Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

WEDNESDAY, 5/18/16

iCultures Grown:

- pSMART-yibDp-GFPuv
  - "GFP" or "yib"
- pSMART-EV
  - "pSMART" or "PSM"
- DLF-00286
  - "286"
- E-cloni

Results:

(5/19) All 8 Cultures grew successfully

Notes:

Taxol_G-Blocks.docx

Mini Prep Protocol

Introduction

sdfghjkl;

Materials

- Mini Prep Kit
- Spin Columns
- Neutralize (B3) Buffer - 4 C fridge
- Epi Tubes

Procedure

Before Beginning

1. All centrifugation steps should be carried out at 13,000 RPM
2. Add 4 volumes of ethanol (>= 95%) to one volume of Plasmid Wash Buffer 2.
3. If precipitate has formed in Lysis Buffer (B2), incubate at 30-37 degrees C, inverting periodically to dissolve.
4. Store Plasmid Neutralization Buffer (B3) at 4 degrees C.

Miniprep

5. Pellet 1-5 ml bacterial culture by centrifugation for 8 minutes at 3500 rpm. Discard supernatant.
6. Resuspend pellet in 200 ul Plasmid Resuspension Buffer (B1 - pink). Vortex or pipet to ensure cells are completely resuspended. There should be no visible clumps.
7. Add 200 ul Plasmid Lysis Buffer (B2 - blue).
8. Gently invert tube 5-6 times.
9. Incubate at room temperature for 1 minute.

00:01:00

10. Color should change to dark pink, and solution will become transparent and viscous. Do not vortex.
11. Add 400 ul of of Plasmid Neutralization Buffer (B3 - yellow).
12. Gently invert tube until neutralized.
13. Incubate at room temperature for 2 minutes.

00:02:00

14. Sample is neutralized when color is uniformly yellow and precipitate forms. Do not vortex.

15. Centrifuge lysate for 2-5 minutes.

16. Carefully transfer supernatant to the spin column.

17. Centrifuge for 1 minute. Discard flow-through.

18. Re-insert column in the collection tube and add 200 uL of Plasmid Wash Buffer 1.

19. Centrifuge for 1 minute. Discarding the flow-through is optional.

20. Add 400 uL of Plasmid Wash Buffer 2 and centrifuge for 1 minute.

21. Transfer column to a clean 1.5 mL microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If there is any doubt, re-spin the column for 1 minute.

    When centrifuging with the epi tubes, cross the lids together to prevent them from breaking off. If there is an odd number then the last one should stick out sideways. Don't place the lids up or down as they can break off during centrifugation.

22. CRITICAL Add >= 30 uL DNA Elution Buffer to the center of the matrix. Wait for 1 minutes.

00:01:00

23. Spin for 1 minute to elute DNA.

    Usually add 50 uL of Elution Buffer
    Nuclease-free water (pH 7-8.5) can also be used to elute the DNA
    Yield may slightly increase if a larger volume of DNA Elution Buffer is used. But the DNA will be less concentrated. For larger size DNA, (>= 10 kb) heating the elution buffer to 50 degrees C to use can improve yield.

Gibson Protocol

Introduction

Gibsons ligate plasmids and vectors together.

Materials

- Vector
- Insert
- Water
- 2X Hi-Fi
- PCR Tubes

Procedure

Amounts

1. 1.5 uL of vector
2. 5 uL of mix (2X Hi-fi Gibson Master Mix)
3. 1.5 uL of inserts
4. Add enough water so that the total volume becomes 10uL

Order

5. Mix in the PCR Tube

    Insert
    Vector
    Mix
    Water

Colony PCR

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

›

Procedure

1. Prepare econotaq master mix with oligos to amplify the region of interest (https://www.lucigen.com/docs/manuals/MA038-EconoTaq-PLUS.pdf (https://www.lucigen.com/docs/manuals/MA038-EconoTaq-PLUS.pdf))

Table1

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	10
3	EconoTaq	12.5	125
4	SL1	0.25	2.5
5	SR2	0.25	2.5
6	H2O	12	120
7	Total	25	250

CRITICAL COPY AND PASTE TABLE, INSERT NUMBER OF REACTIONS DESIRED AND TABLE WILL AUTO CALC VALUES

2. Aliquot out master mix + oligos into a sufficient number of PCR tubes to test colonies
3. If your backbone + insert plates have many more colonies than your backbone only or insert only, you will need to test fewer colonies than if you have lots of colonies on your control plates
4. Also include a control reaction with parent plasmid only
5. Aliquot out some LB (+ antibiotic) into another set of tubes,one for each colony you are testing
6. Pick a colony from your transformation plate with a sterile pipette tip
7. Dab the colony into the PCR tube with the econotaq master mix
8. Dab it again in the tube with LB
9. Run the PCR according to manufacturer's protocol, but with a long (5 min) initial melting step at 95-98

NOTE: If you are including miniprepped DNA in the PCR reaction, dilute to 50 ng/uL.

Thermocycler Protocol

10. Start: 98C for 10 min.
11. Cycle (x35)
12. Melt 98C for 45s
13. Anneal 50C for 45s
14. Extend 72C for ___
15. Time = (1 min / kbase)*length(longest amplificant)
16. 4C for inf

NOTE: When dealing with DNA samples instead of colony samples. Use 3 uL of 1ng/uL DNA

If DNA sample is poor, use at most 50 ng/uL concentration

Freezer Stocks Protocol

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- Cryotubes
- 20% Glycerol
- Culture Samples
  -

Procedure

Labeling And Database

1. Label each cryotube tube with pGEM___

   The number after the pGEM corresponds to the plasmid database

2. Update the Plasmid Database with the number of the tube, the sample name, and where the sample came from

Stocks

3. In a cryotube, add 750 mL of culture solution to 750 mL of 20% glycerol
4. Gently pipette up and down
5. Store in -80 C freezer in the yellow box (if storage location changes update the database)

Preparation for Sequencing

Introduction

How to prepare mini prepped DNA to be sent off for sequencing. The sequencing place used will do overnight sequencing. If the sample is got to them by 5 pm, they will have sequences back by 9 am the next morning.

Materials

- Mini prepped DNA
- Epi tubes (medium sized)

Procedure

Preparation

1. Verify concentrations of the plasmid DNA samples

DNA:

2. 8 uL of 100 ng/uL DNA per reaction
3. 2.2 x 8uL DNA per sample goes into each sequencing tube

   Reason: we have two primers

Primers:

4. Two tubes, 1 per primer
5. Per reaction: 5 uL of 5 uMol
6. Calculate final volumes and concentrations to reach the above specifications and pipette
7. Send off for sequencing

Digest

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- Restriction Enzymes
- CutSmart (10x)
- dH_2O
- DNA
- Ice

Procedure

Restriction Digest

1. Create Master Mix **ON ICE** (25 uL total= 5 reactions)

   5 uL of CutSmart
   0.5 uL of Enzyme 1
   0.5 uL of Enzyme 2
   19 uL of dH_2O

2. Mix with DNA **ON ICE** (total of 8 uL)

   4 uL of DNA
   4 uL of Master Mix

3. Digest for 30 minutes at 37°C

4. Heat kill enzymes for 20 minutes at 80°C

Example: pSB1C3

5. Create Master Mix **ON ICE** (25 uL total= 5 reactions)

   5 uL of CutSmart
   0.5 uL of EcoRI-HF
   0.5 uL of PstI-HF
   0.5 uL of DpnI (to specially methylate the RFP insert)
   18.5 uL of dH_2O

6. Mix with DNA **ON ICE** (total of 8 uL)

   4 uL of Backbone
   4 uL of Master Mix

7. Digest for 30 minutes at 37°C

8. Heat kill enzymes for 20 minutes at 80°C

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

WEDNESDAY, 5/18/16

Made 2 L of LB Broth

LB Broth/Media Protocol

Autoclaved for 30 min. -- One liter was made between two sealable 1 L bottles (each filled with 500 mL of media)

Made 1 L of LB Agar and LB Agar + KAN Plates

LB Agar Plates

Autoclaved for 30 min.

Made 1 L of 10% Glycerol and 500 mL of 50% Glycerol

Glycerol Stocks

Autoclaved for 30 min.

Made Kanamycin Stock

Antibiotic Stocks

Used 350 mg of Kanamycin to make a 1000X stock of 35 ug/mL
Aliquoted into 1 mL epi tubes.

Notes:

- Shaker ("Max-Q 4000") set to 38; thermometer reads 33.2

LB Broth/Media Protocol

Introduction

LB Broth or Media is used to grow cells. Throughout the iGem Project, we used a low salt media because ..... The recipe below is for 1 L of Broth

Materials

- 10 g Tryptone
- 5 g Yeast Extract
- 5 g Sodium Chloride (NaCl or salt)
  - for a high salt media use 10 g of Sodium Chloride
- Deionized Water

Procedure

Make Broth

1. In a 2000 mL Erlenmeyer flask mix the tryptone, yeast extract, sodium chloride.
2. Use a graduated cylinder to measure out 1 L of deionized water
3. Add the water to the flask and mix well
4. Autoclave on a liquid cycle for 15 min

   Make sure to add water or ice to the bucket before autoclaving.

LB Agar Plates

Introduction

This is a recipe for 1 L of LB for plates that are a low salt LB

Materials

- 15 g Agar
- 10 g Tryptone
- 5 g Yeast Extract
- 5 g Sodium Chloride
  - For a high salt agar, use 10 g of Sodium Chloride
- Deionized Water

Procedure

Media

1. In a 2000 mL Erlenmeyer Flask, add the agar, tryptone, yeast extract, and sodium chloride.
2. In a graduated cylinder, measure out 1000 mL of deionized water.
3. Add it to the flask and mix.
4. Autoclave on a liquid cycle for 15 min

   Make sure to add water or ice to the bin before autoclaving.

KAN (Kanamycin) Plates

5. Once out of the autoclave, allow media to cool.
6. Add as many uL of KAN 1000X Stock as there is mL of media

   For a 1 L media batch, add 1 mL of KAN 1000X stock.
7. Mix the flask well.
8. Pour plates.

   Make sure the cover the entire bottom of the plate and remove as many bubbles as possible.

CM (Chloramphenicol) Plates

9. Once media is out of the autoclave, allow it to cool.
10. Add as many uL of CM 1000X Stock as there is mL of media

    For a 1 L media batch, add 1 mL of CM 1000X stock.
11. Mix the flask well.

12. Pour plates.

    Make sure the cover the entire bottom of the plate and remove as many bubbles as possible.

AMP (Ampicillin) Plates

13. Once media is out of the autoclave, allow it to cool.

14. Add as many uL of AMP 1000X Stock as there is mL of media

    For a 1 L media batch, add 1 mL of AMP 1000X stock.

15. Mix the flask well.

16. Pour plates.

    Make sure the cover the entire bottom of the plate and remove as many bubbles as possible.

Glycerol Stocks

Introduction

Three concentrations of glycerol stocks.

Materials

- Glycerol
- pico Water
  - Autoclaved deionized water

Procedure

10% Glycerol Stock

1. Add 50 mL of 100% glycerol to liter bottle
2. Fill bottle to 500 mL with pH2O
3. Autoclave on liquid cycle for 15 min
4. Store at room temp

50% Glycerol Stock

5. Add 250 mL of 100% glycerol to liter bottle
6. Fill bottle to 500 mL with pH2O
7. Autoclave on liquid cycle for 15 min
8. Store at room temp

Made 20% Glycerol Stocks

9. Add 40 mL of 100% Glycerol to a 500 mL bottle
10. Add 160 mL of pH2O.
11. Autoclave on liquid cycle for 15 min.
12. Store at room temp

Antibiotic Stocks

Introduction

Three kinds of antibiotic stocks (1000x). These recipes make 1000X stocks that follow the recommendations for plates.

Materials

- Antibiotic Powder
  - Stored in -20 C freezer
- Pico Water

Procedure

Kanamycin Stocks (10 mL)

1. Use 10 mL of pico water (autoclaved dH2O)
2. Add 500 mg of Kanamycin powder (-20 C freezer)
3. Mix well
4. Filter [using antibiotic filters and syringes (in drawer underneath middle bench)] into aliquots of 1 ml epi tubes
5. Store aliquots labeled with concentration (1000x), date, and KAN in -20 C freezer

Ampicillin Stock (10 mL)

6. Use 10 mL of pico water (autoclaved dH2O)
7. Add 1 g of Ampicillin powder (-20 C freezer)
8. Mix well
9. Filter using antibiotic filters into 1 mL aliquots (epi tubes)
10. Store aliquots labeled with concentration (1000x), date, and AMP in -20 C freezer

Chloramphenicol Stock (10 mL)

11. Use 10 mL of ethanol
12. Add 250 mg of Chloramphenicol powder (-20 C freezer)
13. Mix well
14. Filter using antibiotic filters into 1 mL aliquots (epi tubes)
15. Store aliquots labeled with concentration (1000x), date, and CM in -20 C freezer

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 5/19/16

Miniprep pSMART and yib

Used a 30 mL elution

Tested the concentration using UV/Absorbance and found that at 280 nm, the tib had an absorbance of 0.006 and at 260 nm it had an absorbance of 0.019. Using the ratio of Abs 1= 50 ug/mL this leads to the tib miniprep resulting in DNA of a concentration 95 ug/mL. The pSMART had an absorbance of 0.0037 at 260 nm and an absorbance of 0.025 at 280 nm. the ratio of 260:280 of 1.48. This leads to a concentration of 135 ug/mL.

Protocols:

- Miniprep Protocol NEB
- Testing Miniprep Concentration

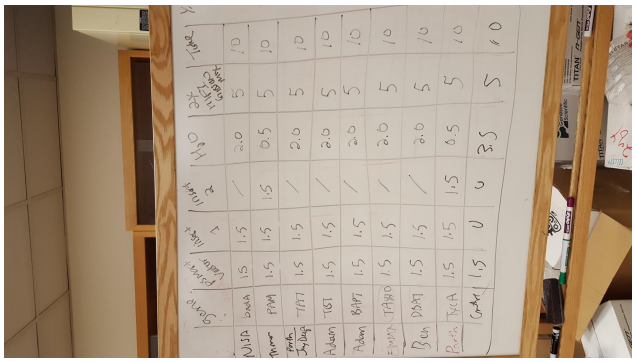
Gibsons

Performed Gibson Assemblies on:

- BadA (Nisa)
- PAM (Thomas)
- TAT (Parth)
- TBT (Adam)
- BAPT (Adam)
- TAX10 (Emma)
- DBAT (Ben)
- TycA (Parth)

Used recipes below

GibsonPlan_-_Bootcamp_20160519.jpg



Protocol:

- Gibson Protocol

Prepared Electrocompetent Cells (50 mL) of DLF-00286 and E-cloni

No changes from protocol

Protocol:

- Electrocompetent Cells Protocol

Made Freezer Stocks

4 stocks were made of each strain for now; 4 stocks were made as a back up

Used 50% instead of 20% Glycerol
4 Strains were:

- yib
- pSMART
- 286
- Ecloni

Protocol:

- Freezer Stock Protocol

Transformations of Gibsons

Used electroporation to transform gibsons into competent cells.
Protocol:

- Electroporation Protocol

Used 50 uL of Competent Cells and 1 uL of DNA
Plated the transformations after a one hour recovery.

