were pACT-Tet OA + 52S OA + 85x tetO 1C3 and 52S tetR NRII sFGFP 3K3 + 85x tetO 1C3, ie. the only two transformations that involved 85x tetO 1C3.

This time we used 85x tetO 1C3 MP1 (Box 8 Slot 55), but in the past when we got one colony to successfully transform of pACT-Tet OA + 52S OA + 85x tetO 1C3 in LG3.300 (161006-EMJ) we had used 85x tetO 1C3 MP2 (Box 8 Slot 56).

In light of this we are re-transforming those above two transformations with both MP2 and MP3 of 85x tetO 1C3. Additionally we are transforming 29 1A3 + pTet GFP 1C3 and pTet GFP 1A3 + 29 1C3 in BL21 to characterize the repressor functionality of pBad tetR-mCherry.

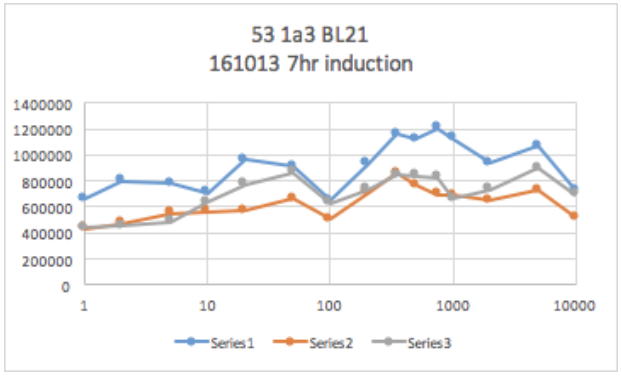
In @ 1:15 AM

The 53 1C3 + 85x tetO 1K3 colonies are at midlog now. I induced them using the same protocol as above, except into tubes instead of Plate.

Induction went in at 2:40 AM

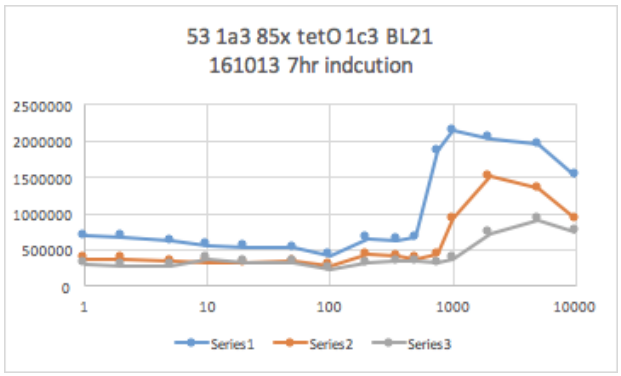
After 7 hr induction the 53 1A3 + 85x tetO 1C3 inductions didn't look too good so I put them back in the Kinetic run for another 7 hrs. Data is in Dropbox / iGEM 2016 / FACS Data / 161013 if you want to look at it

clipboard_2016-10-14_03:00:19.png



w/out array condition didn't induce too well...

clipboard_2016-10-14_03:00:22.png



w/ array kind of did

Out @ 3:00 AM
In @ 3:30 AM

Plating transformations after 3hr outgrowth. Did 50 uL for the 29 + 23 and 100 uL for the synthetic enhancers. Plates went into incubator at 3:30 AM.

