Thomas iGEM2013

From Dueber Lab Wiki

Contents

5/31/13

PCRing GT genes from cDNA

Today we PCR'd 5 glucosyltransferase genes off the Arabidopsis cDNA following the following protocol that the dueberlab website constructed:

Oligos

BS48.oIGEMXIII001	GG3_At_GT_AT4G15550_PCR_1_1
BS49.oIGEMXIII002	GG3_At_GT_AT4G15550_PCR_1_2
BS50.oIGEMXIII003	GG3_At_GT_AT4G15550_PCR_2_1
BS51.oIGEMXIII004	GG3_At_GT_AT4G15550_PCR_2_2
BS52.oIGEMXIII005	GG3_At_GT_AT4G15550_PCR_3_1
BS53.oIGEMXIII006	GG3_At_GT_AT4G15550_PCR_3_2
BS54.oIGEMXIII007	GG3_At_GT__AT1G05680_Anneal_1_1
BS55.oIGEMXIII008	GG3_At_GT__AT1G05680_Anneal_1_2
BS56.oIGEMXIII009	GG3_At_GT__AT1G05680_Anneal_1_3
BS57.oIGEMXIII010	GG3_At_GT__AT1G05680_Anneal_1_4
BS58.oIGEMXIII011	GG3_At_GT__AT1G05680_Anneal_1_5
BS59.oIGEMXIII012	GG3_At_GT__AT1G05680_Anneal_1_6
BS60.oIGEMXIII013	GG3_At_GT__AT1G05680_PCR_1_1
BS61.oIGEMXIII014	GG3_At_GT__AT1G05680_PCR_1_2
BS62.oIGEMXIII015	GG3_At_GT__AT1G05680_PCR_2_1
BS63.oIGEMXIII016	GG3_At_GT__AT1G05680_PCR_2_2
BS64.oIGEMXIII017	GG3_At_GT__AT1G05530_PCR_1_1
BS65.oIGEMXIII018	GG3_At_GT__AT1G05530_PCR_1_2
BS66.oIGEMXIII019	GG3_At_GT_At1g05560_PCR_1_1
BS67.oIGEMXIII020	GG3_At_GT_At1g05560_PCR_1_2
BS68.oIGEMXIII021	GG3_At_GT_AT2G31750_PCR_1_1
BS69.oIGEMXIII022	GG3_At_GT_AT2G31750_PCR_1_2
BS70.oIGEMXIII023	GG3_At_GT_AT2G31750_PCR_2_1
BS71.oIGEMXIII024	GG3_At_GT_AT2G31750_PCR_2_2

Templates

Arabidopsis
pGG001

PCRs

pcr01: BS48/BS49 on Arabidopsis (336bp)
pcr02: BS50/BS51 on Arabidopsis (652bp)
pcr03: BS52/BS53 on Arabidopsis (531bp)
pcr04: BS60/BS61 on Arabidopsis (551bp)
pcr05: BS62/BS63 on Arabidopsis (750bp)
pcr06: BS64/BS65 on Arabidopsis (1410bp)
pcr07: BS66/BS67 on Arabidopsis (1452bp)
pcr08: BS68/BS69 on Arabidopsis (683bp)
pcr09: BS70/BS71 on Arabidopsis (756bp)

Oligo assemblies

anneal01: BS54, BS55, BS56, BS57, BS58, BS59

Golden Gate assembl

⚠ pIGEM13_001 (CamR): pGG001, pcr01, using BsmBI
pIGEM13_002 (CamR): pGG001, pcr04, pc using BsmBI
⚠ pIGEM13_003 (CamR): pGG001, pcr06 u
⚠ pIGEM13_004 (CamR): pGG001, pcr07 u
⚠ pIGEM13_005 (CamR): pGG001, pcr08, BsmBI

.

We also performed an oligo assembly for some of the genes.

To do: construct the parts using GG

6/4/13

This morning we learned about the fold-it program and talked about strategies for rationally designing the GLU enzyme.

Roy and I tried to use foldit in the afternoon and it didn't seem to work, crashing every time we tried to load the PDB structure. I saw on an online forum that deleting the every line in the PDB file that did not start with "ATOM" could help, however I tried this to no avail.

Did a two PCRs to make:

pIGEM13_016	p15A_Kan_sfGFP	Circular		KanR	gcatggtctccacagctgcaggcggc cgcgcgcGagcggagtgtatactggc	2773	Designed
pIGEM13_015	p15A_Amp_sfGFP	Circular		AmpR	gcatggtctccacagctgcaggcggc cgcgcgcGagcggagtgtatactggc	2868	Designed
pIGEM13_014	pTET_strongRBS	2	ColE1	CamR	TCGGTCTCCTCTAGAgatccgtttcc atttaggtgggtacgttggagccgca	2644	Designed

following these protocols:

PCRs labeled 1,2,3,4,5 , T N R

Panels | Table Show/Hide all sequences.

Oligos

BT31.oIGEMXIII047_R_1_1	GGCircular_p15A_Amp_sfGFP_PC
BT32.oIGEMXIII048_R_1_2	GGCircular_p15A_Amp_sfGFP_PC
BT33.oIGEMXIII049_R_2_1	GGCircular_p15A_Amp_sfGFP_PC
BT34.oIGEMXIII050_R_2_2	GGCircular_p15A_Amp_sfGFP_PC
BT35.oIGEMXIII051_R_1_1	GGCircular_p15A_Kan_sfGFP_PC
BT36.oIGEMXIII052_R_2_2	GGCircular_p15A_Kan_sfGFP_PC
BT37.oIGEMXIII053_R_3_1	GGCircular_p15A_Kan_sfGFP_PC
BT38.oIGEMXIII054_R_3_2	GGCircular_p15A_Kan_sfGFP_PC

Templates

pGG096
pWCD0502
pZNR0418

PCRs

pcr01: BT31/BT32 on pZNR0418 (2051bp)
pcr02: BT33/BT34 on pWCD0502 (869bp)
pcr03: BT35/BT32 on pZNR0418 (967bp)
pcr04: BT33/BT36 on pWCD0502 (869bp)
pcr05: BT37/BT38 on pGG096 (1015bp)

Golden Gate assembl

pIGEM13_015 (AmpR): pcr01, pcr02 using E
pIGEM13_016 (KanR): pcr03, pcr04, pcr05 u

and

PCR labeled pTet

Oligos

BT12.oIGEMXIII038	GGCustom_type_2a_(custom_overhangs_tcta_and_tagc)_pTet_PCR_1_1
BT30.oIGEMXIII_046	GG2_pTET_strongRBS_PCR_1_2

Templates

pGG001
pZNR0448

PCRs

pcr01: BT12/BT30 on pZNR0448 (1024bp)

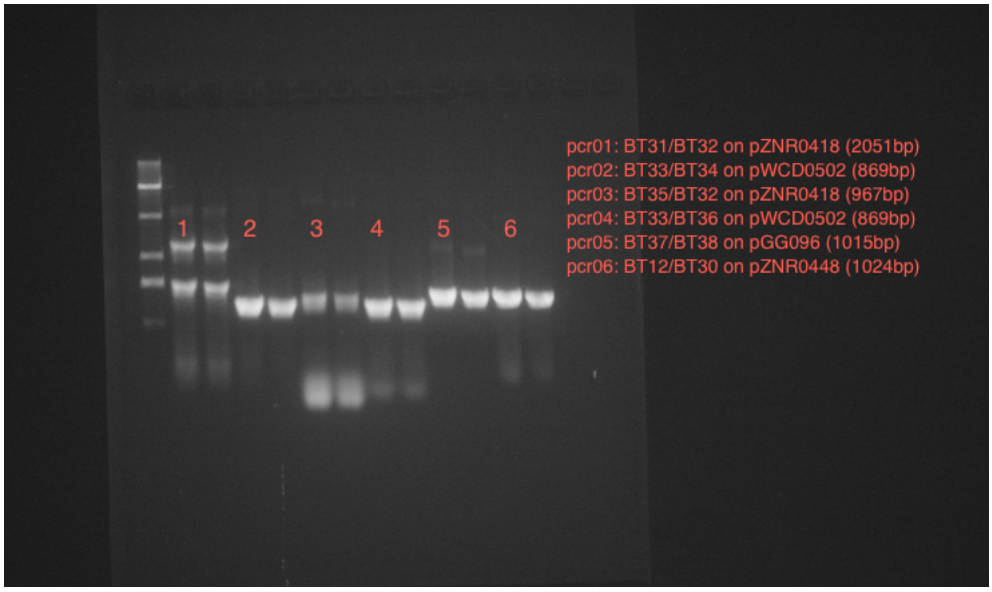
Golden Gate assembli

⚠ pIGEM13_014 (CamR): pGG001, pcr01 us

6/5/13

Prepared pIG002 & pIG013 for sequencing.