Results:

(5/20)
[Name], [10uL colony #], [200uL colony #]

- badA, 1, 6
- PAM, 0, 0
- TAT, 0, 28
- TBT, 0, 0
- BAPT, 23, Lots
- TAX10, 2, 20
- DBAT, 4, 55
- TycA, 0, 0
- Control, 0

- Calculating Efficiency

CFU/ug = [(Colony #)* (Dilution)*(1000 ng/ug)] / X (ng/L) = 4.4×107 CFU/ug for E-cloni

- CFU = “Colony Forming Unit”

Notes:

Possible Projects include:

- Cloning hydroxylase enzymes (x7)
- Fermenting Taxadiene (cannot buy)
- Clone 10 genes in Taxol pathway, map how it works (currently unknown)
- Enzyme Engineering - improve enzymes
- NEB videos
- Computation for CRISPR
- Take pictures to document summer work
- Educational Outreach

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 5/20/16

Colony PCR (Emma; Thomas; Adam; Nisa; Parth)

Protocol:

- Colony PCR Protocol

Table1

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	27
3	MM	12.5	337.5
4	SL1	0.25	6.75
5	SR2	0.25	6.75
6	H2O	12	324

Ran Gel on Colony PCR

No samples worked
Only GFP and pSMART had bands present

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

FRIDAY, 5/20/16

Made Ampicillin and Chloramphenicol Stocks (Nisa; Thomas)

Antibiotic Stocks

Made 7 mL of 200 mg/mL of Ampicillin
Made ~7 mL of 25 mg/mL of Chloramphenicol

Made KAN and CM Plates (Ben; Emma)

50 KAN plates
35 CM plates

LB Agar Plates

Made Gels

Gels

Made 3 gels

Creating Parts

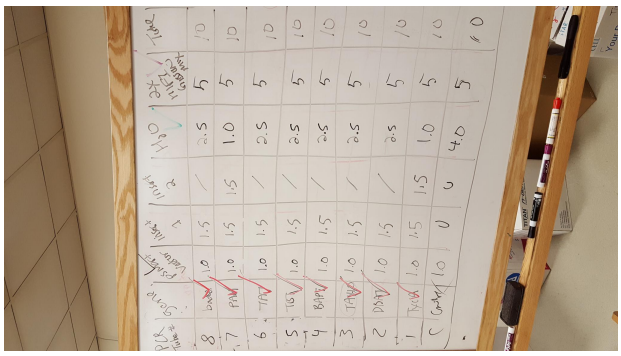
Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

MONDAY, 5/23/16

Gibson Assembly

Performed the Gibson again with a new recipe - less vector and more water (ran out of vector)
Used 1 uL of vector; 5 uL of mix, and 1.5 uL of inserts

Gibson_Plan_for_5_23_.jpg

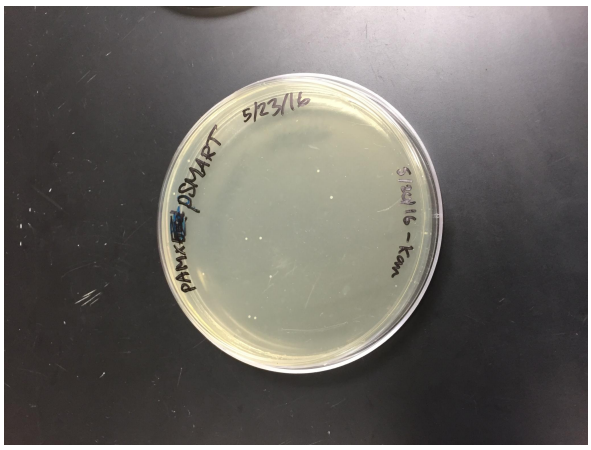


Transformations of Gibsons

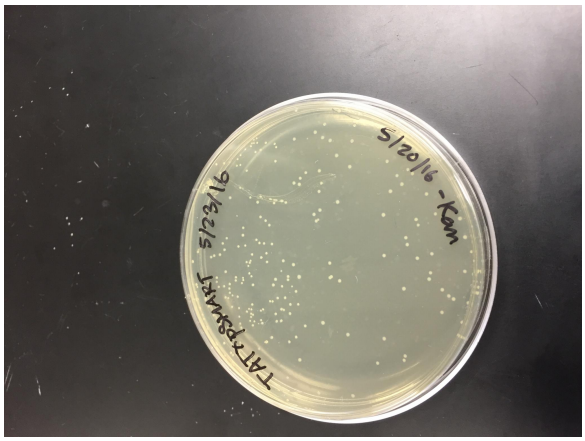
Used 3 uL of DNA; 20 uL of Comp Cells
Recovered for 2-3 hours
All samples electroporated for 654 ms at around 1600 V

Plated Transformation

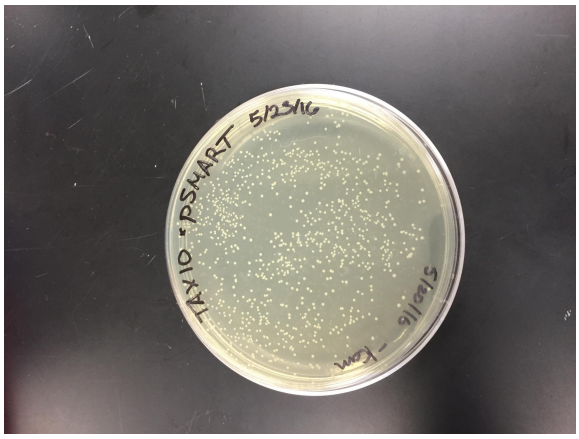
PAM_Transformed_5_23_2016.JPG



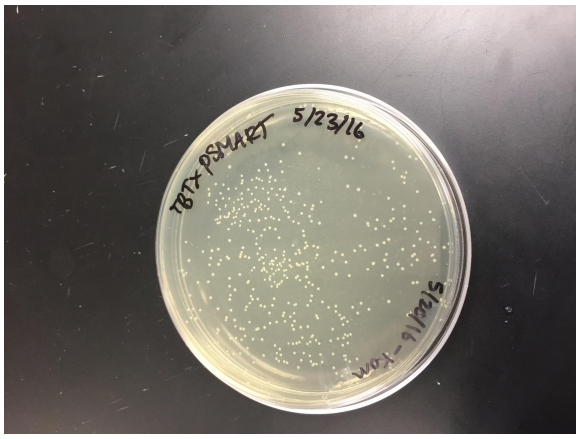
TAT_Transformed_5_23_2016.JPG



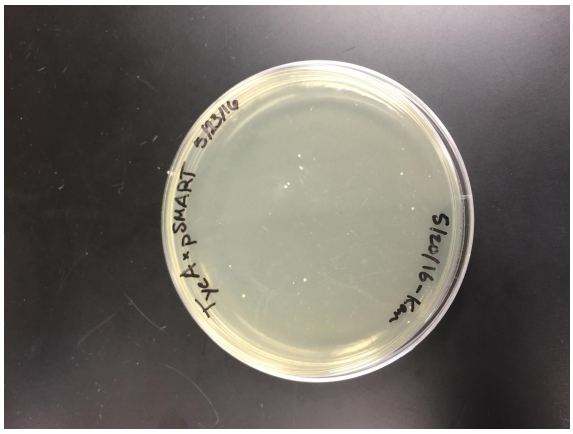
TAX10_Transformed_5_23_2016.JPG



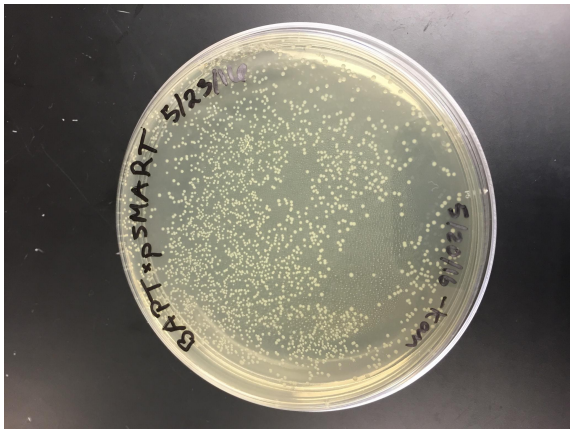
TBT_Transformed_5_23_2016.JPG



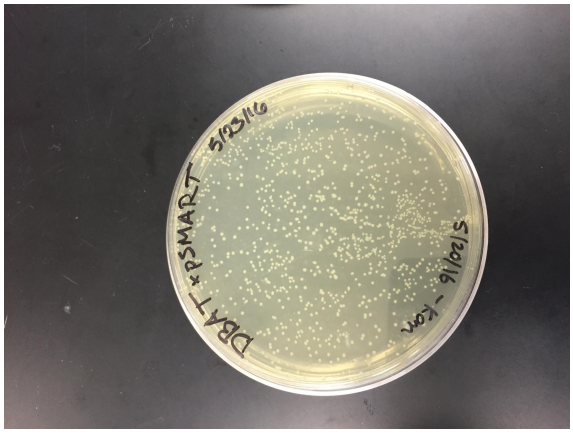
TycA_Transformed_5_23_2016.JPG



BAPT_Transformed_5_23_2016.JPG



DBAT_Transformed_5_23_2016.JPG



BadA did not grow any colonies

Colony PCR of Old Plates

Plates from 5/20/2016

Protocol:

- Colony PCR Protocol

Table2

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	16
3	MM	12.5	200
4	SL1	0.25	10
5	SR2	0.25	10
6	H2O	12	200

Inoculation of E. cloni (Parth; Nisa)

Inoculated a colony of E. cloni in 35 uL of LB broth

Creating Parts

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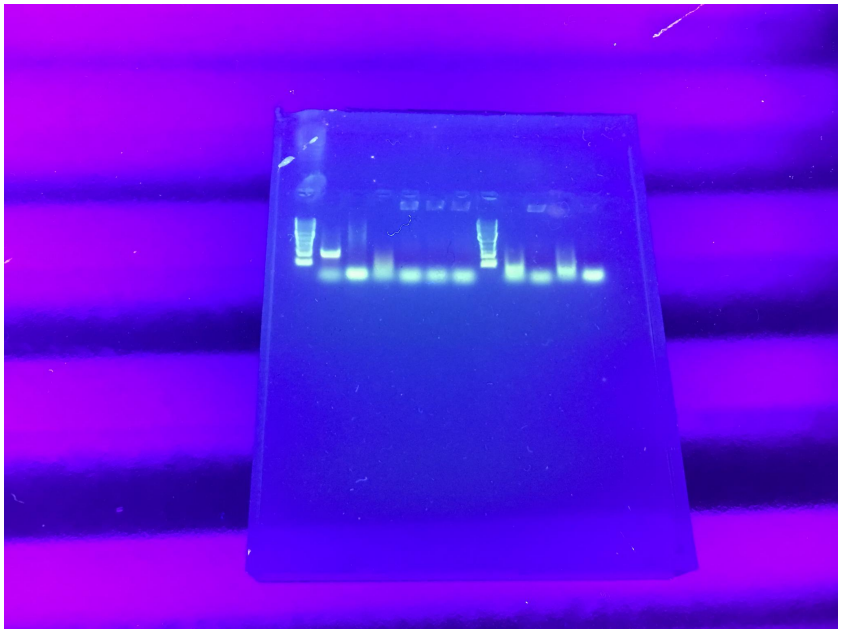
TUESDAY, 5/24/16

Ran Gels on 5/19 Gibsons

8 ul of ladder; 2 ul of DNA stain; 10 ul of DNA; 160 V for 20 min

524_PCR_Colony_Screening_Gel.jpeg



1: Ladder
2: Positive Control
3: Negative Control
4: tycA
5: DBAT
6: TAX10
7: BAPT
8: Ladder
9: TBT
10: TAT
11: PAM
12: BadA
13: Empty

There were no discernible bands; results do not align with expected transformation success
Cause is unknown as there were numerous colonies on plate but no inserts detectd
Because the mini prepped positive control clearly worked, hypothesis is that the econoTaq master mix is not successfully breaking down the cells to allow the DNA inside the nucleus to replicate.

Colony PCR of Gibsons from 5/23 (Ben; Emma)

8 colonies were celected from each plate except BadA
Made LB tubes with 50 mL of KAN + LB
Protocol:

- Colony PCR Protocol

Transformation of badA into Comp Cells

Used badA Gibson from 5/23 and E. cloni from 5/24
Separated Gibson into three different transformations (T1, T2, and T3)
Recovered for 3 hours before plating onto KAN plates
    Left overnight in incubator at 37 C
Electroporation Results:

- T1: 250 V; 654 ms
- T2: 1690 V; 654 ms
- T3: 660 V; 654 ms

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

TUESDAY, 5/24/16

Preparation of E. cloni Comp Cells (Parth; Nisa)

Electrocompetent Cells

Left culture overnight; did not check OD
Washed with glycerol 6 times

Forgot to do the final spin down and resuspension of equal volume of glycerol (these steps were not written in the protocol at the time, protocol has been updated)

Aliquoted 50 uL into 1.7 mL epi tubes

Made Gels

Made 2 more gels; wrapped in cling wrap and stored in 4 C fridge

Gels

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

WEDNESDAY, 5/25/16

Ran Gels for Colony PCR from 5/24

Protocol:

- Gels

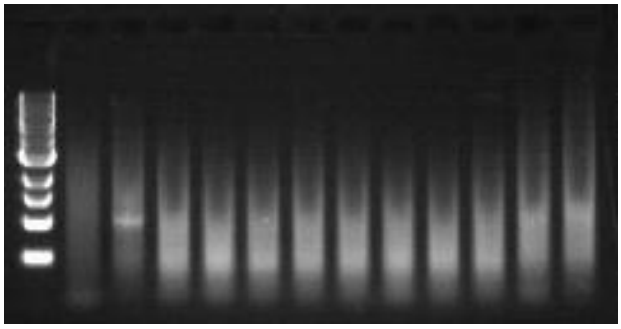
Used 10 uL of DNA instead of 5 uL of H20 and 5 uL of DNA

The Controls were diluted with 7uL of H2O in order to have enough to use in every gel (there was only 25 uL available after the PCR). The controls were also diluted by H2O in each well (5 uL of DNA and 5 uL of H2O). Controls were yibDp-GFP (+) and pSmart, no insert (-)

Two sets of three Gels ran for 35 min at 160 V

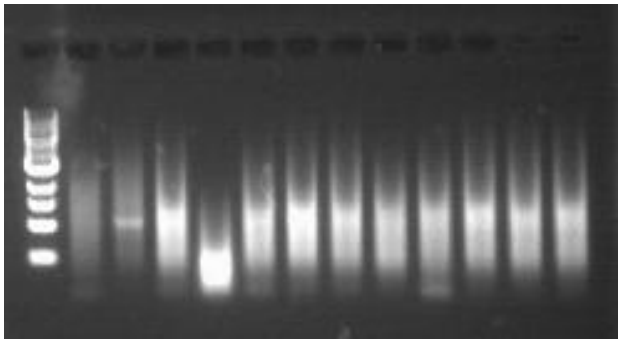
Note: Controls seemed to be the flipped version of what was expected. Assumed that controls were mislabeled

05252016_Angelica1.jpg



1: Ladder
2: Negative Control
3: Positive Control
4-11: PAM 1-8
12-13: BAPT 3-4

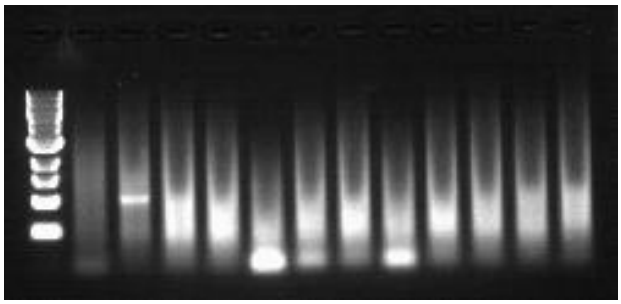
05252016_Brandon1.jpg



1: Ladder
2: Positive Control
3: Negative Control
4-11: DBAT 1-8
12-13: BAPT 1-2

*DBAT 2 (well 5) was a poor band length; less sample was added

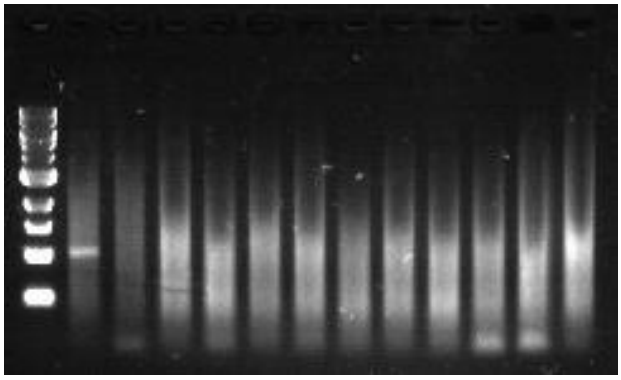
05252016_Suzie1.jpg



1: Ladder
2: Positive Control
3: Negative Control
4-11: TAT 1-8
12-13: BAPT 5-6

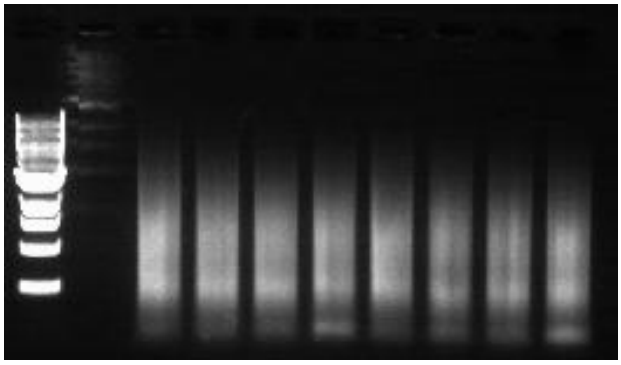
*TAT 3-6 (wells 6-9) had bands too small

05252016_Angelica2.jpg



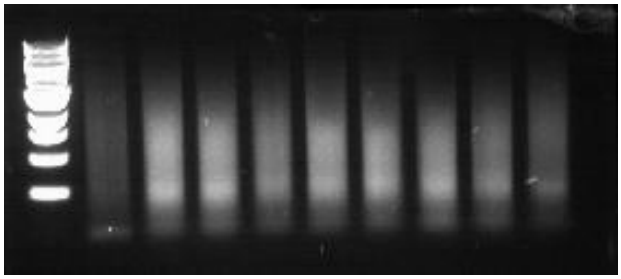
1: Ladder
2: Negative Control
3: Positive Control
4-11: TBT 1-8
12-13: BAPT 7-8

05252016_Brandon2.jpg



1: Ladder
2: Negative Control
3-10: TAX10 1-8

05252016_Suzie2.jpg



1: Ladder
2: Negative Control
3-10: TycA 1-8

DNA PCR (Emma; Thomas)

Goal: To determine if the Gibsons worked; Is the Colony PCR lysing the cells enough?