Out @ 3:40 AM

161014 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

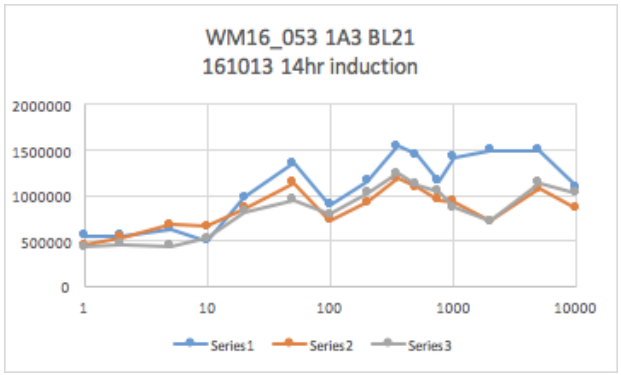
Date: 2016-10-14

FRIDAY, 10/14

In @ 11:30 AM

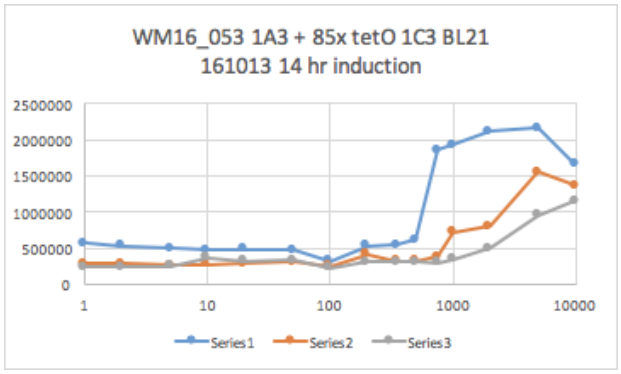
I extracted the data from the 14 hour 53 1A3 +/- 85x tetO 1C3 BL21 inductions. They looked better, but it still looks like the array is inducing a rightward shift:

clipboard_2016-10-14_11:43:03.png



This one is still quite noisy

clipboard_2016-10-14_11:43:06.png



Need to think more about this.

Out @ 11:50 AM

In @ 9:00 PM

Even if we can't pull off the Circuit Control Calculator, we *do* need to have parametrized models for each subsystem. Currently we only have this for the Decoy Binding Array. Math work left to do:

- Create a parametrized model for the Synthetic Enhancer (should follow fairly simply from the DBA kinetic model)
- Tune DBA model to fit observed data
- Tune Synthetic Enhancer model to fit observed data
- Tune RBS parameters to fit observed data

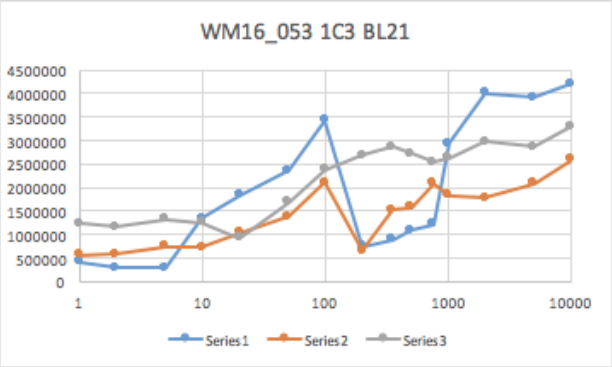
Ideally we would find that the biological parametrization choices we made (ie. 85x tetO vs. another number) lie on the functional end of a critical value, on the other side of which is something that wouldn't work. This way we would be able to support the utility of the models as something that future teams can use to both:

a. Tune their circuits in more precise ways
b. Assist in model-driven design for new Toolbox parts to add to the collection

Working on tuning DBA model to fit observed data now and altering parametrizations to see if we can cross critical thresholds to determine functionality.

Out @ 9:30 PM
In @ 11:30 PM

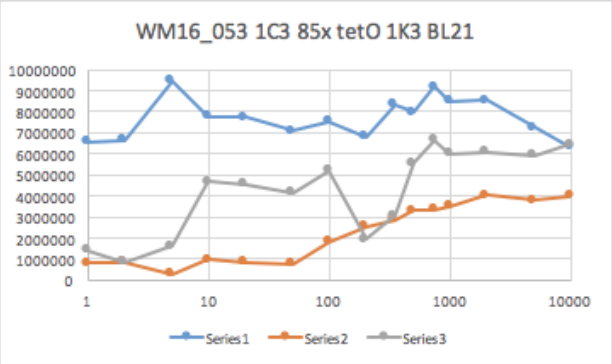
clipboard_2016-10-15_00:06:30.png



The middle zero-induction conditions are likely a result of the atc solution (being low volume at those points) either
(a) not existing because I forgot to add them
(b) they did not mix with the cell culture