Gel extraction of PCRs 1,2,3,4,5,pTet



BsmB1 part assembly of pIG_14,15,16

Followed the instructions found above loaded these onto the thermocycler at 3PM

Designed Cassette Assemblies

Designed cassettes for pIGEM 38-45

Will follow these instructions to build them:

Templates

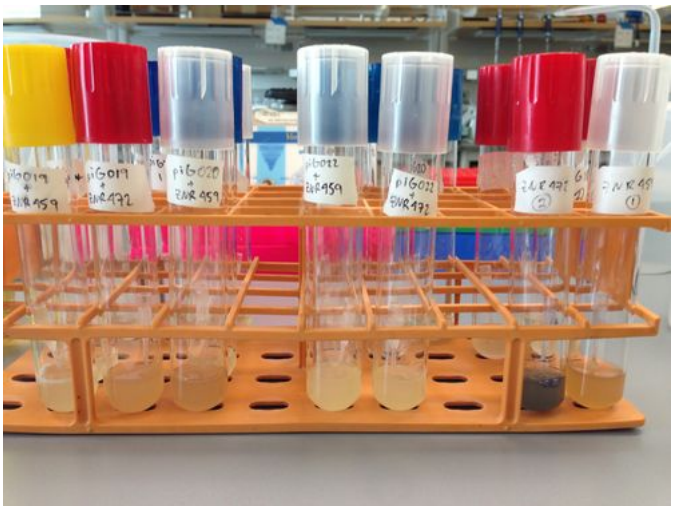
pGG005
pGG006
pGG062
pGG072
pGG078
pGG083
pIGEM13_001
pIGEM13_002
pIGEM13_003
pIGEM13_005
pIGEM13_006
pIGEM13_014
pIGEM13_015
pIGEM13_016
pZNR0423

Golden Gate assemblies

pIGEM13_038 (KanR): pGG006, pIGEM13_014, pIGEM13_001, pGG062, pGG078, pGG083 using BsaI
pIGEM13_039 (KanR): pGG006, pIGEM13_014, pIGEM13_002, pGG062, pGG078, pGG083 using BsaI
pIGEM13_040 (KanR): pGG006, pIGEM13_014, pIGEM13_003, pGG062, pGG078, pGG083 using BsaI
pIGEM13_041 (KanR): pGG006, pIGEM13_014, pIGEM13_005, pGG062, pGG078, pGG083 using BsaI
pIGEM13_042
pIGEM13_043 (AmpR): pGG005, pIGEM13_014, pZNR0423, pGG062, pGG072, pIGEM13_015 using BsaI
pIGEM13_044 (KanR): pGG005, pIGEM13_006, pZNR0423, pGG062, pGG072, pIGEM13_016 using BsaI
pIGEM13_045 (AmpR): pGG005, pIGEM13_006, pZNR0423, pGG062, pGG072, pIGEM13_015 using BsaI

6/6/13

Results from growing the cultures up:



Today Bernie and I ran experiments to check to see if the GT's were actually working by growing the GTs and FMOs under different conditions. We followed the setup found on this spreadsheet:

	A	B	C	D	E	F	G
1	Induced = A	500micromolar Arabinose	**Culture Code**	**Co-Transformant Name**	**A (Induced)**		**C (Repressed)**
2	Uninduced = B		19.459.L.(A,C)	piG019+ZNR459(LW)			
3	Repressed = C	0.2% glucose	19.459.H.(A,C)	piG019+ZNR459(HW)			
4			19.472.L.(A,C)	piG019+ZNR472(LW)			
5	Low Triptophan (LW)	0.37mM Trp	19.472.H.(A,C)	piG019+ZNR472(HW)			
6	High Triptophan (HW)	3.7mM Trp	20.459.L.(A,C)	piG020+ZNR459(LW)			
7			20.459.H.(A,C)	piG020+ZNR459(HW)			
8							
9				**Co-Transformant Name**			
10			22.459.L	piG022+ZNR459(LW)			
11			22.459.H	piG022+ZNR459(HW)			
12			22.472.L	piG022+ZNR472(LW)			
13			22.472.H	piG022+ZNR472(HW)			
14							
15				**Control Plasmids**			
16			19.L	piG019			
17			19.H	piG019			
18			20.L	piG020			
19			20.H	piG020			
20			22.L	piG022			
21			22.H	piG022			
22			459.L	ZNR459			
23			459.H	ZNR459			
24			472.L	ZNR472			
25			472.H	ZNR472			

We decided to repress using .2% glucose because we found that quantity was used in previous papers such as:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC177145/pdf/1774121.pdf

Indican extraction and quantification

Links:

A new HPLC-ELSD method to quantify indican in Polygonum tinctorium L. and to evaluate beta-glucosidase hydrolysis of indican for indigo production. - http://onlinelibrary.wiley.com/store/10.1021/bp0300218/asset/300218_ftp.pdf?v=1&t=hhml2osx&s=fcca5f00e37cf26dfdd659d3780b54f67e7c7ed7

Excerpt: Plant material was extracted in deionized water, and indican was identified and quantified using high performance liquid chromatography (HPLC) coupled to an evaporative light scattering detector (ELSD).

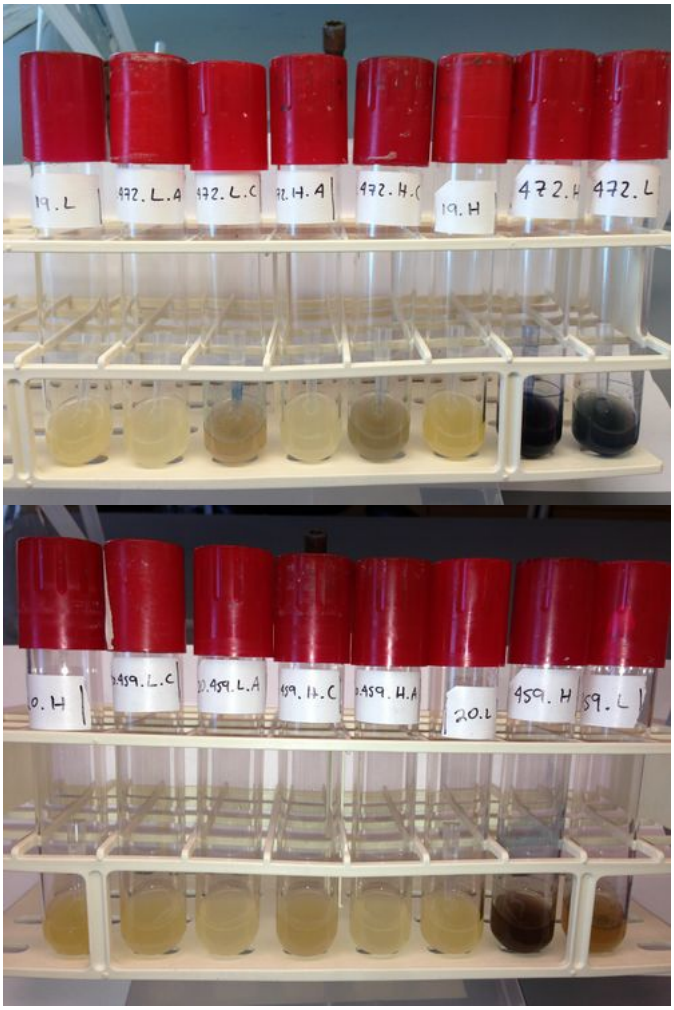
Qualitative Analysis of Indigo Precursors from Woad by HPLC and HPLC-MS - http://onlinelibrary.wiley.com/store/10.1002/(SICI)1099-1565(200001/02)11:1%3C18::AID-PCA483%3E3.0.CO;2-X/asset/483_ftp.pdf?v=1&t=hhmkqds2&s=0d044eec64877d028c07833b64db6a95535cc53a