Used Colony PCR protocol but added 1 uL of DNA instead of 1 colony

For BadA, 2 uL of dH2O was added to the gibson and the entire volume added to the PCR tube

Protocol:

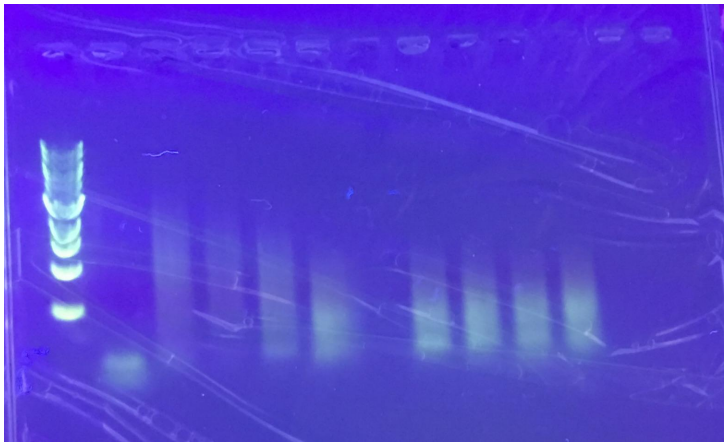
- Colony PCR Protocol

Table3

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	10
3	MM	12.5	125
4	SL1	0.25	2.5
5	SR2	0.25	2.5
6	H2O	12	120

Ran Gel

05252016_PCRAmplificationofGibsonReactions.jpeg



1: Ladder
2: pSMART
3: badA
4: PAM
5: TAT
6: TBT
7: Empty
8: BAPT
9: TAX10
10: DBAT
11: TycA
12-13: Empty

No insert bands appeared
Suspected that the yib and pSMART labels were switched as the control was a pSMART and not the intended yib; labeling has been changed on tubes and on the picture.

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

WEDNESDAY, 5/25/16

Made Gels

Gels

Made 3 gels

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 5/26/16

Econo Taq PCR (Ben)

Goal: Test YibDp and pSmart in freezer to see if they are mislabeled; Is there a better Master Mix Recipe?

Protocol:

- Colony PCR Protocol

Two Master Mixes were made

- MM1 used the protocol given us by Charlie (0.25uL of each primer).
- MM2 used the protocol Adam remembers (1.25uL of each primer in each tube).

The YibDp and the pSmart in the freezer were run on the PCR machine under each of these MM conditions. The mixes are given below:

- Added 25uL EconoTaq / .5uL SL1 / .5uL SR2 / 24uL pH2O to Master Mix 1. 23uL of this was added to two tubes. 1.5uL DNA added after.
    - Each Reaction: 12.5uL EconoTaq / .25uL each primer / 12uL pH2O / Total = 25uL - 2uL to account for potential pipetting error
- Added 25uL EconoTaq / 2.5uL SL1 / 2.5uL SR2 / 20uL pH2O to Master Mix 2. 23uL of this was added to two tubes. 1.5uL DNA added after.
    - Each Reaction: 12.5uL EconoTaq / 1.25uL each primer / 10uL pH2O / Total = 25uL - 2uL to account for potential pipetting error.
- These were run on the same PCR protocol as yesterday. (98C for 5min, then cycle x35: (98C for 30s) / (50C for 30s) / (72C for 3min)

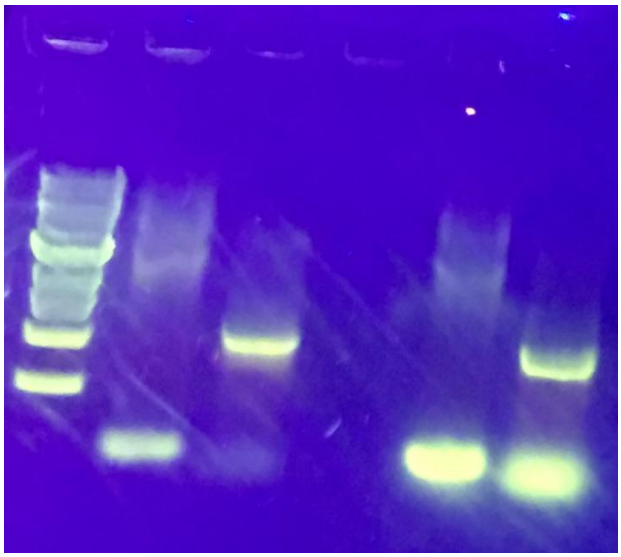
Note: 3min is overkill… Whoops; specifically, the increased extension time is unnecessary due to our insert being ~1kb

The PCR was then ran on a gel

Expectation:

- 1kb band in both yibDp-GFPs, garbage in pSmart (since there is no insert)
- If the results of the samples seem flipped, then the minipreps of the samples located in the fridge have their labels swapped
- If the samples with 1.25 µL of primer are clearer, then the PCR protocol will be updated with this improvement

05/26/2016 psmart vs yibd.jpeg



1: Ladder
2: yibDp-GFP *
3: pSMART *
4: Empty
5: yibDp-GFP **
6: pSMART **

*0.25 uL of primer
** 1.25 uL of primer

The bands indicate that the minipreps had their labels swapped.
PCR machine is proven effective at doing PCR.
0.25 uL of primer is adequate for good PCR results.

Inoculations (Ben)

The following culture samples were inoculated from single colony plates (2 colonies per plate) into 5mL of LB+Kan. The numbers were marked with the corresponding colonies on the plates:

- BAPT-1
- BAPT-2
- DBAT-1
- DBAT-2
- TAT-1
- TAT-2
- TycA-1
- TycA-2
- PAM-1
- PAM-2
- TBT-1
- TBT-2
- BadA-1
- BadA-2
- TAX10-1
- TAX10-2

5/27 All cultures grew except for BadA-1

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 5/27/16

Freezer Stocks/Miniprep/Concentration Check of Inoculations

Protocols:

- Freezer Stocks Protocol
- Miniprep Protocol NEB
- Testing Miniprep Concentrations

Freezer Stock Edit: Used 500 uL of glycerol and culture instead of 750 uL. Stocks were created for each inoculation from 5/26.
Miniprep Edit: Used a 50 uL elution.

Table4

	A	B	C	D	E	F	G	H	I
1	Sample:	PAM-1	PAM-2	DBAT-1	DBAT-2	TBT-1	TBT-2	TAX10-1	TAX10-2
2	ng/uL:	11	300	88	94	57	175	89	96
3	Sample:	BAPT-1	BAPT-2	TycA-1	TycA-2	BadA-1	BadA-2	TAT-1	TAT-2
4	ng/uL:	101	63	11	48	5	146	59	119

PCR Mini prepped DNA

Used a colony PCR protocol
Protocol:

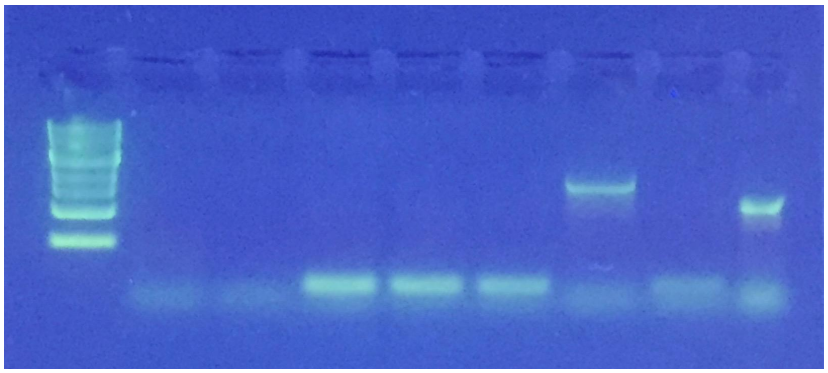
- Colony PCR Protocol

Due to concentration results and limited quantity of Econo Taq, PAM-1, TycA-1, and BadA-1 were not run
Used 0.28 uL of each primer instead of 0.25 uL
Used 1 ng/uL dilutions of the mini prepped DNA
    Dilutions were made is small epi tubes and stored in the -20 C freezer

Gel was run on the PCR Results
Protocol:

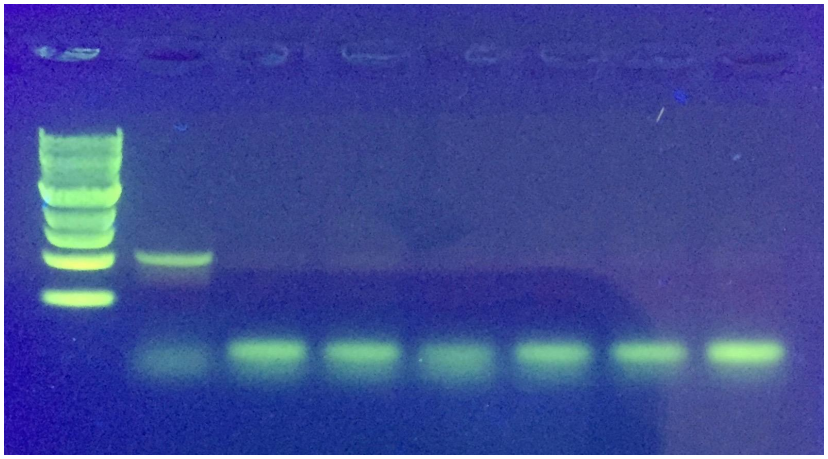
- Gels

5-27-16_Angelica (1).jpg



1: Ladder
2: BadA-2
3: PAM-2
4: TAT-1
5: TAT-2
6: TBT-1
7: TBT-2
8: BAPT-1
8.5: Yib-pD

5-27-16_Brandon (1).jpg



1: Ladder
2: Yib-pD
3: BAPT-2
4: TAX10-1
5: TAX10-2
6: DBAT-1
7: DBAT-2
8: TycA-2

Only TBT-2 showed the expected band size

Sequencing

Total of 16.5 uL of DNA/dilution in each sample tube

Screen Shot 2016-06-05 at 2.11.57 PM.png

Calculations (for 2 samples and primers SL1 and SR2):

- PAM-2: $V_1 = \frac{(100\ ng/\mu L)(16.5\ \mu L)}{(300\ ng/\mu L)} = 5.5\ \mu L\ DNA$ (add 11 uL dH_2O to make dilution)
- TBT-2: $V_1 = \frac{(100\ ng/\mu L)(16.5\ \mu L)}{(175\ ng/\mu L)} = 9.43\ \mu L\ DNA$ (add 7.07 uL dH_2O to make dilution)
- Each Primer: $V_1 = \frac{(5\ \mu Mol)(75\ \mu L)}{(100\ \mu Mol)} = 3.75\ \mu L\ Primer$ (add 71.25 uL dH_2O to make dilution)

5/28: Only TBT-2 and PAM-2 showed positive on the sequencing

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

TUESDAY, 5/31/16

Colony PCR of 5/23 Transformations

Goal: To use probability to find colonies with inserts for those inserts that came back with an empty vector from sequencing

Protocol:

- Colony PCR Protocol

Table5

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	100
3	MM	12.5	1250
4	SL1	0.25	25
5	SR2	0.25	25
6	H2O	12	1200

Ran:

- PAM Colony and Miniprep
- TBT Miniprep
- BAPT 20 colonies
- TAX10 20 colonies
- DBAT 20 colonies
- TAT 14 colonies
- BadA 14 colonies
- pSMART 1 sample
- GFP 1 sample
- ProD 2 colonies

Used an elongation of 3.5 min

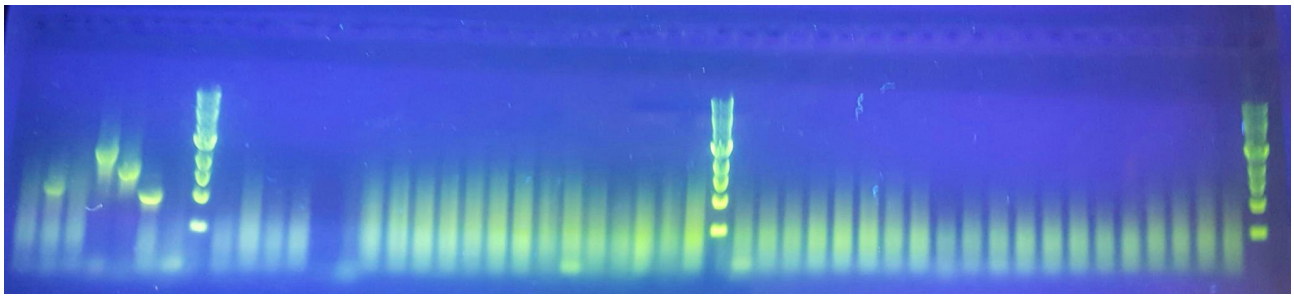
For Gel:

Used a 100 well gell mold
Used 450 mL of agarose; 45 uL of dye
Protocol:

- Gels

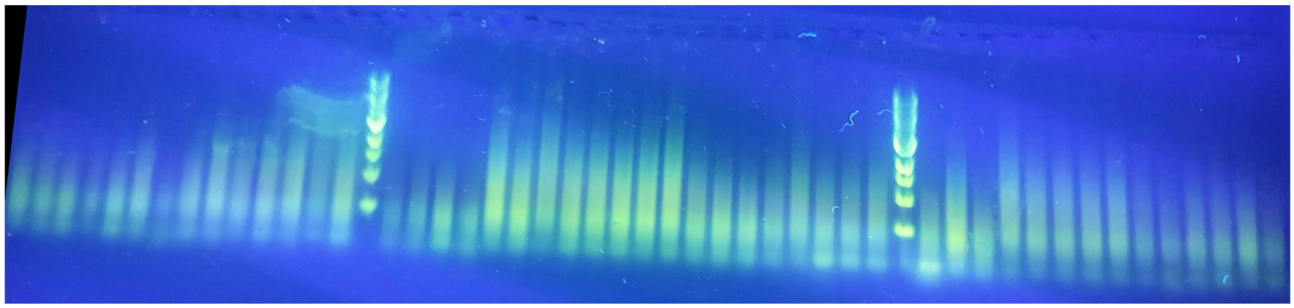
Ran Gels:
Used 10 uL of DNA from PCR; 2 uL of dye
Ran for 35 min at 160 V
During loading, the pipette tips continuously retained some of sample or fell off

5:31:16 Big Bertha Row 1.jpg



1: Pro D
2: Pro D
3: PAM Colony
4: PAM Mini prep
5: TBT Mini prep
6: GFP
7: PSM
8: Ladder
9-29: BAPT 1-20
30: Ladder
31-50: TAX10 1-20

5/31/2016 Big Bertha Row 2.jpg



1-14: TAT 1-14
15: Ladder
16-35: DBAT 1-20
36: Ladder
37-50: BadA 1-14

Only the TBT miniprep, PAM miniprep, TAT colony 9, and TAT colony 10 showed the expected band sizes.