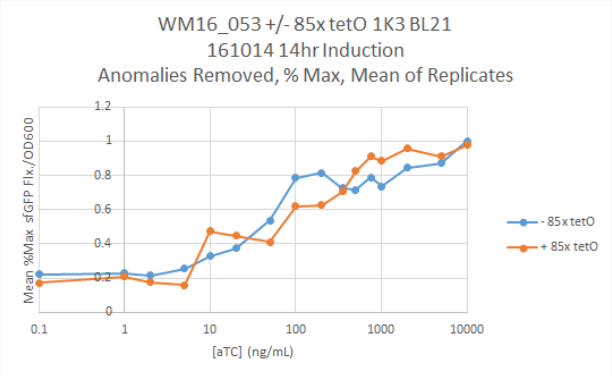
Hence they seem to be anomalous points

clipboard_2016-10-15_00:06:33.png



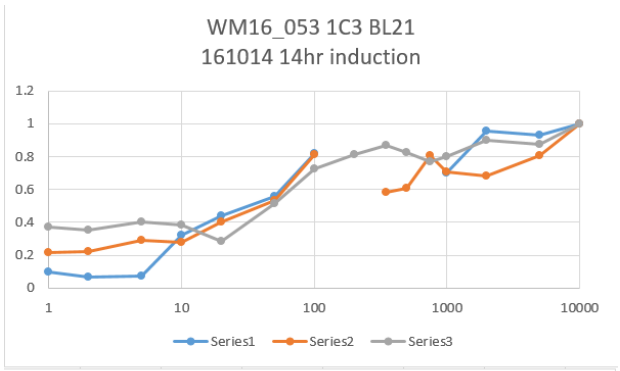
The blue line looks like it is maximally-induced always. The grey line looks like it exhibits the same issue as in the above graph.

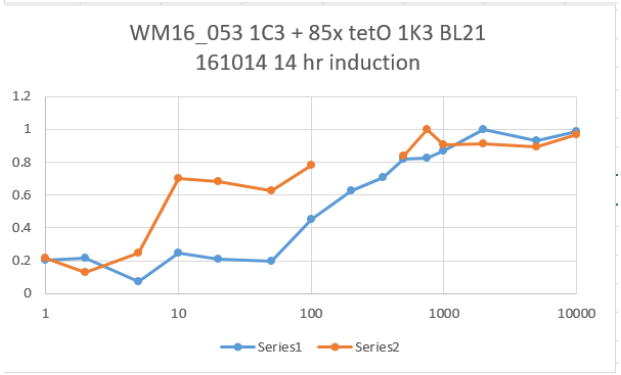
clipboard_2016-10-15_00:28:49.png



But nevermind.

clipboard_2016-10-15_00:30:45.png





If you look at individual replicates on % max, though, Replicate #2 of the +85x tetO 1K3 samples looks like it might have the leftward curve we'd expect to see.

Is this real?

Out @ 1:00 AM
In @ 1:45 AM

Basically none of the colony PCRs worked so Ethan, Kalen and I set up more with 8 colonies per plate.

Out @ 4:30 AM

161015 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-15

SATURDAY, 10/15

In @ 9:30 AM

Made gels to run Colony PCRs

Working on math as in 161014 JPM

Conversion from FLX Plate Reader -> [GFP] is

FLX_PlateReader = 1e11[GFP] - 683.02, where [GFP] is in Moles

so *[GFP] = (FLX_PlateReader + 683.02) / 1e11*

Does this Conversion Equation yield consistent [GFP] values across different experiments?

161010 Kinetic 14 1C3 + 16 3K3 BL21:

Table2

	A	B	C	D	E	F
1	[IPTG]	0	2	5	10	
2	sfGFP/OD600	191172.7749	191847.1986	200867.2131	244935.2014	354216.1
3						
4		191172.77	191847.2	200867.21	244935.2	35421
5	[GFP] / OD600	1.91856e-6	1.9253e-6	2.0155e-6	2.45618e-6	3.54
6	[GFP] / OD600 in nM	1918.557949	1925.302186	2015.502331	2456.182214	3548.99

161010 53 1C3 +/- 85x tetO addGene Amp (BL21):

Table3

	A	B	C	D	E	
1	[aTC]	0	1	2	5	
2	GFP/OD600	97377.21239	95532.69025	124618.5682	115231.3341	868
3		95454.91388	96156	112659.0361	85541.88759	810
4		92145.52606	103714.1463	93492.33912	85527.13987	829
5						
6	Average; no array	94992.55078	98467.6122	110256.6478	95433.45387	836
7						
8	array	57511.68		80142.48366	82354.75578	115
9						
10	no array	0.03634131	0.037670765	0.042180897	0.036509987	0.0
11	array	0.011884	0	0.016560345	0.017017481	0.0
12						
13	[GFP] in M / OD600 no array	9.56756e-7	9.91506e-7	1.1094e-6	9.61165e-7	8.
14	[GFP] in M / Od600 array	5.81947e-7		8.08255e-7	8.30378e-7	1.
15						
16	[GFP] in nM / OD600 no array	956.7557078	991.506322	1109.396678	961.1647387	843
17	[GFP] in nM / Od600 array	581.947	0	808.2550366	830.3777578	116