Tissue and intracellular localization of indican and the purification and characterization of indican synthase from indigo plants.: http://www.ncbi.nlm.nih.gov/pubmed/10795317

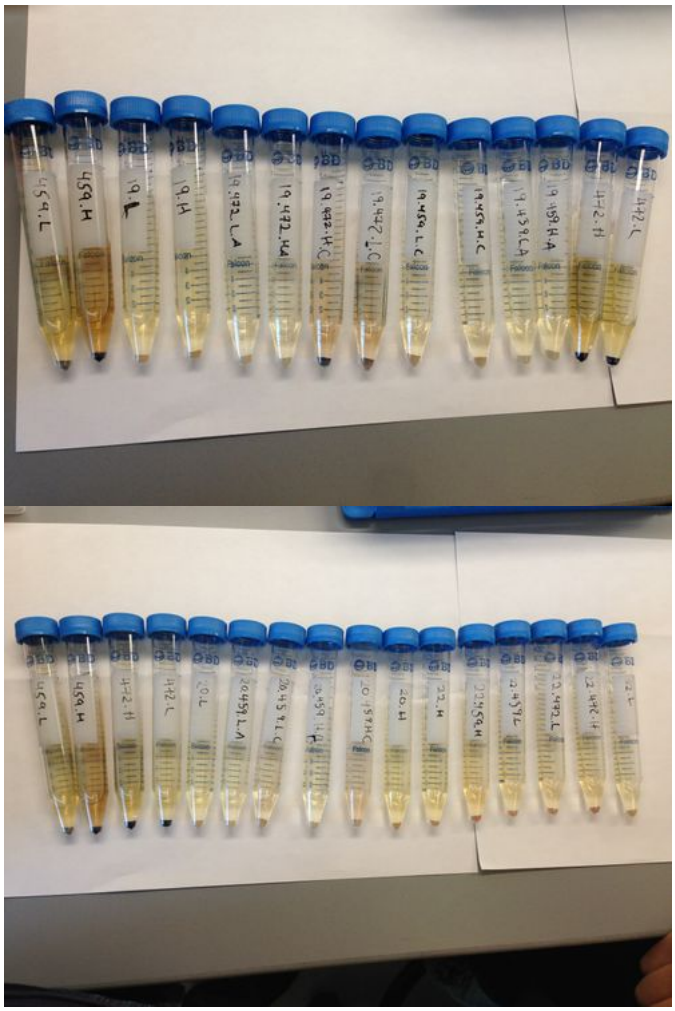
6/7/13

Results of pBAD GT induction experiments

Got results back from yesterdays experiments.

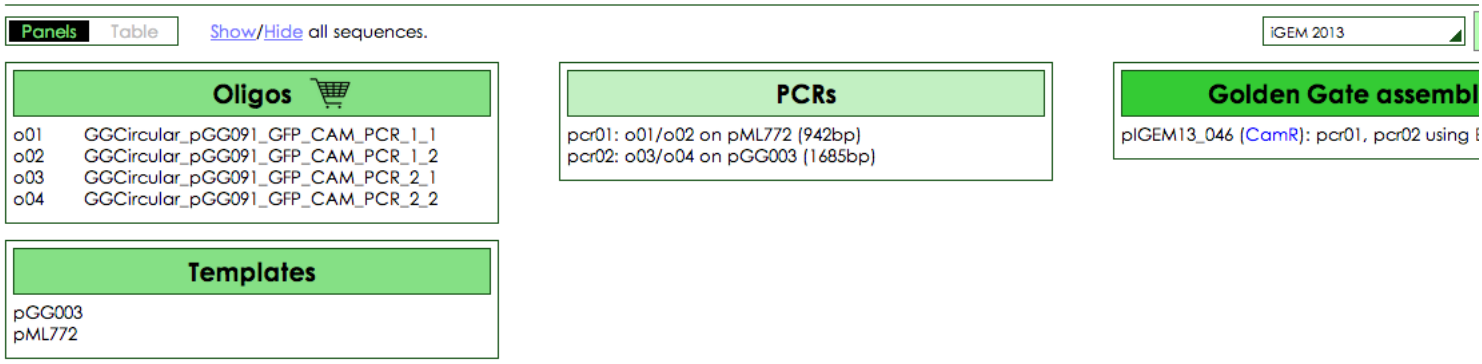


After getting the results of the induced vs uninduced experiments, we decided to spin the cells down in order to check: 1. if the indigo would be in the cells or in the media, and 2. exactly how the cells would look when out of solution.



new mV part Design

I designed a new mV part that combines CAM resistance and a colE1 origin. This part will allow us to do multi-gene assemblies of FMO or p450 cassettes with the various GT cassettes we have. I ordered the oligos that will be used with these parts. Here is the results page with the design instructions I'll be following. Even though it says we'll be using pGG003 and pML772 as templates, it was designed using pZNR0419 and pGG091 on Ape to have CAM, GFP, and colE1:



Using this new mV part, I designed plasmids pIGEM13_046 - 054 that combines all four GT's under pBAD promoters with FMO+mKate (ZNR0318) and p450+mKate (ZNR0418). These assemblies will be built in the next few days when the oligos come in.

I also, unsuccessfully, tried to design 8 more plasmids that would have been the same assembly (GTs + FMO/p450) on a p15a origin, but unfortunately the p15 CAM mV part was previously designed by Kaitlyn C, but not actually made. I deleted those plasmids from the IGEM plasmids page.

6/9/13

At night bernie and I innoculated three 96 well blocks with the Golden Gate parts pGG01-pGG96. We added the correct antibiotic resistances into each well.

6/10/13

In the morning Roy and I combined the three 96 well blocks such that each well had 750mL of culture and then miniprepped that 96 well plate using a Zymo 96-well block miniprep system.

We then went to safety training EHS104 from 2-4 and came back.

I then cleaned tubes.

I haven't been able to get foldit to work on my computer yet so I am going to ask Zach or Chris to help me with that.

6/11/13

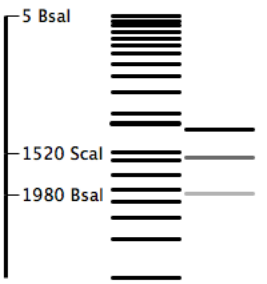
Today we got back results from the new cotransformations of FMO and p450 (fluorescently tagged this time) with pIG19 (a GT). They did not turn out very well and it looked as if Arabinose might surpress ingido production.

At 10AM, I picked colonies that ramya had plated of pIGs 38-45 (all p15 cassette assemblies) and put them in their correct antibiotic + LB to grow up.