Made dilution of sequence confirmed PAM and TBT

Diluted samples to 50 ng/uL

- For TBT Dilution, used 5.7 uL of DNA with 14.3 uL of pico H2O
- For PAM Dilution, used 3.3 uL of DNA with 16.7 uL of pico H2O

Inoculation of ProD Cultures

Picked 2 colonies from proD plate (proD-1 and proD-2)
Inoculated in 10 mL of LB+KAN

Also Inoculated TAT 9, TAT 10, DBAT 16, DBAT 18, and DBAT 20.

Transformations of TBT and TycA

Protocol:

- Electroporation Protocol

TBT was transformed into two cultures

- TBT 1 - 1710 V 3.9 ms
- TBT 2 - 1710 V 4.0 ms

TycA was transformed into one culture

- TycA - 1580 V 654 ms

Recovered for 3 hours

3 uL of DNA and 20 uL of Comp Cells was used

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

WEDNESDAY, 6/1/16

Freezer Stocks of 5/31 Inoculations

Protocol:

- Freezer Stock Protocol

Used 500 uL of glycerol and culture instead of 750 uL

Created stocks of

- TAT 9
- TAT 10
- DBAT 16
- DBAT 18
- DBAT 20

Double Colony PCR

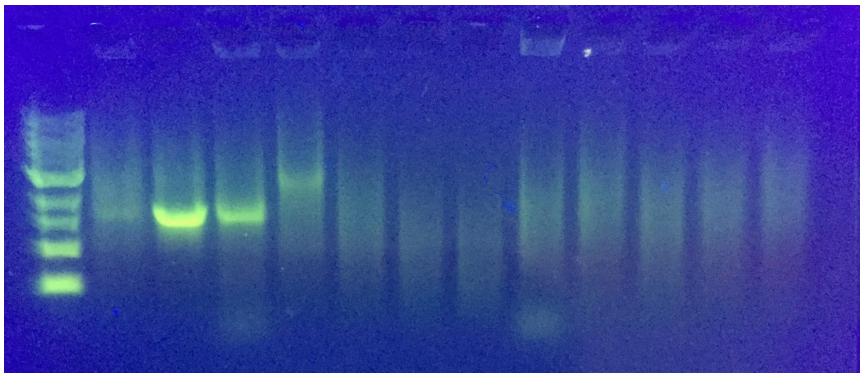
Mike's method of smearing things on the side of the PCR tube before pipetting into the tube was used for all big colonies. The smaller colonies were dipped into the master mix.

- 200uL pipette tips were used to pick and smear the colonies.
- Experimental setup:
    - 8 colonies from: TAX10, BAPT, BadA
        - 2 of each colony, half tested on PCR machine in Teer basement lab and half in Lynch lab. Results will be compared later.
    - 4 controls: PAM-2 Colony, PAM-2 Miniprep, GFP Miniprep, pSmart Miniprep in both lab tests
    - 72 total reactions, made 74 Master mix according to the following:
        - 1x // 74x
        - 12.5 uL Econo // 925 uL
        - .25 uL SL1 // 18.5 uL
        - .25 uL SR2 // 18.5 uL
        - 12 uL H2O // 888 uL
        - 25 uL total // 1850 uL
        - NOTE: WE WERE 7 REACTIONS SHORT. PIPETTES NEED CALIBRATION
    - Protocol used in Teer basement:
        - Start: 98C for 10 min.
        - Cycle (x40)
            - Melt 98C for 45s
            - Anneal 50C for 45s
            - Extend 72C for 3m 30s
                - Time = (1 min / kbase)*length(longest amplificant)
                - Time = 1min/kbase * length(longest amplificant)
        - 4C for inf

Colony PCR

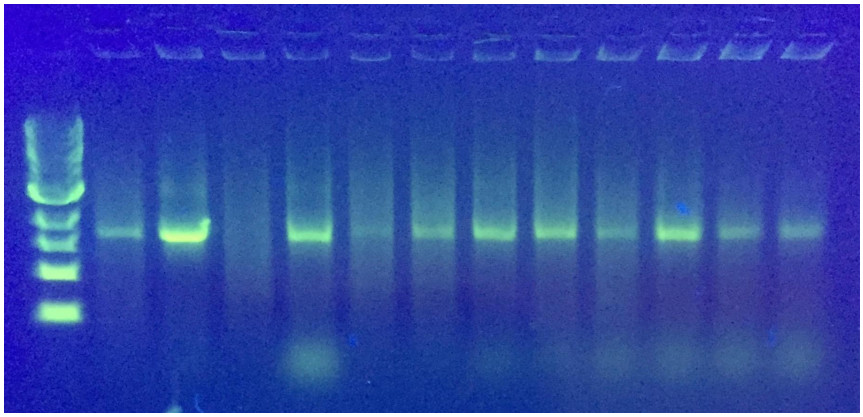
Ran Gel on PCR in both Teer and in Lynch Labs

6-1-16_Angelica.jpg



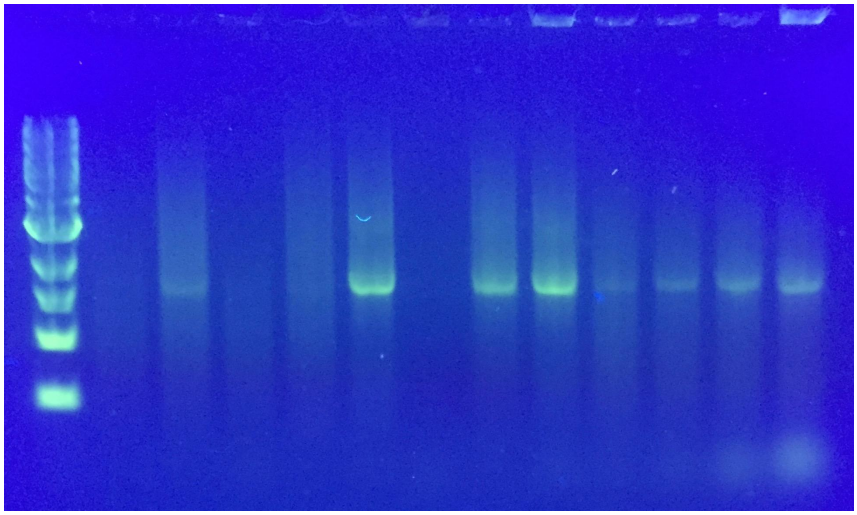
1: Ladder
2: GFP
3: PAM - Mini prep
4: PAM Colony
5: pSMART
6-13: BadA A-H

6-1-16_Brandon.jpg



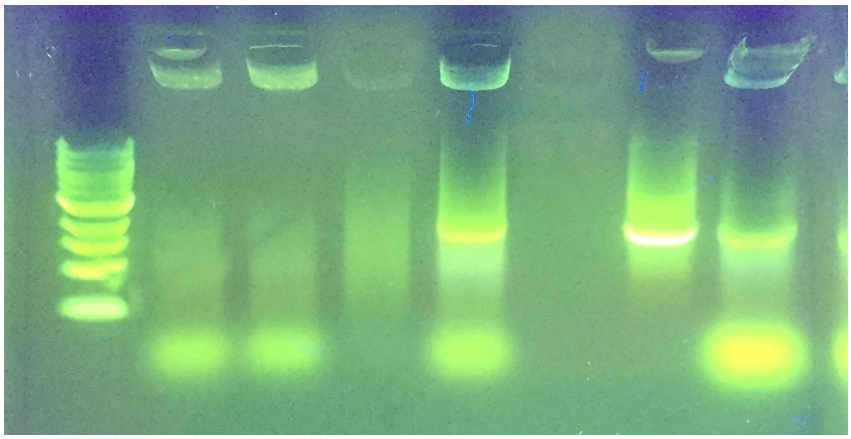
1:Ladder
2-9: TAX10 A-H
10-13: BadA A-D

6-1-16_Suzie.jpg



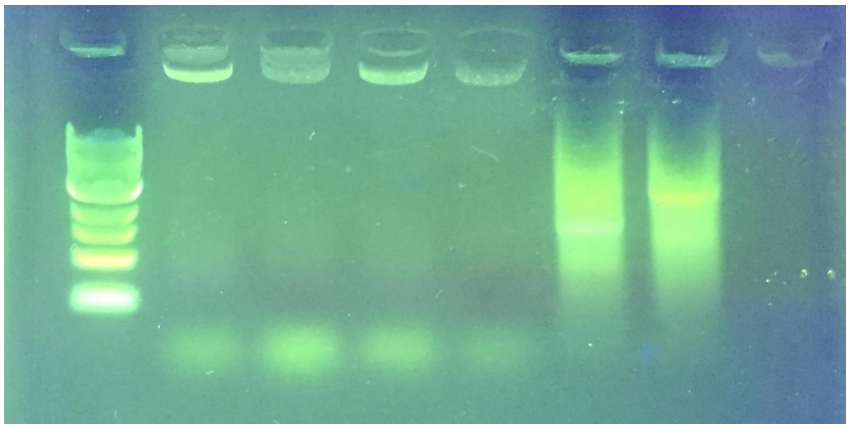
1: Ladder
2-9: BAPT A-H
10-13: BadA E-H

6-1-16_Gel1.jpg



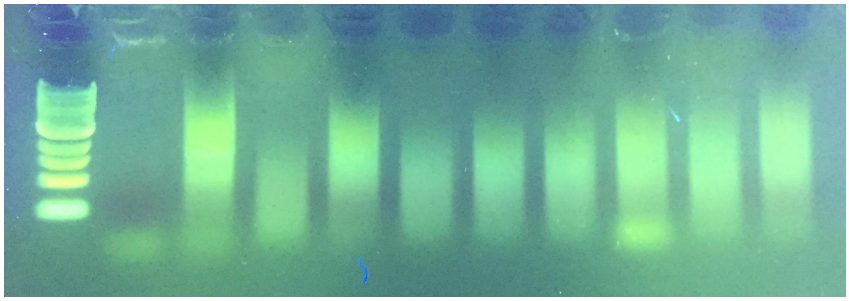
1: Ladder
2-5: BAPT A-D
6: Empty
7: PAM Miniprep
8: PAM Colony

6-1-16_Gel2.jpg



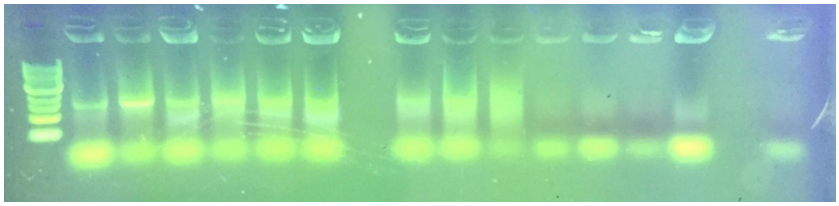
1: Ladder
2-5: BAPT E-H
6: pSMART
7: GFP
8: Empty

6-1-16_Gel3.jpg



1: Ladder
2-3: TAX10 G-H
4-11: BadA A-H

6-1-16_Gel4.jpg



1: Ladder
2-9: BadA A-H
10-15: TAX10 A-F

In the Teer Gels Positive Resutls included:

- TAX10- (A,B,D,E,G,H)
- BAPT- (B, D, E, G, H)

- badA- (A, B, C, D, F, G, H)

Inoculation of Positive Colonies

Colonies denoted in red in the above results were inoculated in 5 mL of LB + KAN and left in the shaker over night

Mini prep/Concentration Check of Successful Transformations from 5/31 (Nisa; Thomas)

Protocols:

- Miniprep Protocol Zyppy
- Testing Miniprep Concentrations

Miniprep Edit: Used 50 uL elution

Table6

	A	B	C	D	E	F
1	Sample	TAT-9	TAT-10	DBAT-16	DBAT-18	DBAT-20
2	ng/uL (Concentration)	121	80	117	72	55

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

WEDNESDAY, 6/1/16

Made KAN Plates

Stored most of KAN plates in bag in 4 C fridge -- the rest stored at room temp on shelf
Made 1 L of Agar for plates - all plates were KAN plate

LB Agar Plates

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 6/2/16

Colony PCR on TycA Plate (Emma; Ben)

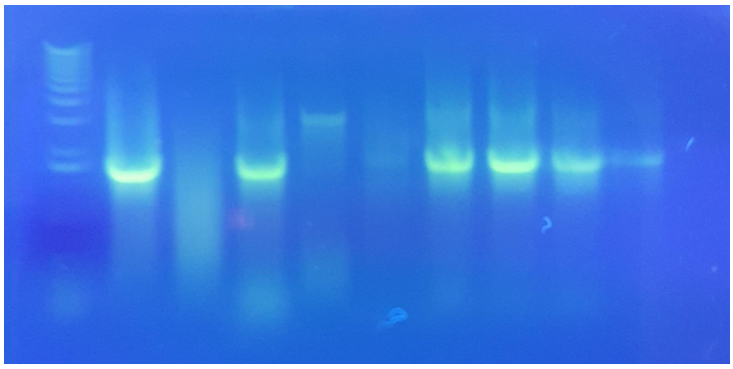
Protocol:

- Colony PCR Protocol

Duplicate NOT run in Lynch Lab
Used an elongation of 4.5 min
Smeared 10 colonies using Mike's method

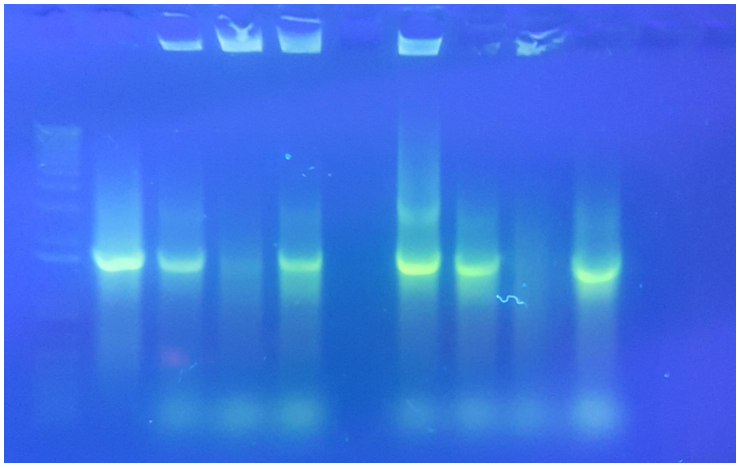
Ran a Gel

6-2-2016 Angelica (TycA).jpg



1: Ladder
2: GFP
3: PAM Mini prep
4: PAM Colony
5: pSMART
6-9: TycA 1-4
10: Spillage from TycA 4

6-2-2016 Brandon (TycA).jpg



1: Ladder
2: GFP
3: PAM Colony
4-5: TycA 9-10
6: Empty
7-10: TycA 5-8

Due to Gel results, TycA Samples 1-6, 9, and 10 were chosen for inoculation

Inoculation of Colonies

TycA Samples 1-6, 9, and 10 were inoculated in 5 mL of LB+KAN

Freezer Stocks/Miniprep/Concentration Check/Sequencing of Successful 6/1 Transformations (Parth, Nisa)

Samples:

- TAX10- (A,B,D,E,G,H)
- BAPT- (B, D, E, G, H)
- badA- (A, B, C, D, F, G, H)

Protocols:

- Freezer Stocks Protocol
- Miniprep Protocol Zyppy
- Testing Miniprep Concentrations
- Preparation for Sequencing

Miniprep Edit: Used a 50 uL elution

Table9

	A	B	C	D	E	F	G	H
1	Sample:	TAX10-A	TAX10-B	TAX10-D	TAX10-E	TAX10-G	TAX10-H	
2	Concentration (ng/uL)	69	203	210	190	132	94	
3	Sample:	BAPT-B	BAPT-D	BAPT-E	BAPT-G	BAPT-H		
4	Concentration (ng/uL)	61	51	146	142	180		
5	Sample:	badA-A	badA-B	badA-C	badA-D	badA-F	badA-G	badA-H
6	Concentration (ng/uL)	52	209	60	100	165	149	130

Results of Testing DNA Concentration

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

THURSDAY, 6/2/16

Made Gels

2 Gels were made

Gels

Autoclaved Tips and Glassware

Autoclaved 10 boxes of 200 tips and 5 boxes of 10 tips on gravity cycle
Autoclaved beakers and flasks

Made 20% Glycerol Stocks

Glycerol Stocks

40 mL of 100% Glycerol was added to 160 mL pH2O

Freezer Purge

The freezer stocks were gone through and all negative sequence stocks were discarded.