161014 53 1C3 +/- 85x tetO 1K3

Table1

	A	B	C	D	E	F	G
1	[GFP] in M / Od600						
2							
3	53 1C3			53 1C3 + 85x tetO 1K3			
4	1	2	3	1	2	3	
5							
6	2.85823e-6	6.49633e-6	1.14333e-5		6.13995e-6	1.29707e-5	
7	4.21701e-6	5.59677e-6	1.23149e-5		8.1963e-6	1.42201e-5	
8	2.89678e-6	5.83004e-6	1.16925e-5		8.7636e-6	8.77236e-6	
9	2.99017e-6	7.5415e-6	1.31921e-5		2.97377e-6	1.63646e-5	
10	1.34809e-5	7.25206e-6	1.26034e-5		9.956e-6	4.68557e-5	
11	1.84021e-5	1.0401e-5	9.27569e-6		8.57375e-6	4.55738e-5	
12	2.35199e-5	1.38456e-5	1.6843e-5		7.92471e-6	4.17371e-5	
13	3.43543e-5	2.10798e-5	2.38486e-5		1.83489e-5	5.21794e-5	
14			2.68255e-5		2.53762e-5		
15		1.51829e-5	2.85951e-5		2.86237e-5		
16		1.57667e-5	2.71365e-5		3.31021e-5	5.56304e-5	
17		2.09021e-5	2.53236e-5		3.34775e-5	6.66096e-5	
18	2.941e-5	1.83334e-5	2.63804e-5		3.51504e-5	6.02577e-5	
19	4.00719e-5	1.77307e-5	2.96839e-5		4.05306e-5	6.09326e-5	
20	3.91652e-5	2.09364e-5	2.87187e-5		3.78142e-5	5.93916e-5	
21	4.20076e-5	2.5915e-5	3.29202e-5		4.01339e-5	6.43164e-5	

It seems like [GFP] seems to range between 1e2 ~ 1e5 nM across indcutions with IPTG and aTC inductions these past few days.

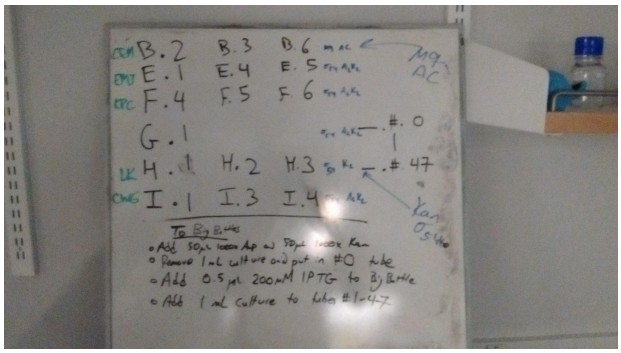
The Bio-Rad people wrote me back after looking at the FCS files. They want more information about the Drop Drive Amplitude during the high-SSC scatter acquisitions... looking into this.

Ethan, Kalen, and Likhitha set up 200 uL overnight culture of the respective colonies that worked on colony PCR (161015 KPC) into 20 mL LB in flasks. In ~12:30 PM.

Out @ 1:00 PM
In @ 2:00 PM

Me, Ethan, Likhitha, Kalen, and Callan are following the 161011 JPM protocol for Synthetic Enhancer induction on:

161015.jpg



Where the key is from 161014 KPC

using the following aTC induction table:

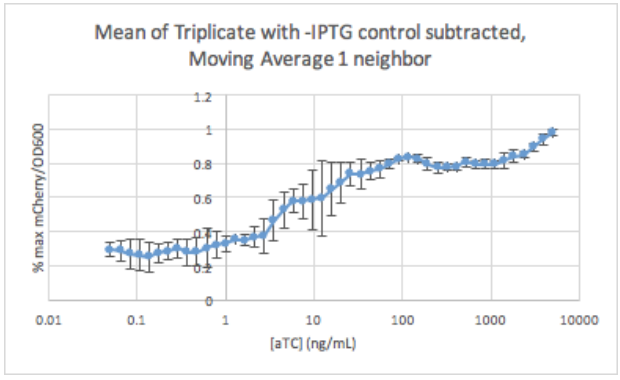
Table4

	A	B	C	D
1	**Number**	**Add __ uL of**	**___ ng/mL aTC**	**to 1 mL Sigma 54 Broth to get ___ ng/mL aTC**
2	**1**	1	200	0.2
3	**2**	1.284386264	200	0.256877253
4	**3**	1.649648074	200	0.329929615
5	**4**	2.118785326	200	0.423757065
6	**5**	2.721338768	200	0.544267754
7	**6**	3.495250133	200	0.699050027
8	**7**	4.489251258	200	0.897850252
9	**8**	5.76593265	200	1.15318653
10	**9**	7.405684692	200	1.481136938
11	**10**	9.511759691	200	1.902351938
12	**11**	12.21677349	200	2.443354698
13	**12**	15.69105606	200	3.138211211
14	**13**	20.15337686	200	4.030675372
15	**14**	25.8847204	200	5.176944081
16	**15**	33.24597932	200	6.649195865
17	**16**	42.70067916	200	8.540135832
18	**17**	54.84416576	200	10.96883315
19	**18**	70.44109314	200	14.08821863
20	**19**	90.47357242	200	18.09471448
21	**20**	1.162030136	20000	23.24060273
22	**21**	1.492495545	20000	29.8499109
23	**22**	1.916940777	20000	38.33881553
24	**23**	2.462092401	20000	49.24184803

25	**24**	3.16227766	20000	63.2455532
26	**25**	4.061585988	20000	81.23171977
27	**26**	5.216645252	20000	104.332905
28	**27**	6.700187504	20000	134.0037501
29	**28**	8.605628793	20000	172.1125759
30	**29**	11.05295141	20000	221.0590282
31	**30**	14.19625896	20000	283.9251793
32	**31**	18.23348001	20000	364.6696002
33	**32**	23.41883126	20000	468.3766252
34	**33**	30.07882518	20000	601.5765036
35	**34**	38.63282989	20000	772.6565977
36	**35**	49.61947603	20000	992.3895206
37	**36**	63.73057342	20000	1274.611468
38	**37**	81.85467307	20000	1637.093461
39	**38**	1.051330177	2000000	2102.660354
40	**39**	1.350314038	2000000	2700.628076
41	**40**	1.734324802	2000000	3468.649604
42	**41**	2.227542952	2000000	4455.085904
43	**42**	2.861025569	2000000	5722.051138
44	**43**	3.674661941	2000000	7349.323881
45	**44**	4.71968532	2000000	9439.37064
46	**45**	6.061898993	2000000	12123.79799
47	**46**	7.785819799	2000000	15571.6396
48	**47**	10	2000000	20000

I also found out that the previous synthetic enhancer induction had true [aTC] off by a factor of 2. I have now fixed this in 161011 JPM and in the Excel file for the Synthetic Enhancer graphs.

clipboard_2016-10-15_20:31:54.png



This is the graph from 161012 JPM but adjusted to have the correct [atc] concentration on the x axis.

Inductions went in periodically between 7:00 PM and 9:00 PM.

Colony PCRs + Inoculations: Instructions for Adam

We are going to re-do inductions on WM16_053 (functional tetR + pTet GFP) +/- 85x tetO because our previous results were extremely noisy but suggestive of an effective array (when individual replicates were examined).

There are four plates from 161011 that contain these transformations: they are

- 53 1A3 BL21 - D
- 53 1A3 + 85x tetO 1C3 BL21 - C
- 53 1C3 BL21 - F
- 53 1C3 + 85x tetO 1K3 BL21 - E

and they are in the Fridge on the top shelf.

You can pick four colonies for the solo transformations, but pick eight for the cotransformations.

All PCRs will use the primers VF2 and VR. Do 10 uL volume Colony PCRs *(recall that this is 5 uL Q5, 0.5 uL VF2, 0.5 uL VR, 3 uL NFW per reaction)* and remember to add at least 10% extra volume to the master mix.