2PM: did a double digest of pIG15 that will be run on a gel by Hojae to determine whether or not the pIG15 cassette assembled correctly. We'll look for bands at:

pIGEM13_015.gb

Size	site1		site2		Mass %
1515	BsaI	5	ScaI	1520	53
893	BsaI	1980	BsaI	5	31
460	ScaI	1520	BsaI	1980	16

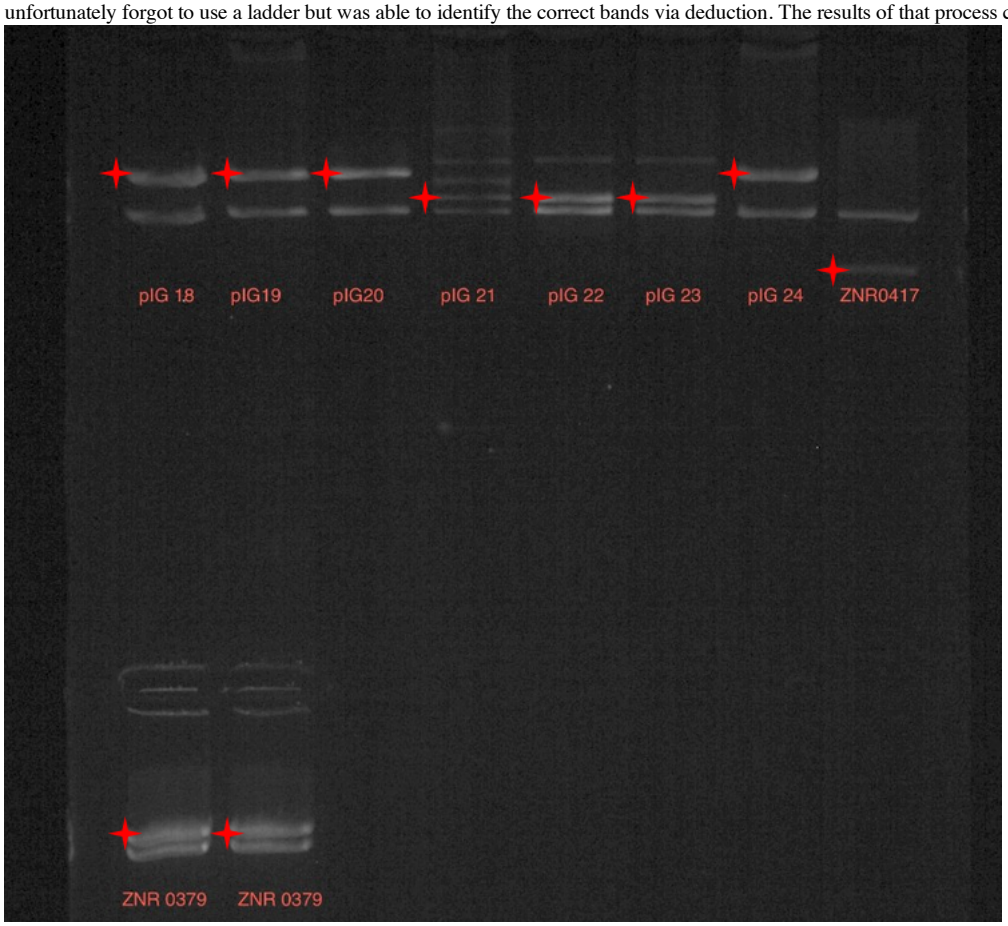


6/12/13

Digest, Ligation, Transformation of pIGs 61-67

Did a digest, ligation, transformation of pIGs 61-67. These are the multi-gene assemblies and the reason for doing the digestion, ligation part was because both the backbone and one of the genes were on plasmids with KAN resistance making Golden Gate multi-gene assembly impossible. Thus, to get around this problem, I digested each part first with BsmB1 for 2 hours at 55C following Vinay's "Digestion" protocol. Zach told me after the fact that I should have used a typical 2uL double digest. I then ran a Gel Extraction. I

unfortunately forgot to use a ladder but was able to identify the correct bands via deduction. The results of that process can be seen here:



I transformed pIGs 61-67 as well as a negative control into BW cells.

GG Assemblies of pIGs 25 & 78

I also set up two GG cassette assemblies for pIGs 25 and 78. I transformed those two into BW cells and plated on LB/KAN.

6/13/13

Positive Results for 61-67

Negative control only had a few colonies, and 61-67 all had about 3-4X more. I picked 2 colonies from each and grew them up in 5mL LB/KAN

Bad Results for 25, 78

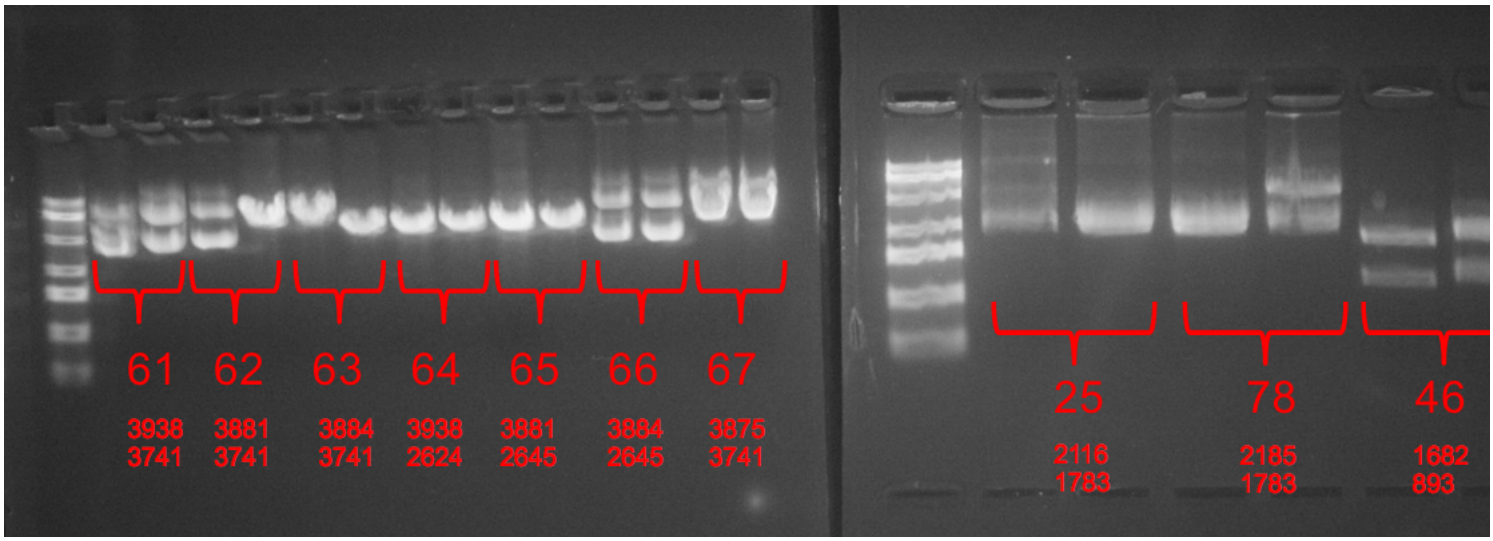
Almost all the colonies of 25 and 78 were fluorescing red. I picked two colonies for each that appeared to be white when viewed with the green light and grew them up in 5mL LB/KAN.

Test digests of 61-67 and 25, 78 and 46

Digested 61-67 multi-cassette assemblies with BglII at 30C for 30 minutes using the Double Digest Set-Up for the Igem2012 protocol page.

Digested 25, 78 and 46 cassette assemblies with BsmB1.

The picture for those digests is here:



The first column for each pIG is the A column and the other column is the B column. It appears that only pIGs 63A, 66AB, 67AB, 25AB, 78AB and 46AB worked. We submitted pIGs 61A,62B,63A,64A,65A,66B,67B,25B,46B and 78B in for sequencing. Roy set up the sequencing reactions.

Bernie and I also set up an experiment with the pIGs 63A, 66B and 67B (the ones that looked like they turned out the best from the test digest) in high tryptophan and high or no arabinose using controls of pZNR0379 and pZNR0380 as well as pIGs 20, 23 and 24.

Here is a picture of the wells of the 24 well plate: File:ArabinoseFMO GT ExperimentsJune13.png

6/14/13

Bernie observed that the second culture of 67- turned out to have a little bit of fluorescence under the microscope.

Had Roy set up Golden Gate multi-gene assemblies for pIGs 104-11 (equivalents of pIGs 61-67 except on a CAM backbone) using the new mV GFP-CAM part.

Re-set up the failed experiment that Bernie I had done last night, this time using the correct antibiotic resistance.

Re-picked colonies for pIGs 61-67. Will grow them up and miniprep and test digest them tonight.