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 6/3/16

Freezer Stocks/Miniprep/Concentration Check of TycA

Labels were pGEM40-48 for samples TycA 1, 2, 3, 4, 5, 6, 8, 9, 10
Protocols:

- Freezer Stocks Protocol
- Miniprep Protocol Zyppy
- Testing Miniprep Concentrations

Miniprep Edit: First step is 3500 rmp for 8 min

Table11

	A	B	C	D
1	Sample:	tycA 1	TycA 2	TycA 3
2	Concentration (ng/uL)	83	25	42
3	Sample:	TycA 4	TycA 5	TycA 6
4	Concentration (ng/uL)	75	36	53
5	Sample:	TycA 8	TycA 9	TycA 10
6	Concentration (ng/uL)	51	51	71

Transformations

Protocol:

- Electroporation Protocol

DBAT, BAPT, TAX10, and TycA were transformed into competent E Cloni Cells

Recovered in Shaker for 2 hours.

Table10

	A	B	C
1	Sample	Volts	milliseconds
2	BAPT	1520	654
3	TAX10	1540	654
4	DBAT 1	1570	0.9
5	DBAT 2	1570	654
6	TycA 1	1610	1.3
7	TycA 2	1630	1.3

Transformations were then plated:

Table12

	A	B	C
1	Sample	10 uL	100 uL
2	BAPT	A	B
3	TAX10	A	B
4	DBAT 1	A	B
5	DBAT 2	A	B

TycA 1 and TycA 2 were both plated with 200 uL

Inoculated TycA

(1-8) small colonies and placed in shaker

Sent for Sequencing

Protocol:

- Preparation for Sequencing

These samples were sent for sequencing:

- TycA 1-6, 8-10
- BadA-B

Did not dilute,

Sent 10 uL of each sample

Only sent in 1 primer

- TycA sent in with SL1
- BadA-B sent in with SR2

Diluted Samples

Diluted samples of BadA-B and TAT-9 to 50 ng/uL

- BadA => 1.2 uL DNA; 3.8 uL pH2O
- TAT => 2.1 uL DNA; 2.9 uL pH2O

Colony PCR

Protocol:

- Colony PCR Protocol

Made a double PCR; ran one set on the old PCR machine; ran the new set on the new PCR machine

Samples run were:

- pSMART
- GFP
- ProD Colony
- ProD Colony
- PAM-2 Colony
- PAM-2 Miniprep
- TBT Miniprep
- BadA-B Miniprep
- TAT-9 Miniprep

Table13

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	24
3	MM	12.5	300
4	SL1	0.25	6
5	SR2	0.25	6
6	H2O	12	288

Added a new protocol to the new PCR machine

- Saved in a new folder "IGEM 2016" as "IGEM COL V1"
    - Ran for 30s at 50, 72, and 98 with an elongation of 4.5 min

Notes:

BadA is Sequence Confirmed

Tax10 and BAPT were blanks

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

FRIDAY, 6/3/16

Made Gels

Made 2 new gels and left in fridge for weekend

Gels

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SATURDAY, 6/4/16

Freezer Stocks of TycA and addition to Database

Protocol:

- Freezer Stock Protocol

Labels were pGEM049-056 for samples TycA 1, 2, 3, 4, 5, 6, 7, 8

Mini Prep Inoculations for TycA

Protocol:

- Miniprep Protocol NEB

The following inoculations were mini prepped:

- TycA 1
- TycA 2
- TycA 3
- TycA 4
- TycA 5
- TycA 7
- TycA 8
- TycA 9

Used a 50 uL elution.

Left minipreps for concentration testing on monday.

Concentrations (from 6/6 for these minipreps):

Protocol:

- Testing Miniprep Concentrations

Tested Concentrations using nanodrop

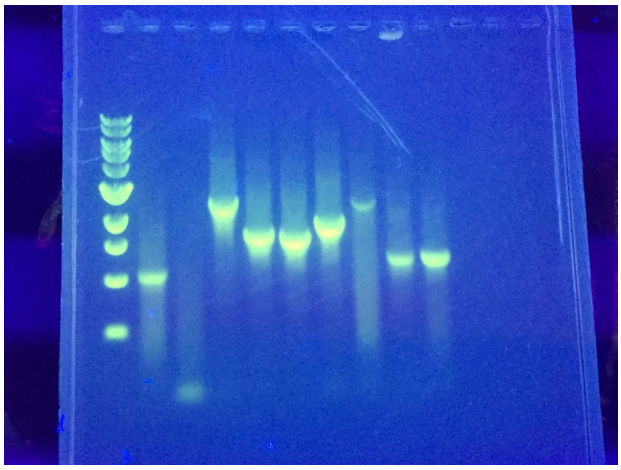
Table17

	A	B	C	D	E	F	G	H
1	Sample	TycA-2	TycA-3	TycA-4	TycA-5	TycA-7	TycA-8	TycA-9
2	Round 1 (ng/uL)	33	10	22	6	3	5	5
3	Round 2 (ng/uL)	13	43	52	3	19	4	22
4	Concentration used:	33	43	52	N/A	19	N/A	22

Ran Gel on 6/3 PCR

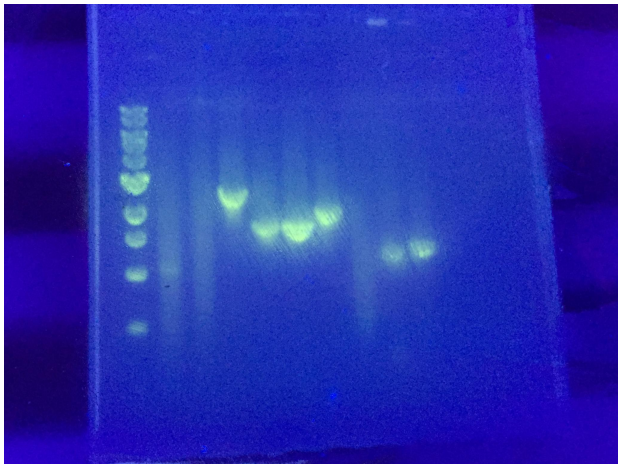
The two PCR on 6/2 were run on two PCR machines. Samples tested in Angelica were from the old PCR machine and samples tested on Brandon were from the new PCR machine.

06/03/2016 Angelica JPG



30s denature, 30s anneal, 3:30 elongation, 35 cycles, oldmachine
1: Ladder
2: GFP
3: pSMART
4: PAM Miniprep
5: TBT Miniprep
6: TAT Miniprep
7: BAdA Miniprep
8: PAM Colony
9: ProD 1
10: ProD2
11-13: Empty

06/03/2016 Brandon.JPG



30s denature, 30s anneal, 3:30 elongation, 35 cycles, new machine
1: Ladder
2: GFP
3: pSMART
4: PAM Miniprep
5: TBT Miniprep
6: TAT Miniprep
7: BAdA Miniprep
8: PAM Colony
9: ProD 1
10: ProD2
11-13: Empty

Plated Gibson Transformations (Parth)

DBAT, TAX10, and BAPT were all plated on to Kan plates with the following uL amounts.

- 10 uL, 50 uL, 100 uL of transformation per enzyme

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SUNDAY, 6/5/16

Colony PCR of Controls

Goal: Testing if the 45 second annealing and denature steps affected controls

Protocol:

- Colony PCR Protocol

PCR of controls:

- Standard PCR mix used
- Miniprepped DNA diluted to 50ng/ul concentration

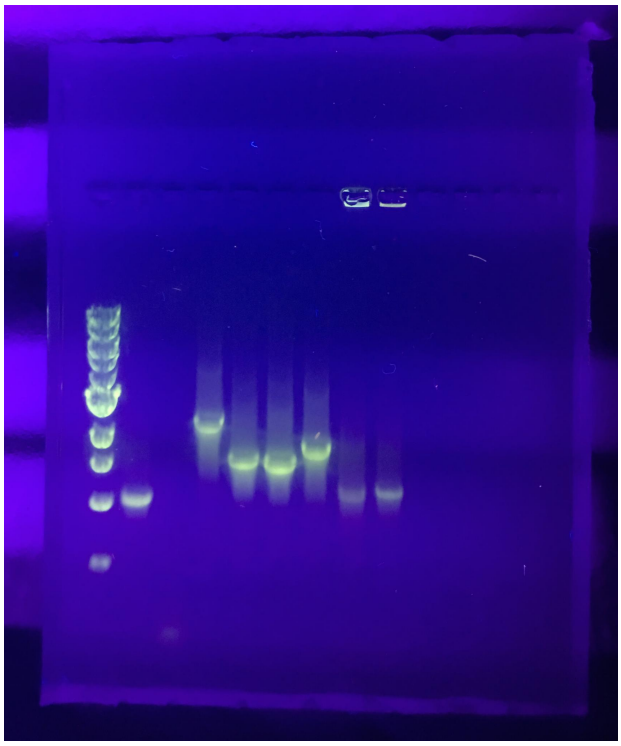
Samples: GFP, Psmart, Pam Mini, TBT mini, TAT mini, BADA Mini, PAM colony, ProD 1, Prod D2

Table16

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	24
3	MM	12.5	300
4	SL1	0.25	6
5	SR2	0.25	6
6	H2O	12	288
7	Total	25	600

Ran Gel

image (4).jpeg



45s denature, 45s anneal 3:30 elongation 35 cycles, Old machine
1:Ladder
2:GFP
3:pSMART
4:PAM miniprep
5:TBT miniprep
6:TAT miniprep
7:badA miniprep
8:proD colony
9:proD colony

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

SUNDAY, 6/5/16

Gels for PCR (Jaydeep)

Made 4 gels, 200mL total

Gels

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

MONDAY, 6/6/16

Colony PCR (Ben; Nisa)

Goal: Try different times and protocols to see where our controls stopped working

Notes:
-Running 4:30 elongation time on old PCR (Mike) and new PCRA (Charlie). 3:30 on new PCRB (NEB)
- Ran with 40x cycles

Samples used:
- PSM, GFP, PAM C, PAM M , BadA M, TBT M, TAT M, ProD C, ProD C.

(Time in: About 10:20a)

Table14

	A	B	C	D	E
1			(# col +1 control)	Using Old MM (Used in the first 6 that went into Mike)	New MM to make
2	Thing	1 rxn	30	6	24
3	MM	12.5	375	75	300
4	SL1	0.25	7.5	1.5	6
5	SR2	0.25	7.5	1.5	6
6	H2O	12	360	72	288
7	Total	25	750	150	600

Left Gels for tomorrow

Miniprep of DBAT, TAX10, and BAPT (Jay > Ben, Parth)

Goal: Miniprep....

Protocol:

- Miniprep Protocol NEB

50 uL elution of dH2O

Inoculation samples used:

- TAX10 (1-6)
- DBAT (1-6)
- BAPT (1-6)

Concentrations:

Protocol:

- Testing Miniprep Concentrations

Table18

	A	B	C	D	E	F	G
1	TAX10 Sample	1	2	3	4	5	6
2	Concentration (ng/uL)	127	119	-12	40	98	84
3	DBAT Sample	1	2	3	4	5	6
4	Concentration (ng/uL)	-2	59	122	13	116	63
5	BAPT Sample	1	2	3	4	5	6
6	Concentration (ng/uL)	24	41	23	14	73	100

Transformation of Sequence Confirmed Mini prep into 286 Cells(Thomas; Emma)

Goal: That our miniprepped sequence confirmed cells can be transformed into the more robust 286 cells

Protocol:

- Electroporation Protocol

Details:

- Transformed: sequence confirmed minipreps of PAM-2, TBT-2, TAT-9, BadA-B
- Used 500ul tubes
- because of a shortage of 1.5ml tubes

left incubator for 2.5 hours

Recorded volts during electroporation

Table15

	A	B	C
1	Sample	Volts	Miliseconds
2	TAT -9 A	610	654
3	TAT-9 B	1690	3.5
4	BadA B	1680	3.8
5	PAM-2	1680	3.4
6	TBT-2	1710	4

Sequencing (Parth, Thomas)

Protocol:

- Preparation for Sequencing

Sent the following samples for sequencing along with SL1 primer:

- TycA- (2,3,4,7,9)

- TAX10- (1,2,4,5,6)
- DBAT- (2,4,5,6)*
    - Accidentally sent sample 4 instead of 3
- BAPT- (2,5,6)

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

MONDAY, 6/6/16

Gels for PCR (Ben; Jaydeep)

Made 4 gels, 200mL tot

Gels

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

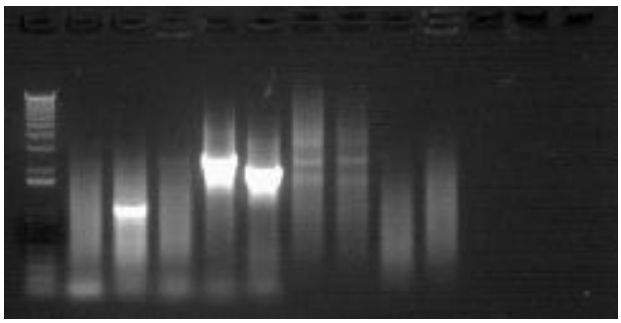
TUESDAY, 6/7/16

Ran Gels for PCR Colony on 6/6

Protocol:

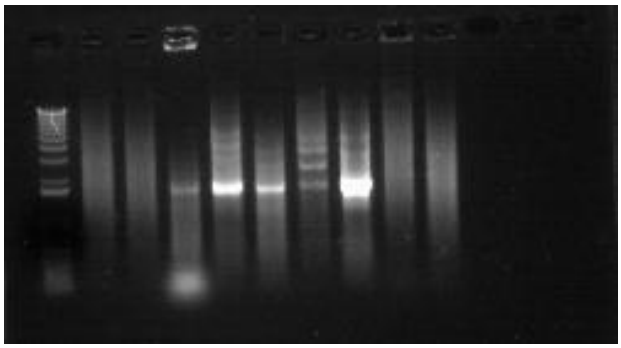
- Gels

Mike-4m30s_elong.jpg



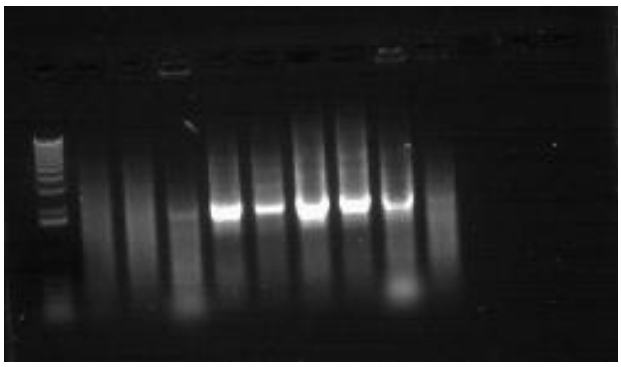
denature 45s, anneal 45s, elongation 4:30, 40 cycles, old machine
1: Ladder
2: pSMART
3: GFP
4: PAM Colony
5: PAM Miniprep
6: BadA Miniprep
7: TBT Miniprep
8:TAT Miniprep
9: ProD Colony
10: ProD Colony
11-13: Empty

Charlie-4m30s_elong.jpg



denature 45s, anneal 45s, elongation 4:30, 40 cycles, new machine
1: Ladder
2: pSMART
3: GFP
4: PAM Colony
5: PAM Miniprep
6: BadA Miniprep
7: TBT Miniprep
8:TAT Miniprep
9: ProD Colony
10: ProD Colony
11-13: Empty

NEB-3m30s_elong.jpg



denature 45s, anneal 45s, elongation 3:30, 40 cycles, new machine
1: Ladder
2: pSMART
3: GFP
4: PAM Colony
5: PAM Miniprep
6: BadA Miniprep
7: TBT Miniprep
8:TAT Miniprep
9: ProD Colony
10: ProD Colony
11-13: Empty

Well that didn't work quite right... Time for the next experiment!

PCR again: Find working Protocol for long TycA inserts

Protocol:

- Colony PCR Protocol

GFP, Psm, PAM-M, PAM-C, TBT-M, TBT-C, TAT-M, TAT-C, BadA-M, BadA-C, ProD-1, ProD-2

Note: Add in 1 uL of 20-50ng/uL DNA.