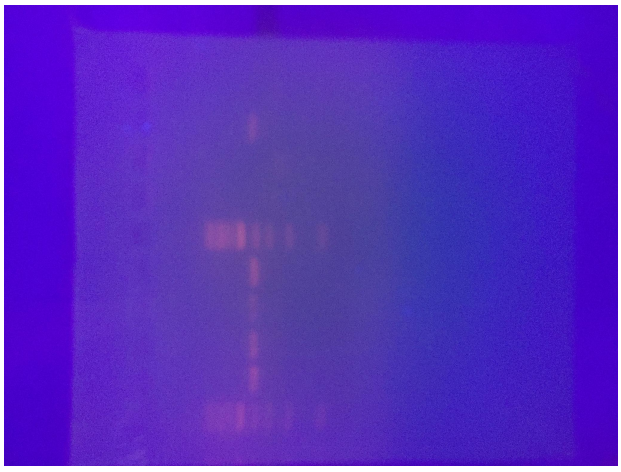
Annealing temperature for VF2 VR is 66C.
The extension time for the solo transformations is 1:00 as the insert for 53 will be ~2kb (remember that VF2 and VR will add a little extra to the insert on the part page)
The extension time for the cotransformations is 1:30 as the 85x tetO array will be ~3kb.

All gel materials are now in the back room with the shaker. Please leave the light off in the room as aTC inductions are occurring.

Pick three good solo transformation colonies to inoculate in M9 Glycerol with low-dosage antibiotic
Pick up to six good cotransformation colonies to inoculate in M9 Glycerol with low-dosage antibiotic

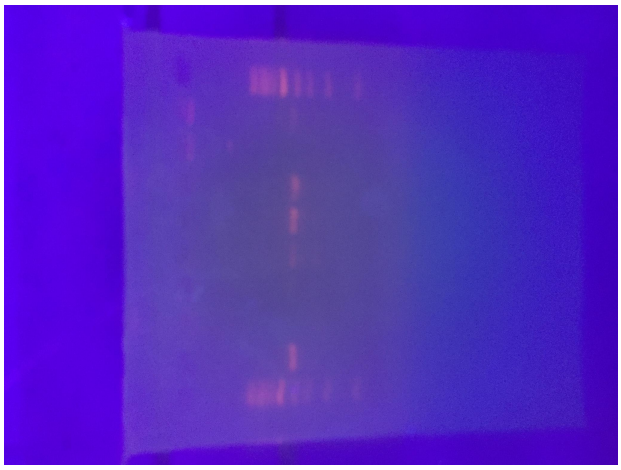
> If there is no room in the shaker you can go upstairs to the 3rd floor... the shaker by the elevator on the autoclave side should have space and is set to 37C 250 rpm.

IMG_8423.JPG



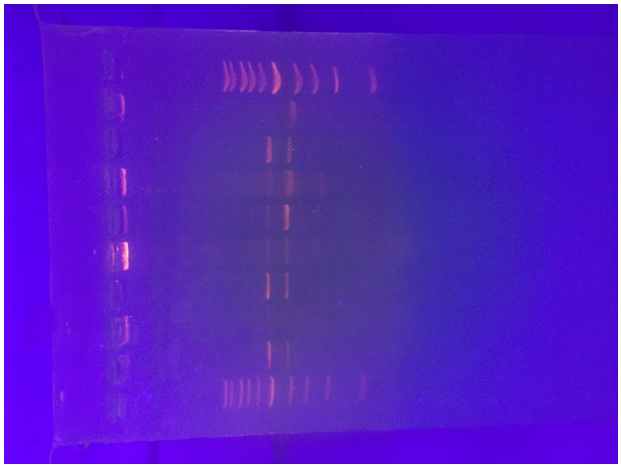
D1,2,4; F3

IMG_8424.JPG



E1,4,5,6

IMG_8425.JPG



C1,3,4,5,6,7

Please let me know when you put the inoculations in so I can estimate when they'll be at midlog for induction tomorrow.

Innoculations of above colonies are in at 0038 hours

Thanks!
John

Out @ 9:10 PM

161016 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

Date: 2016-10-16

SUNDAY, 10/16

In @ 2:30 PM

Callan, Kalen and I set up inductions for the inoculations of 53 (1C3 or 1A3) +/- 85x tetO (1K3 or 1C3) in BL21 that Adam inoculated last night (161015 JPM) in Tubes. Used 250 uL volume so we can consistently extract 125 uL for measurement tomorrow.