6/16/13

Designed Anderson Promoter Series Oligos

Ordered the oligos seen here:

Oligos

o01	GG2_BBa_J23100_100_Anneal_1_1
o02	GG2_BBa_J23100_100_Anneal_1_2
o03	GG2_BBa_J23100_100_Anneal_1_3
o04	GG2_BBa_J23100_100_Anneal_1_4
o05	GG2_BBa_J23102_86_Anneal_1_1
o06	GG2_BBa_J23102_86_Anneal_1_2
o07	GG2_BBa_J23102_86_Anneal_1_4
o08	GG2_BBa_J23101_70_Anneal_1_1
o09	GG2_BBa_J23101_70_Anneal_1_2
o10	GG2_BBa_J23101_70_Anneal_1_3
o11	GG2_BBa_J23101_70_Anneal_1_4
o12	GG2_BBa_J23108_51_Anneal_1_1
o13	GG2_BBa_J23108_51_Anneal_1_2
o14	GG2_BBa_J23108_51_Anneal_1_4
o15	GG2_BBa_J23110_33_Anneal_1_1
o16	GG2_BBa_J23110_33_Anneal_1_2
o17	GG2_BBa_J23110_33_Anneal_1_4

Templates

pGG001

Oligo assemblies

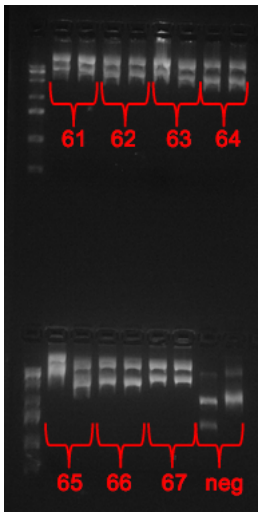
anneal01: o01, o02, o03, o04
anneal02: o05, o06, o03, o07
anneal03: o08, o09, o10, o11
anneal04: o12, o13, o10, o14
anneal05: o15, o16, o10, o17

Golden Gate assembl

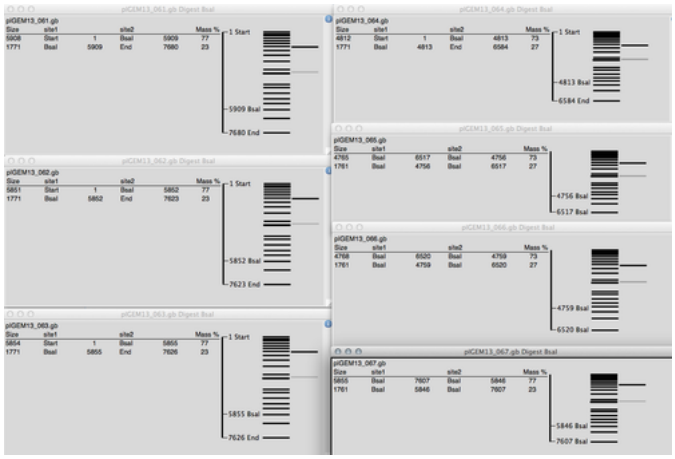
⚠ pIGEM13_112 (CamR): pGG001, anneal
pIGEM13_113 (CamR): pGG001, anneal02
pIGEM13_114 (CamR): pGG001, anneal03
pIGEM13_115 (CamR): pGG001, anneal04
pIGEM13_116 (CamR): pGG001, anneal05

Test Digest of 61-67 redos

Followed the Double Digest protocol using only Bsa1.



It should look like:



Unfortunately, none of the samples appear to have digested correctly; 61-67 will be abandoned because we now have the multi-gene backbone part on Cam that will allow us to build multi-gene assemblies in a simpler fashion.

6/17/13

Roy miniprepped grow-ups of pIGs 104-110 and I prepared a 2uL test digest using BamH1 to cut.

Test Digest of pIGs104-110 with BamH1 should look like:

6/18/13

Met up with University of Nevada Reno around 10:30 and discussed our two projects.

Came back in at 9pm and miniprepped pGG001, pIGs 9,79,80,123-128. Unfortunately the grow ups of 125-128 were fluorescing green and so I repicked colonies for those.

6/19/13

In the morning I miniprepped the repicked colonies of 125-128. Roy is going to set up test digests for everything: pGG001, pIGs 9,79,80,123-128.

Now that we have the GTs on a pTET promoter, I am going to design multi-gene assemblies of FMOs (on pglpt) with all of the GTs (on pTET).