Table7

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	38
3	MM	12.5	475
4	SL1	0.25	9.5
5	SR2	0.25	9.5
6	H2O	12	456
7	Total	25	950

Labels:

Table8

	A	B	C	D	E	F	G	H	I	J	K	L
1	1	2	3	4	5	6	7	8	9	10	11	12
2	GFP	P-Smart	PAM-C	TBT_C	TAT-C	BadA-C	ProD-1	ProD-2	PAM-M	TBT-M	TAT-M	BadA-M

Old PCR machine on Angelica

(98C for 30s / 50C for 30s / 72C for 4m30s) x35

Left (New PCR A) on Suzie

(98C for 30s / 50C for 30s / 72C for 4m30s) x35

Right (New PCR B) on Brandon

(98C for 30s / 50C for 30s / 72C for 3m30s) x40

Inoculations (Adam)

Made liquid cultures of:

- TycA (small colonies)

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

TUESDAY, 6/7/16

Made KAN Plates (Nisa; Parth; Thomas)

Made 52 KAN plates

LB Agar Plates

iGEM Oligos

Project: Duke iGEM 2016
Authors: Adam Yaseen
Date: 2016-06-07

TUESDAY, 6/7/16

Table1

	A	B	C
1	ID#	Date	Description
2	oIG01	6/7/2016	PAM additional sequencing primer
3	oIG02	6/7/2016	TycA additional sequencing primer B
4	oIG03	6/7/2016	TycA additional sequencing primer A
5	oIG04	6/7/2016	TycA additional sequencing primer C
6	oIG05	6/7/2016	badA additional sequencing primer
7	oIG06	6/21/2016	badA point mutagenic forward primer
8	oIG07	6/21/2016	badA supporting mutagenic reverse primer
9	oIG08	6/21/2016	TBT insert mutagenic forward primer
10	oIG09	6/21/2016	TBT supporting mutagenic reversre primer
11	oIG10	6/22/2016	pSMART 1/2 Gibson forward primer
12	oIG11	6/22/2016	pSMART 1/2 Gibson reverse primer
13	oIG12	6/22/2016	pSMART 2/2 Gibson forward primer
14	oIG13	6/22/2016	pSMART 2/2 Gibson reverse primer
15	oIG14	7/11/2016	G-block F
16	oIG15	7/11/2016	G-block R
17	oIG16	7/18/2016	TycA 1/2 R
18	oIG17	7/18/2016	TycA 2/2 F
19	oIG18	7/18/2016	TycA M1 F, fixing for golden gate
20	oIG19	7/18/2016	TycA M1 R
21	oIG20	7/18/2016	TycA M2 F, fixing for golden gate
22	oIG21	7/18/2016	TycA M2 R
23	oIG22	7/20/2016	G-Block M F
24	oIG23	7/20/2016	G-Block M R
25	oIG24	7/20/2016	Tax10 M R
26	oIG25	7/22/2016	pSB1C3 F
27	oIG26	7/22/2016	pSB1C3 R

28	oIG27	7/26/2016	Amp2 F
29	oIG28	7/26/2016	Amp1 R
30	oIG29	9/21/2016	pSB1C3 5' M F
31	oIG30	9/21/2016	pSB1C3 5' M R
32	oIG31	9/21/2016	badA RBS M F
33	oIG32	9/21/2016	bada RBS M R
34	oIG33	9/21/2016	pSB1C3 3' M F
35	oIG34	9/21/2016	pSB1C3 3' M R
36	oIG35	9/21/2016	DBAT RBS M F
37	oIG36	9/21/2016	DBAT RBS M R
38	oIG37	9/21/2016	BAPT RBS M F
39	oIG38	9/21/2016	BAPT RBS M R
40			

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

WEDNESDAY, 6/8/16

Miniprep TycA Inoculations from 6/7 (Ben; Nisa; Ben)

Protocol:

- Mini Prep Protocol NEB

50 uL elution used

Inoculation samples used:

- TycA 1, 3, 4, 9

Concentrations:

Tested concentrations using nanodrop (Testing Concentrations Protocol)

Table20

	A	B	C	D	E
1	TycA Sample	1	3	4	9
2	Concentration (ng/uL)	13	13	9	4

Colony PCR: Testing overnight PCRs

Protocol:

- Colony PCR Protocol

Each will be run with:

1. GFP
2. Psmart
3. PAM-C
4. PAM-M
5. TBT-C
6. TBT-M
7. ProD

Each Protocol:
[98C for X sec / 50C for X sec / 72C for 3m30s] x 35

Old: X = 45s
Left: X = 45s
Right: X = 30s

Table19

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	23
3	MM	12.5	287.5
4	SL1	0.25	5.75
5	SR2	0.25	5.75
6	H2O	12	276
7	Total	25	575

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 6/9/16

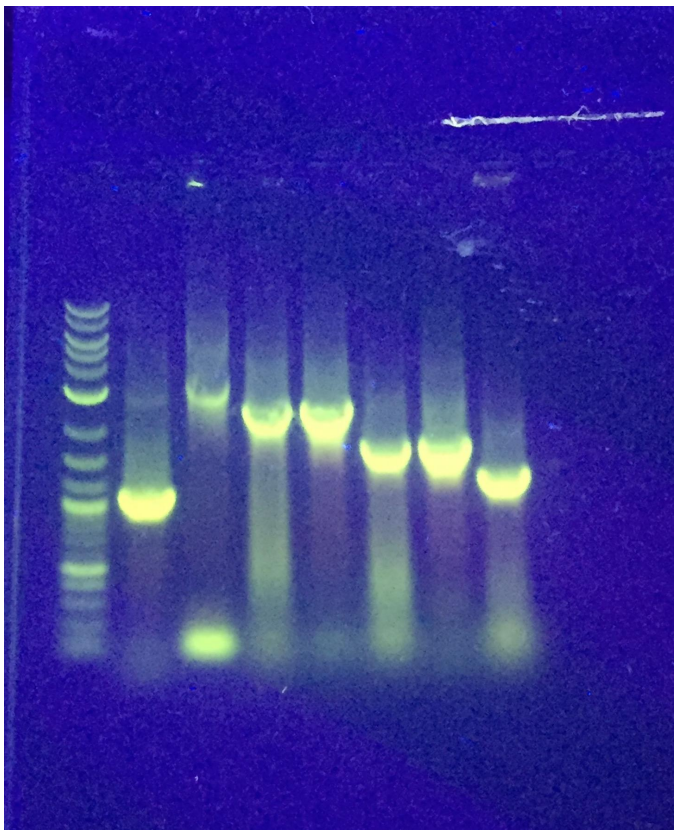
Ran Gel on Overnight PCR from 6/8

Protocol:

- Gels

Ran gels for

Suzie 6/9

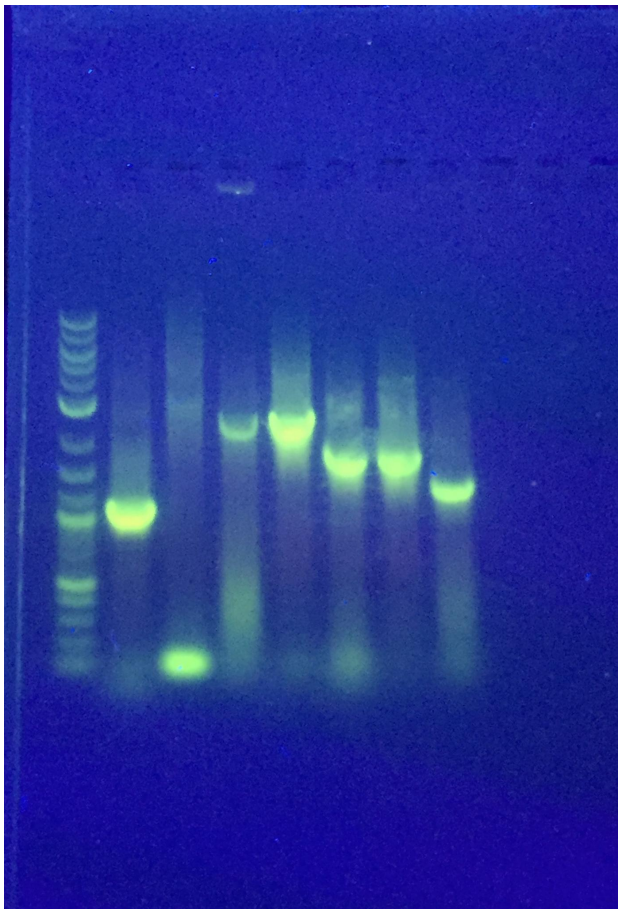


RIGHT: [98C for 30 sec / 50C for 30 sec / 72C for 3m30s] x 35

NOTE: overnight caused some resideue bands to appear in the negative control

1. Ladder
2. GFP
3. Psmart
4. PAM-C
5. PAM-M
6. TBT-C
7. TBT-M
8. ProD

Brandon 6/9

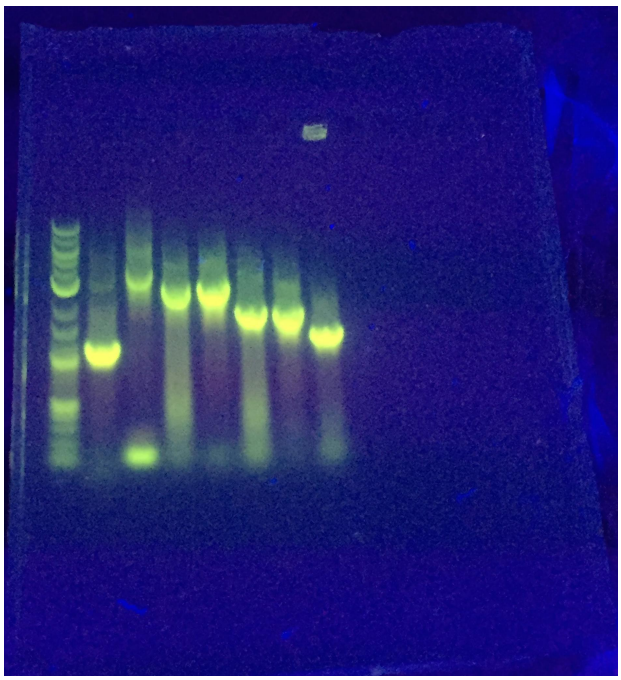


OLD: [98C for 45 sec / 50C for 45 sec / 72C for 3m30s] x 35

NOTE: Faint band on negative control likely due to overnights. What is the long crap on PAM-C?

1. Ladder
2. GFP
3. Psmart
4. PAM-C
5. PAM-M
6. TBT-C
7. TBT-M
8. ProD

Angelica 6/9



LEFT: [98C for 45 sec / 50C for 45 sec / 72C for 3m30s] x 35

NOTE: Bright band on negative control likely due to overnight at 105C. What is the bright band over ProD?

1. Ladder
2. GFP
3. Psmart
4. PAM-C
5. PAM-M
6. TBT-C
7. TBT-M
8. ProD

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 6/10/16

Gibson Attempt Three (Changing concentrations)

We found that the lucigen p-smart is 742ng/ul
Our DBAT gblock is 40ng/ul
We diluted the p-smart at 17ng/ul in a separate aliquot

Attempted the gibson again, used Comp Cells from the Lynch Lab; 1 uL of DNA 20 uL of comp cells

DBAT in several trials

Protocol:

- Gibson Protocol

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SATURDAY, 6/11/16

Transformation Results

- Nothing grew ☹ 😭
- Potentially due to low times on the electroporation

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

MONDAY, 6/13/16

Transformation Take Two

We performed the Gibson Again, on DBAT

Used two variables;

- 4 uL of DNA
- And then diluted with water to 1 uL of H2O and 1 uL of DNA

Results (6/14)

Most plates grew

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

MONDAY, 6/13/16

Made LB +KAN

LB Broth/Media Protocol

Made 4 bottles (250mL) of 125 mL aliquots of LB+KAN broth
    Used 1/8 of the LB media recipe in each bottle (0.625 g of Salt and Yeast and 1.25 g of tryptone)
The old stocks had been contaminated
Used smaller bottles and smaller aliquots to limit contamination

Made Aliquots of Commonly Used Materials

Made conical tube aliquots (~50 mL) of 10%, 20%, and 50% Glycerol as well as pico water.
Made aliquots to help limit contamination and to clean up bench space.

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

TUESDAY, 6/14/16

Colony PCR of DBAT Plates

Protocol:

- Colony PCR Protocol

Edits:

- 8 colonies picked per plate (DBAT -A, DBAT-B, DBAT-3)
- Controls: (GFP, PSmart, TBT-M, TBT-C)
- PCR run on old machine
    - (98 C for 30s/ 50C for 30s/72c for 2m45s)x35

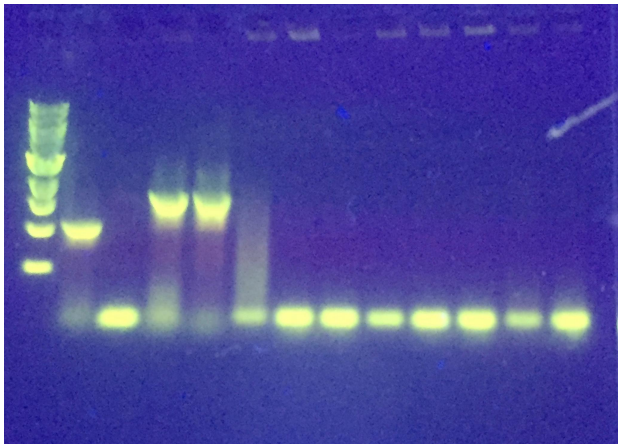
Table21

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	30
3	MM	12.5	375
4	SL1	0.25	7.5
5	SR2	0.25	7.5
6	H2O	12	360

Gel of colony PCR

Angelica

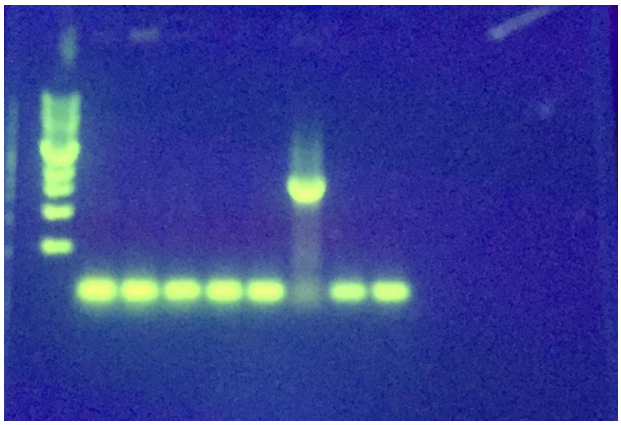
IMG_1235.jpg



1. Ladder
2-5. GFP, pSMART, TBT Miniprep, TBT Miniprep
6-13. DBAT-B (colonies 1-8)

Brandon

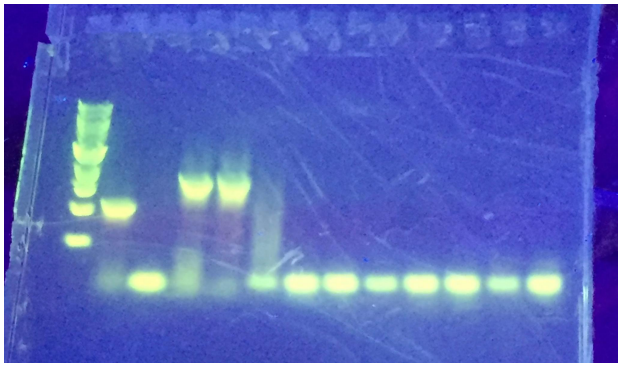
IMG_1236.jpg



1. Ladder
2-9. DBAT-A (colonies 1-8)

Suzie

IMG_1239.jpg



1. Ladder
2-9. DBAT-3 (colonies 1-8)

Restriction Digest

Protocol:

- Digest

Edits:

- Digested pSB1C3 (45 ng/uL) and TAT9 (121ng/uL) with EcoRI-HF and PstI-HF
- Added DpnI in the Master Mix for pSB1C3
- Mixed 3 uL of insert DNA with 5 uL of Master mix instead of 4 uL and 4 uL

Ligation

Protocol:

- Ligation

Edits:

- Ligated EcoRI/PstI digested pSB1C3 with EcoRI/PstI digested TAT9
- 2 uL of each were used

Transformation

Protocol:

- Electroporation Protocol

Edits:

- 3uL of ligation DNA was used as well as 20 uL of competent cells

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

TUESDAY, 6/14/16

Made CM Plates

Made 0.5 L of CM Plates - 23 plates

LB Agar Plates

Made CM LB Stock

Made 0.5 L of LB Broth + CM

LB Broth/Media Protocol

Autoclaved Glassware

New half liter and liter bottles came in. All were autoclaved.

Made KAN Stock

Made KAN Stocks using 40 ng/ul.