Table1

	A	B	C	D	E	F	G	H	I	J	K	L
1	16	Add 250 ul of 20000 ng/mL aTc to 250 ul diluted culture to make 10000 ng/mL aTc								Need __ uL solution for 1		
2	15	Add 125 ul of 20000 ng/mL aTc to 250 ul diluted culture to make 5000 ng/mL aTc								495		
3	14	Add 50 ul of 20000 ng/mL aTc to 250 ul diluted culture to make 2000 ng/mL aTc										
4	13	Add 25 ul of 20000 ng/mL aTc to 250 ul diluted culture to make 1000 ng/mL aTc								Need __ uL o solution for		
5	12	Add 18.75 ul of 20000 ng/mL aTC to 250 ul diluted culture to make 750 ng/mL aTC								470		
6	11	Add 12.5 ul of 20000 ng/mL aTc to 250 ul diluted culture to make 500 ng/mL aTc										
7	10	Add 8.75 ul of 20000 ng/mL aTC to 250 ul diluted culture to make 350 ng/mL aTC										
8	9	Add 5 ul of 200000 ng/mL aTc to 250 ul diluted culture to make 200 ng/mL aTc										
9	8	Add 250 ul of 200 ng/mL aTc to 250 ul diluted culture to make 100 ng/mL aTc										
10	7	Add 125 ul of 200 ng/mL aTc to 250 ul diluted culture to make 50 ng/mL aTc										
11	6	Add 50 ul of 200 ng/mL aTc to 250 ul diluted culture to make 20 ng/mL aTc										
12	5	Add 25 ul of 200 ng/mL aTc to 250 ul diluted culture to make 10 ng/mL aTc										
13	4	Add 12.5 ul of 200 ng/mL aTc to 250 ul diluted culture to make 5 ng/mL aTc										
14	3	Add 5 ul of 200 ng/mL aTc to 250 ul diluted culture to make 2 ng/mL aTc										
15	2	Add 2.5 ul of 200 ng/mL aTc to 250 ul diluted culture to make 1 ng/mL aTc										
16	1	Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc										

aTC inoculation conditions

These went in at 5:00 PM (all except ChlorKan #2,3,4; Amp #3; ChlorAmp #6) or 6:45 PM

Kalen, Ethan, and Likhitha prepped Plates with Synthetic Enhancer inductions. Inductions were 20 +/- 2 hours.

Recall that the key for the Synthetic Enahancers is given in 161014 KPC as

Table2

	A	B	C	D	E
1	Key	Part	Primers	Ta	Length
2	A1-8	029 1A3 + 023 1C3	8/9	64	029:2775 023: 1017
3	B1-8	029 1C3 + 023 1A3	8/9	64	029:2775 023: 1017
4	C1-8	029 1A3 + 023 3K3	8/9	64	029:2775 023: 1017
5	E1-8	52S (OA) Kan + pACT-Tet (OA) Amp	44/45	59	3kb
6	e1-8	52S (OA) Kan + pACT-Tet (OA) Amp	42/43	63	3kb
7	F1-8	UNS 52S + NRII (3K3) + TetR (Amp)	8/9	64	
8	G1	UNS 52S sfGFP NRII (3K3) + TetR (Amp)	8/9	64	
9	H1-8	UNS 52S tetR NRII sfGFP (3K3)	8/9	64	
10	I1-8	52S (OA) Kan + pACT-Tet (OA) Amp	44/45	59	3kb
11	i1-8	52S (OA) Kan + pACT-Tet (OA) Amp	42/43	63	3kb