6/20/13

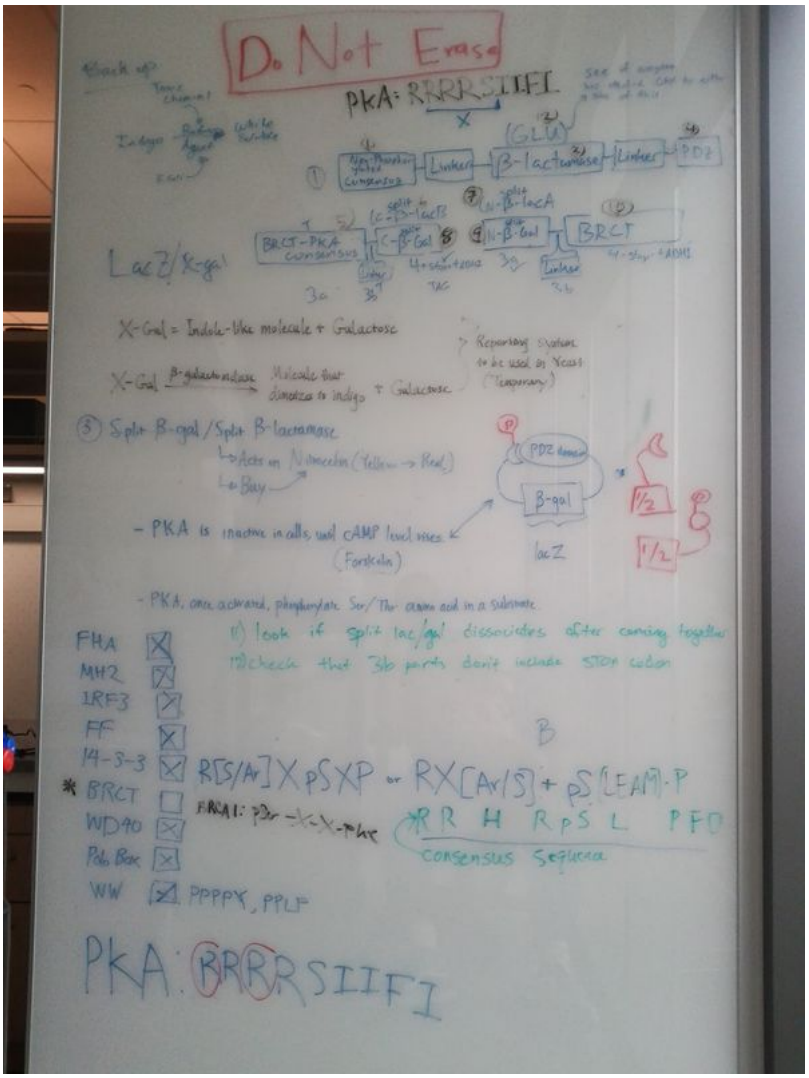
Cassette Designs

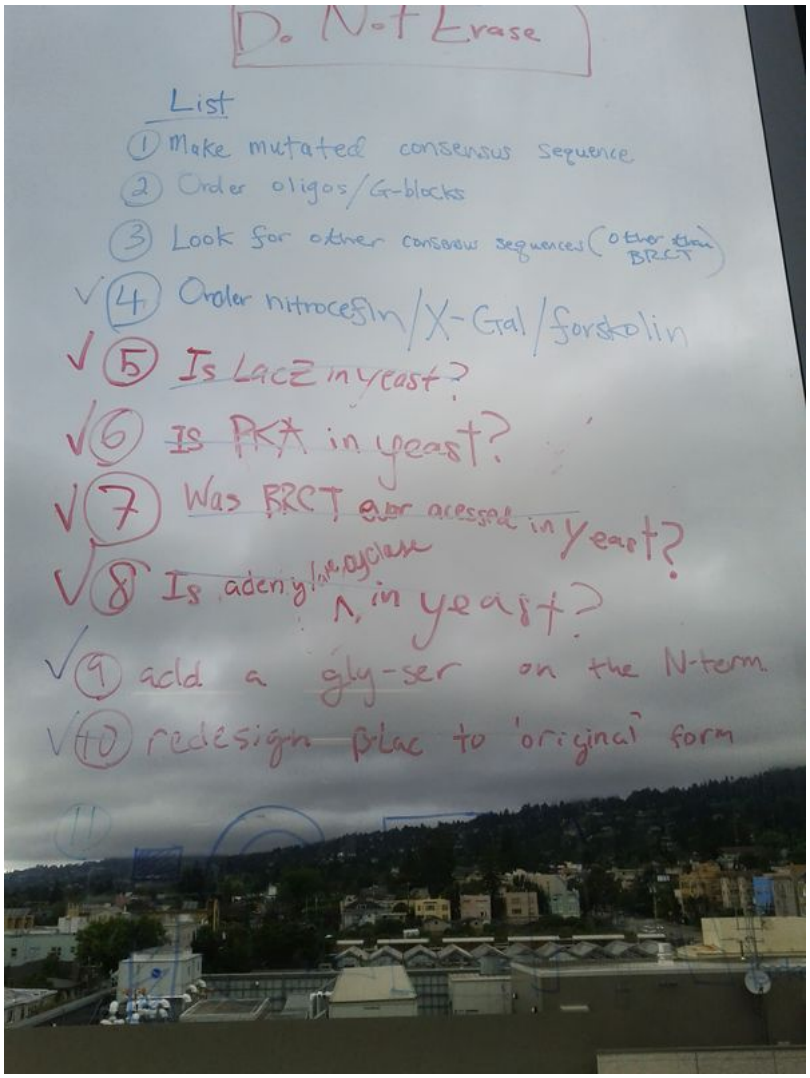
Because we are currently assembling the mutant GTs and mutant FMO's as parts, as well as the Anderson promoter series, we decided to design the GTs on the Anderson Promoters and put the mutant GTs/FMOs on just the Anderson 100% promoter.

6/24-25/13

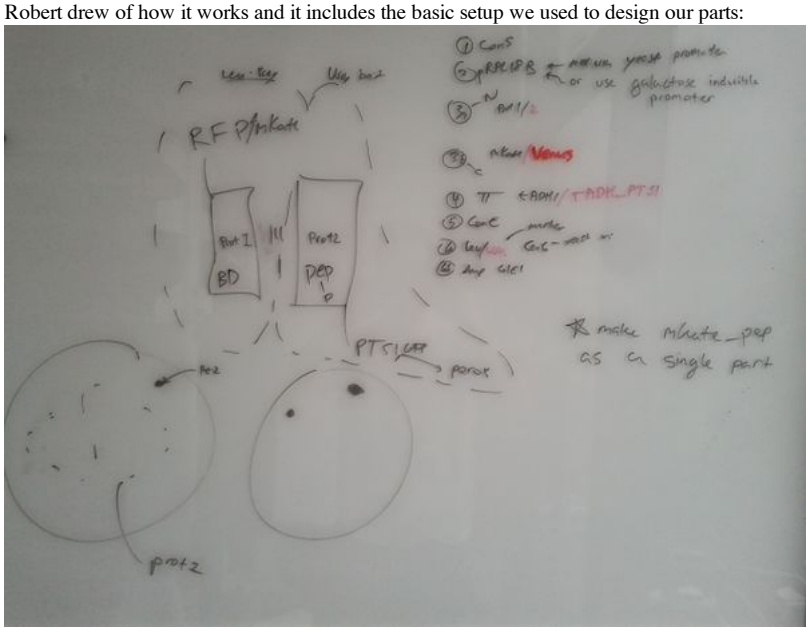
Brainstormed The Enzyme Caging Side of the project (beta-galactosidase and beta-lactamase)

We decided that engineering a single enzyme whose activity would be regulated by an attached phosphorylatable peptide and a peptide-binding domain would be more difficult than having a split version of the enzyme that is already know to work when some intracellular interaction pulls them together. Thus, we had to find a way to bring two halves of an enzyme together only upon phosphorylation. To do this we looked at a lot of SH2-like domains which are known to bind phospho-peptides. Most of the info we found can be found in the "Alternative Reporter Systems Discussion" page. Here is a photo of what some of our findings and some of the questions we had to answer along the way:

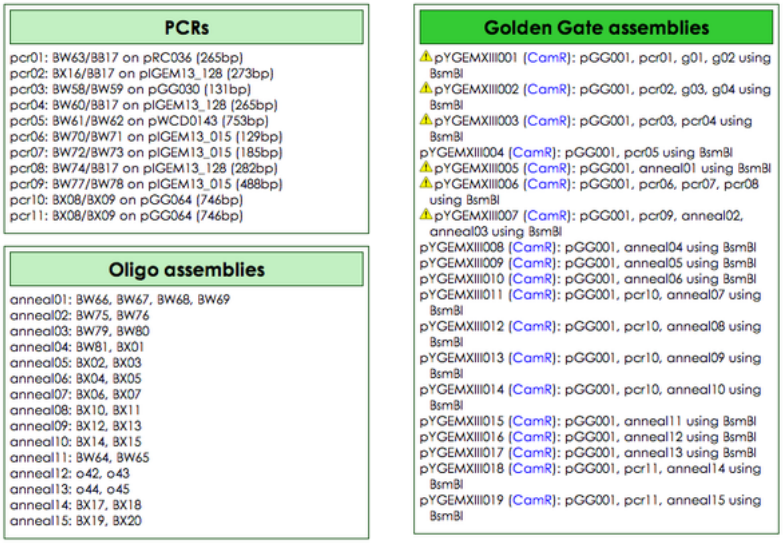




We realized that it would make sense to have a way to test if our Binding domains were truly binding upon phosphorylation. I thought of doing a yeast two hybrid assay to do so, and when I talked to Zach he redirected me to Robert who suggested we use the "in-house" system which essentially tags one of the proteins with mKate and the other with GFP and a peroxisome locating signal and if the proteins interact then you see a build up of Red in the peroxisome and not in other places in the cell. Here is the rough diagram Robert drew of how it works and it includes the basic setup we used to design our parts:



We ordered GBlocks and primers for the parts we decided would be worthwhile to check:



6/28/13

- I grew up and made aliquots of competent BW cells with Robert's help. Finished the whole process on Saturday the 29th, labeled them and put them into the correct competent cell box.
- Asked Zach why the split Gal 3a part didn't work and realized that it was just a regular part 3. Redesigned it as an actual 3a part on 6/30.

- Went to a qualcomm meeting in the morning.
- Began designing Matlab code to make graphs from the HPLC data, but Ramya took that job over and did most of the coding.
- Researched where to find Woad
- Realized we hadn't ordered some primers we needed to make part pYGEM16 & 17.

Peroxisome Y2H and split-gal/lac testing

- Designed most of the peroxisome-Y2H parts. We will be testing whether or not BRCT and 1433 will bind the ligands that PKA will (hopefully phosphorylate).

Keio Collection Knockouts

- Emailed Kelly from the Arkin lab to ask her to streak a LacY knockout and a wild-type from the Keio Collection to use to test whether x-gal uptake is affected by the presence of LacY.

6/30/13

Designed pYGEMXIII035-038 (redesign of beta-galactosidase N terminus into an actual 3a part)

7/1/13

- Picked colonies of FMO and GTs on pGLPT for Ramya and FMO on pGLPT for Bernie into LB/CAM.

Peroxisome Y2H and split-gal/lac testing

7/2/13

We've already made pYGEM001, and the pcr02 for pYGEM002

I put the PCRs 3-7 into the thermocycler at 5pm.