Antibiotic Stocks

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

WEDNESDAY, 6/15/16

Colony PCR of DBAT small colonies

Protocol:

- Colony PCR Protocol

DBAT-A 1:11
DBAT-B 1:4
DBAT-3 1:8, 12

Table22

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	28
3	EconoTaq	12.5	350
4	SL1	0.25	7
5	SR2	0.25	7
6	H2O	12	336
7	Total	25	700

Ran Gels:

pic order:
angelica
brandon
suzie

image-2.jpeg

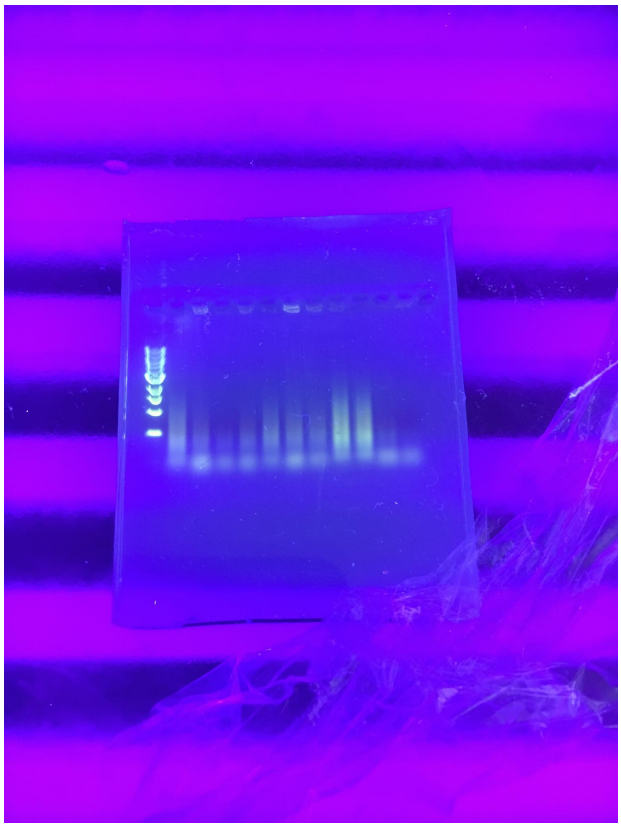


image-3.jpeg

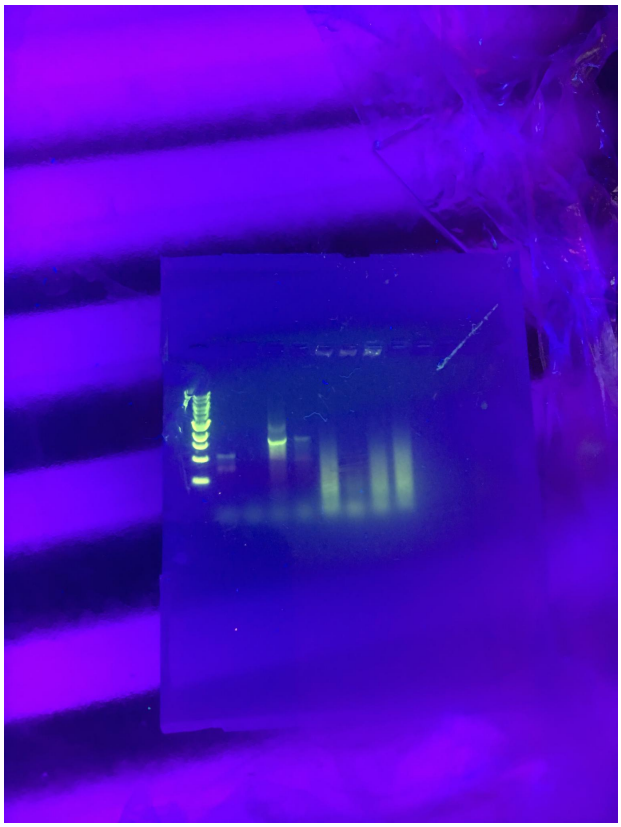
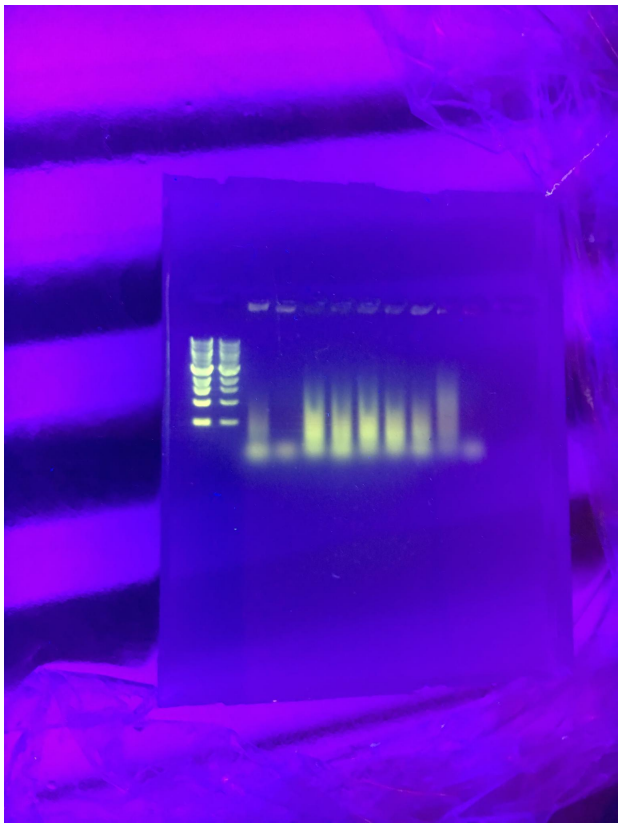


image-4.jpeg



Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

WEDNESDAY, 6/15/16

Made E-cloni Stocks

Took old plate and inoculated two 50 mL LB flasks.

- The plate had several infections

Took a long time to grow - suspicious

Decided to discard

Made new E-cloni Plate

Because the old plate had several infections, a chip of stock Ecloni was plated onto a new plate and grown overnight

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 6/16/16

Colony PCR for Small DBAT Colonies

Protocols:

- Colony PCR Protocol

Controls:
GFP x100, 2.5 uL
pSMART x100, 2.5 uL
TBT Colony

Colonies:
Kan 6/7/16 DBAT A 75 uL: 1, 2, 3, 4, 5, 7, 8, 9

Table23

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	12
3	EconoTaq	12.5	150
4	SL1	0.25	3
5	SR2	0.25	3
6	H2O	12	144
7	Total	25	300

Ran Gels:

Inoculated DBAT

DBAT A6 came back sequence confirmed.
It was inoculated in 5 mL of LB + KAN

Checked gBlock Concentrations

Diluted to 4x (0.5 uL gBlock and 1.5uL pH20. Vol total = 2uL)
Multiplied read by 4 to get the following concentrations

Table24

	A	B
1	**gBlock**	**Real Concentration (ng/uL)**
2	TycA-1	48
3	TycA-2	40
4	Tax10	96
5	BAPT	32

Freezer Stocks/Minipreping/Concentration Check/Sequencing

Protocols:

- Freezer Stocks Protocol
- Miniprep Protocol Zyppy
- Testing Miniprep Concentration
- Preparation for Sequencing

Preformed on:

- TAT9-pSB1C3 1-1
- TAT9-pSB1C3 1-2
- TAT9-pSB1C3 2-1
- TAT9-pSB1C3 2-2
- TAT9-pSB1C3 2-3
- TAT9-pSB1C3 2-4
    - Note that there is a slim chance that 2-3 and 2-4 may have been switched within the minipreping procedure

Concentration Results:

Table25

	A	B	C	D	E	F	G
1	Sample	1-1	1-2	2-1	2-2	2-3	2-4
2	Concentration (ng/uL)	128	85	160	207	140	53

Sequencing:

- Used primers meant for the pSB1C3 backbone (Amp 1, Amp 2)
    - Amp 1 Concentration: 51.2 µM
    - Amp 2 Concentration: 47.99 µM
- Results (6/17): All were sequence confirmed except for TAT9-pSB1C3 1-2

Compontent Cells

Protocol:

- Electrocompetent Cells

Edits:

- Incorrently measured OD using 420nm instead of 600nm

- Prematurely took out cells resulting in only one tube of comp. cells
- Redo 6/17

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

THURSDAY, 6/16/16

Made E-cloni Stocks

Inoculated two flasks of 50 mL LB
Let grow

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 6/17/16

Control PCR

Everyone to do their own PCR to check for human error.
GFP/pSM/TBT-M/TBT-C/PAM-M/PAM-C/ProD/ProD
Make own master mix - focus on what you are doing and why

Emma

Master Mix:

Table26

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	10
3	EconoTaq	12.5	125
4	SL1	0.25	2.5
5	SR2	0.25	2.5
6	H2O	12	120
7	Total	25	250

H20 1st; SL1 2nd; SR2 3rd; ET 4th

Samples:
1=GFP
2=pSMART
3=TBT Miniprep
4=TBT Colony
5=Pam Miniprep
6=PAM Colony
7=ProD 1
8=ProD2

Master Mix was mixing by pipetting up and down
The Master Mix was not aliquoted until all four rows were being aliquoted simultaneously

Parth

Samples:

- pSMART miniprep 1:100 dilution
- GFP miniprep 1:100 dilution
- TBT miniprep
- TBT colony
- PAM miniprep
- PAM colony

- ProD colony 1
- ProD colony 2

Protocol:

- Colony PCR Protocol

Table28

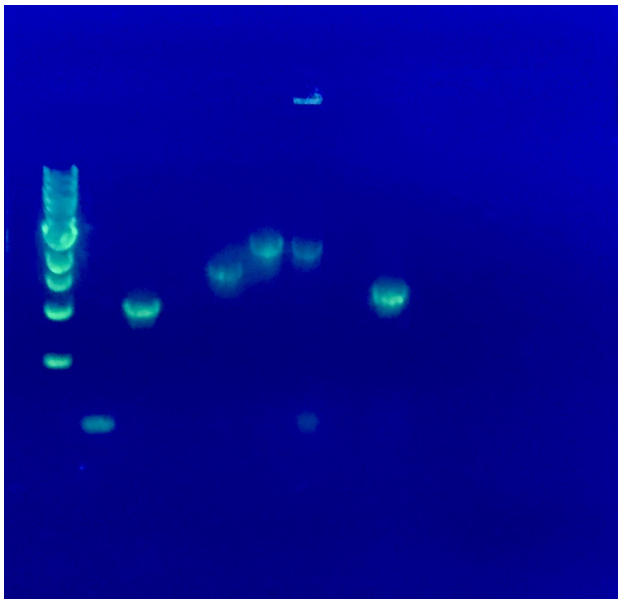
	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	9
3	EconoTaq	12.5	112.5
4	SL1	0.25	2.25
5	SR2	0.25	2.25
6	H2O	12	108
7	Total	25	225

Reaction Mix

Edits to procedure: No LB was aliquoted and used, the purpose of this test was to check consistency of PCR procedure. Longest amplificant is PAM (2.5 kb); therefore, extension time was 3.5 minutes.

Results:

IMG_1939.jpg



Samples going in the same order from left to right as listed previously. Note: ProD colony 1 did not PCR correctly as there was no liquid, thus there is a gap before ProD-2.

All sizes match correctly.

Jay

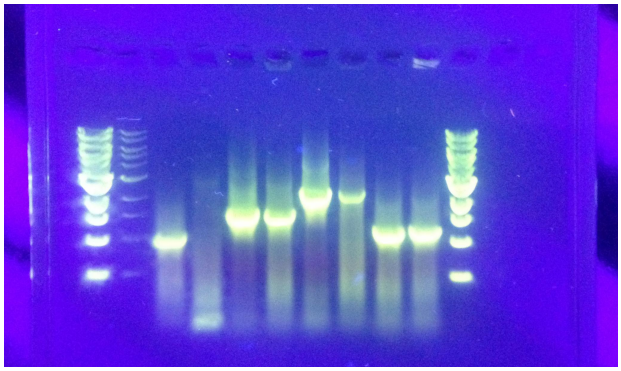
Protocol:

- Colony PCR Protocol (Edit: Extension time 3 1/2 minutes)

Table27

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	9
3	EconoTaq	12.5	112.5
4	SL1	0.25	2.25
5	SR2	0.25	2.25
6	H2O	12	108
7	Total	25	225

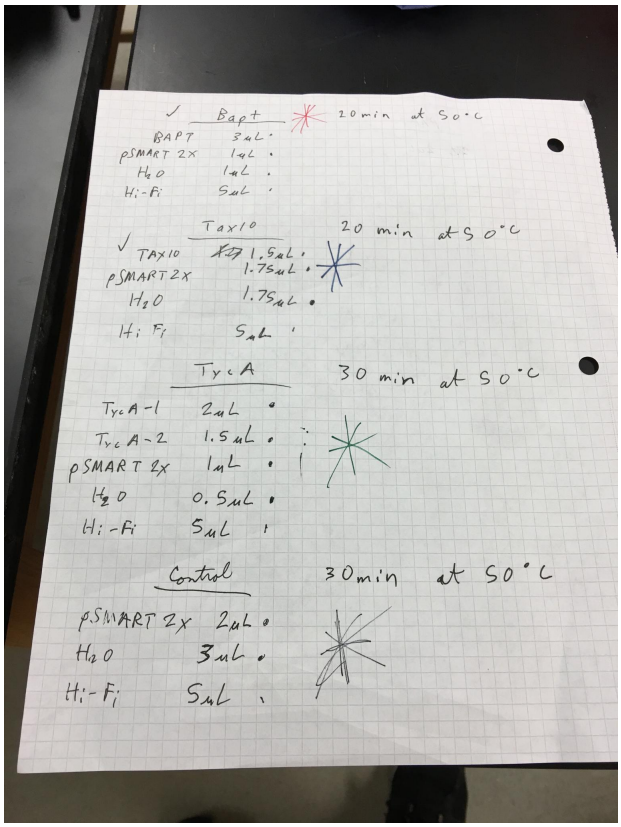
061716Gel.jpg



1. Ladder
2. Blank (Ladder contaminated)
3. GFP 1:100 dilution
4. pSMART 1:100 dilution
5. TBT-Miniprep (50ng/uL)
6. TBT-Colony
7. PAM-Miniprep (50ng/uL)
8. PAM-Colony
9. ProD-Colony 1
10. ProD-Colony 2
11. Ladder

Gibsons

image-5.jpeg


✓ Bapt 20min at 50°C
BAPT 3uL
pSMART 2X 1uL
H2O 1uL
Hi-Fi 5uL
Tax10 20 min at 50°C
✓ TAX10 1.5uL
pSMART 2X 1.75uL
H2O 1.75uL
Hi Fi 5uL
TycA 30 min at 50°C
TycA-1 2uL
TycA-2 1.5uL
pSMART 2X 1uL
H2O 0.5uL
Hi-Fi 5uL
Control 30min at 50°C
pSMART 2X 2uL
H2O 3uL
Hi-Fi 5uL

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

FRIDAY, 6/17/16

Comp Cells were made

Electrocompetent Cells	Not yet tested

End of Week Check

Made more 70% ethanol
Made sure pipette tips were everywhere
Cleaned all counters

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SATURDAY, 6/18/16

Competent Cell Test

Used 5 concentrations of DNA in 2 transformations each for a total of transformations

White (W) = 0.5 pg/ul
Red (R) = 5 pg/ul
Green (G) = 10 pg/ul
Orange (O) = 20 pg/ul
Yellow (Y) = 50 pg/ul

Transformed Cells using the Electroporation protocol. Used 1 uL of DNA in 20 ul of Comp Cells. Plated after recovering for three hours.

Protocols:

- Electroporation Protocol

Transformation Results

Table30

	A	B	C
1	Sample	Voltage (V)	Time (ms)
2	W1	1710	3.9
3	W2	1710	3.8
4	R1	1700	0.7
5	R2	1710	654
6	G1	1720	4.1
7	G2	1720	4.3
8	O1	1700	0.7
9	O2	1710	654
10	Y1	1710	3.8
11	Y2	1700	654

Plated each transformation on a CM plate with 25 ul of transformed cells. The Y1 and Y2 samples were plated three times at dilutions of 25 ul, 75 ul, and 100 ul.

Results (6.19)
The colonies on each plate were counted and tabulated. The data was then transformed using the CFU equation.