Out @ 8:20 PM

161017 JPM

Made with Benchling

Project: iGEM 2016

Authors: John Marken

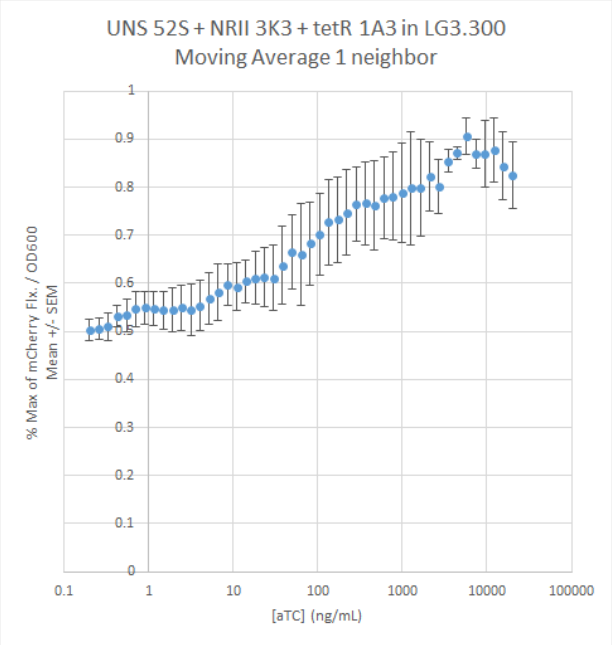
Date: 2016-10-17

MONDAY, 10/17

In @ 1:00 PM

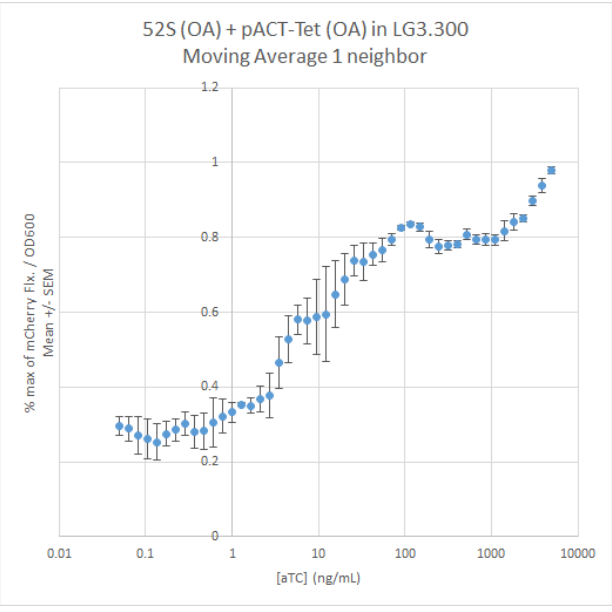
Analyzed the UNS 52S+NRII 3K3 and tetR 1A3 in LG3.300 data from yesterday. Turns out when you apply the same data trasnformations we applied to the original 52S OA + pACT-Tet OA characterization, you get:

clipboard_2016-10-17_21:53:10.png



Noisy and doesn't go low but is there!!

clipboard_2016-10-17_21:52:34.png



Originial construct characterization

This data is in Dropbox/iGEM 2016/FACS Data/161016 Synthetic Enhancers/ Plate 9

Out @ 3: 15 PM

Adam made graphs from the 53 +/- 85x tetO data but they either didn't shift or shifted to the right. Oh well.

In @ 9:00 PM

Writing up advice to Braod Run iGEM on their parameter optimization problem

Andy pointed out that my SEM calculations on the above graphs are erroneously calculated with a divisor of sqrt(47) instaed of sqrt(3)... re-doing those. Fixed and correct charts are included in the top of this benchling.

Compiling all relevant excel sheets graph data into a new folder 000 Final Data in Dropbox/iGEM 2016/FACS Data

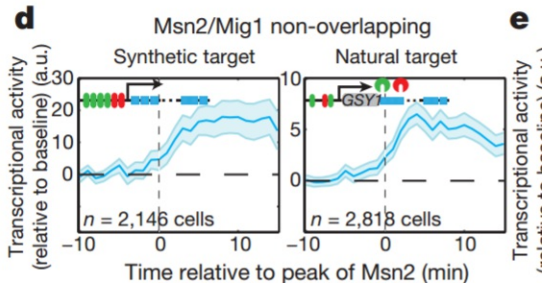
Writing up outlines for Wiki Content portions with general framework of argument, papers to reference, location of graphs to post, further instructions to do...

The idea is to crowdsource the Wiki writing by having me come up with the outlines and instructions and having team members flesh them out to submission quality.

Completed Ribozymes, RBS Tuning, and Interlab

Andy and I are working on determing the best way to visualize the synthetic enhancer data-- the UNS 52S 3-replicate mean-across replicates looks best, but right now the error bars are so stark and contrasted with the rest of the plot that it distracts from the important message (the mean has a staircase induction!). Right now Andy and I are thinking of making something like this:

clipboard_2016-10-18_02:27:02.png



where the shaded region would represent SEM.

Out @ 2:15 AM

161018 JPM

Made with Benchling
Project: iGEM 2016
Authors: John Marken
Date: 2016-10-18

TUESDAY, 10/18

In @ 9:35 AM

Andy made Interlab plots which look quite good in the plotting package Seaborne. Despite its quality the activation barrier looks fairly high for learning how to use it, so we may have to default to Excel graphs if Andy can't pull it off for every plot before the Freeze.

Making more Wiki Content outlines:

- Collaborations
- Education and Public Engagement
- Medal Requirements

Made master Wiki spreadsheet to track progress and completion of each content page

Out @ 12:15 PM
In @ 2:00 PM