Oligos

BW63.oiGEMXIII124 GG4_BRTC__PCR_1_1
BB17.opZNRADH1-2 use to make ADH1 TT modular
BX16.oiGEMXIII158 GG4_1433T_Human_tADH1_PCR_1_1
BX60.oiGEMXIII170 GG3a_14-3-3_PKA_Consensus_Sequence_Anneal_1_1
BX61.oiGEMXIII171 GG3a_14-3-3_PKA_Consensus_Sequence_Anneal_1_2
BX62.oiGEMXIII172 GG3a_Mutant_14-3-3_PKA_Consensus_Sequence_Anneal_1_1
BX63.oiGEMXIII173 GG3a_Mutant_14-3-3_PKA_Consensus_Sequence_Anneal_1_2
BW61.oiGEMXIII122 GG3_BGAL_C_terminus_PCR_1_1
BX64.oiGEMXIII174 GG3a_remake_of_B-Gal_N_terminus_PCR_1_2
BX65.oiGEMXIII175 GG3_lacZ_full_PCR_1_1
BX66.oiGEMXIII176 GG3_lacZ_full_PCR_1_2
BX67.oiGEMXIII177 GG3_lacZ_full_PCR_2_1
BX68.oiGEMXIII178 GG3_lacZ_full_PCR_2_2
BX69.oiGEMXIII179 GG3_lacZ_full_PCR_3_1
BW62.oiGEMXIII123 GG3_BGAL_C_terminus_PCR_1_2
BX70.oiGEMXIII180 GG3_B-Lactamase_Full_PCR_1_1
BX71.oiGEMXIII181 GG3_B-Lactamase_Full_PCR_1_2

gBlocks

g01 GG4_BRTC__gBlock_1
g02 GG4_BRTC__gBlock_2
g03 GG4_1433T_Human_tADH1_gBlock_1
g04 GG4_1433T_Human_tADH1_gBlock_2

PCRs

pcr01: BW63/BB17 on pRC036 (265bp)
pcr02: BX16/BB17 on pIGEM13_128 (273bp)
pcr03: BW61/BX64 on pWCD0143 (752bp)
pcr04: BX65/BX66 on pWCD0143 (673bp)
pcr05: BX67/BX68 on pWCD0143 (978bp)
pcr06: BX69/BW62 on pWCD0143 (1518bp)
pcr07: BX70/BX71 on pRC237 (702bp)

Oligo assemblies

anneal01: BX60, BX61
anneal02: BX62, BX63

Golden Gate assemblies

⚠ pYGEMXIII001 (CamR): pGG001, pcr01, g01, g02 using BsmBI
⚠ pYGEMXIII002 (CamR): pGG001, pcr02, g03, g04 using BsmBI
pYGEMXIII016 (CamR): pGG001, anneal01 using BsmBI
pYGEMXIII017 (CamR): pGG001, anneal02 using BsmBI
pYGEMXIII034 (CamR): pGG001, pcr03 using BsmBI
pYGEMXIII039 (CamR): pGG001, pcr04, pcr05, pcr06 using BsmBI
pYGEMXIII040 (CamR): pGG001, pcr07 using BsmBI

Keio Collection Knockouts

Plate	Location	Gene Name	Knockout Name	Annotation
5	e5	pgm	JW0675	Phosphoglucomutas
43	e8	fre	JW3820	flavin reductase
45	c6	galF	JW2027	UDP-glucose pyrophosphorylase
49	b9	lacI	JW0336	Lactose operon repressor
49	c12	galU	JW1224	UDP-glucose pyrophosphorylase
49	f10	galE	JW0742	UDP-glucose 4-epimerase
51	g12	bglB	JW3699	phospho-beta-glucosidase
51	a4	bglX	JW2120	phospho-beta-glucosidase
51	d7	ascB	JW2686	phospho-beta-glucosidase
51	h2	chbF	JW1723	phospho-beta-glucosidase
55	h7	chbA	JW1725	cellobiose transporter
55	b8	chbB	JW1727	cellobiose transporter
57	f10	ascF	JW5435	cellobiose transporter
61	g1	bglA	JW2869	phospho-beta-glucosidase
63	e9	tnaA	JW3686	Tryptophanase
85	g5	lacY	JW0334	lactose transporter

Positive Mutant Consensus Sequence

- I looked into a way of designing a peptide sequence such that it would act as if it were always phosphorylated. I came across several articles that I added to the iGEM wiki under the Progress tab in the link "Sensor kinase peptide discussion".
- Basically it seems as if substituting Glutamate (E) for Serine would mimic the phosphorylated serine state. They both have the same "chain length".

Getting Woad

I called several places in vain, in a search for the Woad plant. Eventually I stumbled across the UC Berkeley Botanical Garden's website, and a simple search of their database revealed that they had Woad growing there. Over the course of the next few days we filled out a plant petition form and on Monday 7/8 we took a trip to the Botanical Garden and picked up the specimen in question.

7/10/13

Oligos

BZ24.oiGEMXIII206	GG4_SYNZIP21+tADH1_PCR_1_1
BZ25.oiGEMXIII207	GG4_SYNZIP21+tADH1_PCR_1_2
BW60.oiGEMXIII121	GG4_B-Gal_N_terminus_PCR_2_1
BB17.opZNRADH1-2	use to make ADH1 TT modular

Templates

pGG001
pMRY149

PCRs

pcr01: BZ24/BZ25 on pMRY149 (164bp)
pcr02: BW60/BB17 on pMRY149 (265bp)

Golden Gate assemb

⚠ pYGEMXIII067 (CamR): pGG001, pcr01, BsmBI

Oligos

BW63.oiGEMXIII124	GG4_BRTC__PCR_1_1
BB17.opZNRADH1-2	use to make ADH1 TT modular
BX16.oiGEMXIII158	GG4_1433T_Human_tADH1_PCR_1_1
BW58.oiGEMXIII119	GG4_B-Gal_N_terminus_PCR_1_1
BW59.oiGEMXIII120	GG4_B-Gal_N_terminus_PCR_1_2
BW60.oiGEMXIII121	GG4_B-Gal_N_terminus_PCR_2_1
BW66.oiGEMXIII127	GG3b_15_residue_linker_Anneal_1_1
BW67.oiGEMXIII128	GG3b_15_residue_linker_Anneal_1_2
BW68.oiGEMXIII129	GG3b_15_residue_linker_Anneal_1_3
BW69.oiGEMXIII130	GG3b_15_residue_linker_Anneal_1_4

gBlocks

g01	GG4_BRTC__gBlock_1
g02	GG4_BRTC__gBlock_2
g03	GG4_1433T_Human_tADH1_gBlock_1
g04	GG4_1433T_Human_tADH1_gBlock_2

Templates

pGG001
pGG030
pIGEM13_128
pRC036

PCRs

pcr01: BW63/BB17 on pRC036 (265bp)
pcr02: BX16/BB17 on pIGEM13_128 (273bp)
pcr03: BW58/BW59 on pGG030 (131bp)
pcr04: BW60/BB17 on pIGEM13_128 (265bp)

Oligo assemblies

anneal01: BW66, BW67, BW68, BW69

Golden Gate assembli

⚠ pYGEMXIII001 (CamR): pGG001, pcr01, g BsmBI
⚠ pYGEMXIII002 (CamR): pGG001, pcr02, g BsmBI
⚠ pYGEMXIII003 (CamR): pGG001, pcr03, p BsmBI
⚠ pYGEMXIII005 (CamR): pGG001, anneal0

7/11/13

Bicistronic RBS Design

I made 4 Promoter + Bicistronic RBS parts using the sequences found in the "Precise and reliable gene expression via standard transcription and translation initiation elements" Vivek (http://www.nature.com/nmeth/journal/v10/n4/full/nmeth.2404.html) . I used P3 and concatenated it with BCD2, BCD9, BCD18, and BCD21. I changed the end of the BCD to match the "type 2" 3' overhang. Instead of ending in TAATG, it now ends TAatATG such that tATG will be the correct 3' type 2 overhang. The part plasmids are pIGEMXIII306-309.

RFP	P3	P7	P5	P1	P6	P2	P8	P14	P11	P10	P12	P9	P13	P4
BCD2	634.54	422.83	546.55	463.87	478.01	117.45	161.65	132.13	168.00	106.93	101.08	82.15	53.56	57.63
BCD1	559.40	404.10	522.63	441.90	448.22	222.87	143.44	124.41	122.59	96.84	91.35	69.62	48.98	50.75
BCD7	424.14	428.65	368.94	315.04	314.94	325.73	120.42	118.04	105.27	85.00	78.59	59.92	45.07	27.00
BCD6	356.48	282.03	250.12	194.52	209.17	124.41	90.94	74.97	70.26	63.11	56.35	44.99	34.71	10.77
BCD5	418.77	457.78	375.44	336.62	339.73	188.99	127.67	112.05	105.43	89.56	84.77	62.27	46.97	48.32
BCD9	357.18	357.58	312.60	270.79	266.57	157.21	102.44	90.37	82.71	71.89	66.43	47.20	37.90	41.12
BCD11	331.91	365.38	322.07	215.08	287.74	163.22	113.98	96.95	88.32	79.02	106.16	56.75	41.46	42.29
BCD10	450.63	487.96	408.50	350.64	344.06	197.99	129.98	109.44	111.53	90.29	81.56	58.15	45.12	46.05
BCD12	362.90	359.81	337.34	283.42	265.47	162.52	104.69	98.11	85.25	76.60	65.82	54.92	38.62	44.78
BCD14	252.75	270.14	233.13	195.48	189.73	109.33	88.98	131.73	59.53	69.97	70.58	45.43	61.29	28.38
BCD13	301.33	304.55	286.52	236.27	220.41	132.27	98.79	96.71	84.12	66.94	84.29	51.00	42.85	30.06
BCD18	174.68	191.95	177.17	136.33	147.55	83.44	73.19	69.23	58.65	54.75	47.75	39.88	31.06	23.92
BCD15	277.88	293.65	252.81	195.39	213.48	118.03	98.38	90.17	78.62	78.07	26.68	49.62	38.97	27.89
BCD17	208.68	233.60	96.93	166.37	152.52	97.24	71.08	72.24	65.36	52.31	48.07	36.38	31.10	26.82
BCD19	245.44	253.60	197.23	205.29	156.48	104.71	74.80	71.21	63.72	64.09	46.84	36.31	29.59	28.90
BCD20	194.84	202.92	202.65	154.97	148.43	82.26	75.74	66.03	53.13	54.54	43.05	35.66	29.58	21.82
BCD21	121.56	138.62	123.14	95.91	100.37	64.18	57.62	51.15	42.74	41.03	32.89	30.26	24.40	18.45
BCD16	93.89	94.83	87.40	86.53	74.03	49.07	40.26	40.40	34.99	29.71	27.36	20.91	16.29	14.85
BCD23	108.39	150.01	126.76	106.43	110.99	47.85	63.03	52.63	43.76	45.13	38.18	67.55	25.96	17.73
BCD24	44.44	56.14	51.94	44.05	46.63	26.28	28.04	24.08	19.61	21.25	17.48	16.61	13.69	11.12
BCD8	39.16	52.11	46.67	38.62	39.32	24.60	22.55	19.33	16.40	17.06	14.22	14.22	6.14	10.62
BCD22	21.32	16.80	16.06	14.49	14.24	11.08	10.56	10.19	9.42	9.35	8.50	8.16	7.53	7.62

Future Tests with P3BCDs

I will put the P3BCDs in front of a Flag-tagged GFP and a Flag-tagged RFP and test the expression levels of the different RBSs.

7/15/13

3 more Keio Knockouts

- asked Kelly Wetmore to plate three more keio knockouts: pgi, galF, yeeO

Building the Bicistronic RBSs

- did PCRs of pigs 306-309

Leucine Zippers as positive controls

- designed leucine zipper (Synzip21) to be a part 4 so we could use it as a positive control in our cell lysate experiments

7/16/13

- emailed Kelley 3 more knockouts that we want. Put 3 kan plates in the fridge for her
- moved the w.t. glycerol strain into its position in the -80
- planned competent cell stuff for keio knockouts

Building the Bicistronic RBSs

- Gel purified PCR products for pIGs 306-309 and added gelpic to the dropbox gelpic folder
- set up BsmB1 for pis 306-309
- designed flag-tagged GFP and RFP to use to check bicistronic expression

7/17/13

Verifying the Keio Knockouts

- designed colony PCRs to verify that the knockouts are correct
- for each gene I ordered a primer that would bind outside the gene in the "forward" direction (e.g. "pgm of" as determined in comparison to the annotated genome) and one that would bind inside the gene in the reverse direction.
- between the primer pair is a ~700bp gap.
- also ordered primers that would bind to the KanR transposon cassette.

Building the Bicistronic RBSs

Miniprepped pIGs 306-309

Week of 7/18/13

- Test digested pIGs 306-309 and sent them for sequencing
- Miniprepped pIGs 59-69
- It turned out our split lacZ constructs had been designed incorrectly. We cloned the wrong deletion mutants.
- Redesigned lacZ
- Continue work on bicistronic promoter-rbs parts
- Got the Keio Knockouts from Kelley. Ran an inconclusive colony PCR of the the KO's. There may have been bubbles within the PCR tube that could have thrown off some of the results giving false negatives. But for the most part, it looked like the knockouts she gave us weren't actually KO's.
- Thought about MuA transposon stuff - is there anything that determines the insertion site of the transposon. Met with Caleb from the Savage lab to discuss possible transposon strategies for designing a split B-Glu. I worked on making an initial design for the MuA system with beta-glu. But, in the end we decided that this wouldn't be the most productive use of our time because it didn't really fit with out story, especially if we abandoned the sensor idea.

Week of 7/25/13

- Looked into BRCT and 14-3-3 domains
- Made competent cells of tg1 for the lab
- Continued to troubleshoot the keio-knockouts. Colony PCRs returned strange results that suggested possible contamination. Eventually did genome preps and sent them for sequencing to ensure that the genes were actually knocked out. Unfortunately, the genome-sequencing reactions failed suggesting that they may have been contaminated from the get-go. A few of them did seem to work. Namely pgi
- Made competent cells of PGI
- check sequencing results of pY76, 78, 79 . py76 has a possible deletion. found a primer to re-read the portion in question.
- insolubility of GFP could be an issue for PKA phosphorylation
- set up constructs to test BCDs

Week of 8/01/13

- Ran a tecan of the split GFP constructs
- redid py77 sequencing
- constructed the GTs with BCDs and pAnd100_strong RBS
- did a yeast transformation of the GT constructs to test if they are coagulating in yeast as well as in e coli
- redesigned the mV part to remove the unwanted not1 sites and allow for subcloning with the GTs

Week of 8/08/13

- did a colony pcr to test if the GT transformations worked correctly
- did mutagenesis PCRs of the GTs - followed Davis' 2011 iGEM protocol
- looked into fluorescent in-vivo lactamase assay
- started reconstructing split lactamase parts

Week of 8/15/13

- made a multigene with tnaA, tnaB and FMO
- looked into NNK saturation mutagenesis for oleD
- started con
- ran a tecan with the lactamase parts. This time we induced PKA with forsolin, but once again we got inconclusive results. We will probably have to abandon the split enzyme biosensor pathway.

Week of 8/22/13

- started to construct FMO and oleD both his-tagged and on a t7 expression system for purification
- began to help Roy carry out the indigo titer experiments
- To mutagenize OLED, and generate all of the the OleD asp mutations, we designed an OleD with a GFP dropout replacing the middle of the gene. In the place of the GFP dropout, we can ligate in oligo anneals with the various desired mutations on them.

Week of 9/1/13

- Continued to make oleD GFP dropout parts
- Ran a hasty anaerobic experiment with FMO, but got inconclusive results. I wanted to test whether or not FMO would create indole which couldn't oxidize in the anearobic conditions. This idea is potentially flawed because indole is toxic to e coli at relatively low levels

Week of 9/8/13

- started to ligate oligo anneals into the GFP dropout

Week of 9/15/13

- lots of data crunching of FMO, Glu and OleD kinetics. Roy running the experiments. I'm parsing the data on matlab and generating the Km's and Vmax's.

Week of 9/22/13

- lab work becoming less of a priority
- started to work on the Presentation and wiki