Table31

	A	B
1	Sample	Colony #
2	W1	2
3	W2	4
4	R1	14
5	R2	6
6	G1	80
7	G2	115
8	O1	9
9	O2	38

Colonies/Plate for all but Y1 and Y2

Table32

	A	B	C
1	Dilution	Y1	Y2
2	25	20	52
3	75	53	29
4	100	33	233

Colonies/Plate of Yellow Concentration

Table33

	A	B	C	D	E
1	Sample	Colony #	Dilution (ul)	DNA Concentration (ng/ul)	CFU
2	W1	2	25	0.005	10000000
3	W2	4	25	0.005	20000000
4	R1	14	25	0.005	70000000
5	R2	6	25	0.005	30000000
6	G1	80	25	0.01	200000000
7	G2	115	25	0.01	287500000
8	O1	9	25	0.02	11250000
9	O2	38	25	0.02	47500000
10	Y1a	20	25	0.05	10000000
11	Y1b	53	75	0.05	79500000
12	Y1c	33	100	0.05	66000000
13	Y2a	52	25	0.05	26000000
14	Y2b	29	75	0.05	43500000
15	Y2c	233	100	0.05	466000000
16	Average				97660714.285714

Transformed Data

Plates Chilled

Plates in the incubator were taken out

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

MONDAY, 6/20

Colony PCR

Emma

samples (color, letter/number)

Table34

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	17
3	EconoTaq	12.5	212.5
4	SL1	0.25	4.25
5	SR2	0.25	4.25
6	H2O	12	204
7	Total	25	425

Thomas

samples (color, letter/number)

Table36

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	35
3	EconoTaq	12.5	437.5
4	SL1	0.25	8.75
5	SR2	0.25	8.75
6	H2O	12	420
7	Total	25	875

Parth

samples (color, letter/number)

Table35

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	35
3	EconoTaq	12.5	437.5
4	SL1	0.25	8.75
5	SR2	0.25	8.75
6	H2O	12	420
7	Total	25	875

PCR 30@98C/30@50C/3:00@72C
Controls: GFP, psmart, TBT-M, TBT-C, ProD
Master Mix for Emma's control made by Thomas
PCR machine for Emma's Samples was turned off after ten seconds due to Jay's forgotten samples

Gel Electrophoresis

Table37

	A	B	C	D	E	F	G	H	I	
1	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Wells 7-26	Well 27	Wells 28-35	Wel
2	GFP (PP)	ProD (PP)	TBT-Minipre p (PP)	TBT-Colony (PP)	PSmart (PP)	Ladder	Green Numbers 1-20; BAPT #2 (TL)	Ladder	Red Letters A-H; TAX10 #1 (TL)	Blac 1-15 (JC

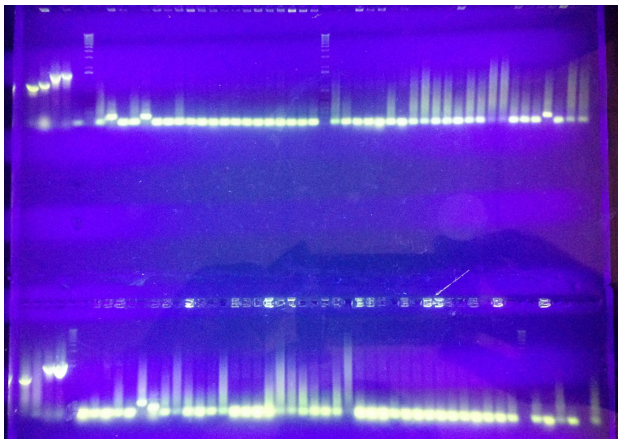
Big Bertha Row 1

Table38

	A	B	C	D	E	F	G	H	I	
1	Well 1	Well 2	Well 3	Well 4	Well 5	Wells 6-29	Wells 30-43	Well 44	Wells 45-48	Wel
2	GFP (EM)	PSma rt (EM)	TBT-Colony (EM)	TBT-Miniprep (EM)	Ladder	Green Letters A-X; BAPT #2 (EM)	Black Letters A-N; BAPT #1 (EM)	Ladder	Blue Letters A-D; TAX10 #2 (EM)	(Eva Inte Lett #2 (

Big Bertha Row 2

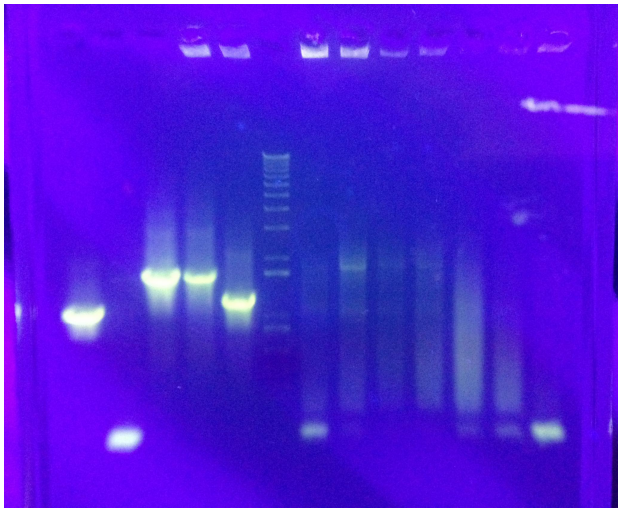
Big Bertha.JPG



See Tables above

Angelica

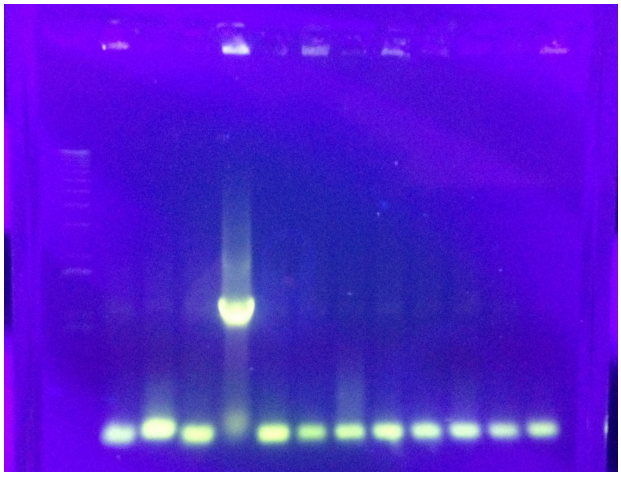
Angelica.JPG



1. GFP (Version: Thomas's)
2. pSMART (Version: Thomas's)
3. TBT-Miniprep (Version: Thomas's)
4. TBT-Colony (Version: Thomas's)
5. ProD (Version: Thomas's)
6. Ladder
7-13. TAX10#2 1-7 (Blue Numbers)

Brandon

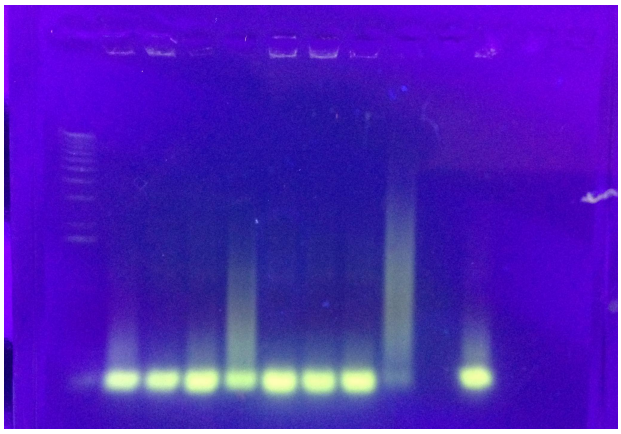
Brandon.JPG



1. Ladder
2-4. TAX10#2 8-10 (Blue Numbers)
5. ProD (Version: Emma's)
6-13. TAX10#1 1-8 (Red Numbers)

Suzie

Suzie.JPG



1. Ladder
2-9. TAX10#1 10-17 (Red Numbers)
10. Blank
11. TAX10#1 9 (Red Number)

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

TUESDAY, 6/21

Minipreps/Concentration Check/Sequencing

Protocols:

- Miniprep Protocol Zyppy
- Testing Miniprep Concentration
- Preparation for Sequencing

Samples:

- BAPT #1 (A, B)
- TAX10 #2 (2, 3, 4)
- TAX10 #2 (A, F)
- BAPT #2 (C, D, F, R, X)

Results

Table42

	A	B	C	D	E	F	G	H	I	J
1	Sample	BAPT#1-A	BAPT#1-B	BAPT#2-C	BAPT#2-D	BAPT#2-F	BAPT#2-R	BAPT#2-X	TAX10#2-A	TAX10#2-
2	Conc	123	60	67	61	66	56	54	50	5

Sent in for sequencing

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

TUESDAY, 6/21

Made CM and KAN plates

Made one liter of CM and one liter of KAN agar.
The plates set out for two hours and were still not set up - they were left overnight but were still not set in the morning.
All of the plates were discarded.

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

WEDNESDAY, 6/22

Colony PCR on TycA

4:30 elongation 30 s Elongation/Denaturation

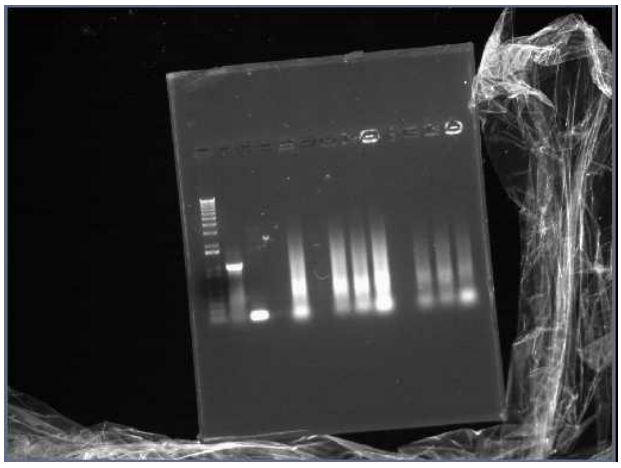
Table43

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	53
3	EconoTaq	12.5	662.5
4	SL1	0.25	13.25
5	SR2	0.25	13.25
6	H2O	12	636
7	Total	25	1325

Gel Electrophoresis for TycA

Angelica

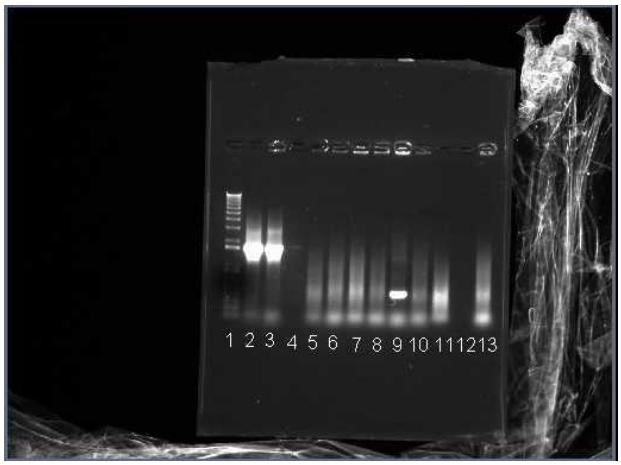
TycA62216Angelica.jpg



1. Ladder
2. GFP
3. pSMART
4-10: TycA yi 1-7
11-13: TycA san 11-13

Brandon

TycA62216Brandon.jpg



1. Ladder
2. TBT Mini Prep
3. TBT Colony
4-11. TycA er 1-8
12. Empty
13. TycA san 14

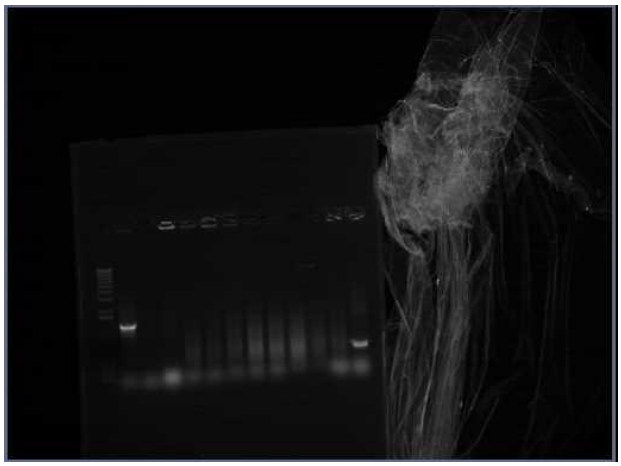
Suzie

TycA62216Suzie.jpg

1. Ladder
2. PAM Mini Prep
3. PAM Colony
4-11. TycA #2 1-8

Mike

TycA62216Mike.jpg



1. Ladder
2. ProD
3. ProD
4-13. TycA san 1-10

Inoculations were made of samples:

- TycA san: 1, 10
- TycA er: 6, 8
- TycA yi: 4, 2

- TycA #2: 4, 5

Inoculations placed in 5 mL of LB+KAN and

Transformations

Transformed several samples into cells. All samples used 1ul of DNA and 20 ul of Comp Cells.
Transformations were recovered for 2 hours and then 75 uL was plated onto a KAN Plate. Another set of plates was made after 4 hours of recovery with a 5:95 ratio of cells to LB.

Table44

	A	B	C	D
1	DNA Sample	Cell Sample	Volts	Tims (ms)
2	DBAT #1	286	1700	3.7
3	DBAT #2	286	1710	3.8
4	DBAT EV #1	286	1700	3.6
5	DBAT EV #2	286	1710	3.8
6	BadA EV #1	286	1710	3.7
7	BadA EV #2	286	1710	3.7
8	DBAT #1	E.Cloni	1720	4
9	DBAT #2	E.Cloni	1720	4

Restriction Digest

Protocol:

- Digest

Edits:

- Digested pSB1C3 (45 ng/uL) and DBAT (70ng/uL) with EcoRI-HF and PstI-HF
- Added DpnI in the Master Mix for pSB1C3
- Mixed 3 uL of insert DNA with 5 uL of Master mix instead of 4 uL and 4 uL

Ligation

Protocol:

- Ligation

Edits:

- Ligated EcoRI/PstI digested pSB1C3 with EcoRI/PstI digested DBAT
- 2 uL of each were used

Transformation

Protocol:

- Electroporation Protocol

Edits:

- 3uL of ligation DNA was used as well as 20 uL of competent cells

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

WEDNESDAY, 6/22

Made CM and KAN plates

Made one liter of CM and one liter of KAN agar.
Stored in the 4C fridge

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 6/23

Minipreps/Concentration Check/Sequencing (Nisa)

Protocols:

- Miniprep Protocol Zyppy
- Testing Miniprep Concentration
- Preparation for Sequencing

Samples:

- TycA EV-6
- TycA San 1, 10

Results

Table45

	A	B	C	D
1	Sample	TycA EV-6	TycA San-1	TycA San-10
2	Conc	41	9	55

Made Plates of 286 (Parth & Nisa)

Made plates of 286

- 1 with 25 uL concentration spread out
- 2 by streaking into single colonies

Inoculations (Parth)

Made Inoculations of DBAT-pSB1C3 #1 (A-E)

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

THURSDAY, 6/23

Made DLF-00286 Competent Cells

Total of 29 aliquots of 50 uL were stored in the -80°C freezer

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 6/24

Freezer Stocks/Miniprep/Concentration Check/Sequencing (Nisa)

Protocols:

- Freezer Stocks Protocol (750 uL)
- Miniprep Protocol Zyppy (50 uL elution buffer)
- Testing Miniprep Concentrations
- Preparation for Sequencing

Samples:

- DBAT-pSB1C3 #1 (A-E)

Results

Table29

	A	B	C	D	E	F
1	Sample	DBAT-pSB1C3#1-A	DBAT-pSB1C3#1-B	DBAT-pSB1C3#1-C	DBAT-pSB1C3#1-D	DBAT-pSB1C3#1-E
2	Conc	31	84	51	47	56

Gibson Transformations

Samples:

- TAX10-1B
- TAX10-2B

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SATURDAY, 6/25

Colony PCR on TAX10 Plates 1B & 2B

3:00 elongation
38 Reactions: 17 colonies from 1B, 13 colonies from 2B, 1 uL GFP, 1 uL pSMART, 1 uL TBT miniprep, 1 TBT colony, 1 uL BadA miniprep, 1 BadA colony, 2 ProD colonies

Table39

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	40
3	EconoTaq	12.5	500
4	SL1	0.25	10
5	SR2	0.25	10
6	H2O	12	480
7	Total	25	1000

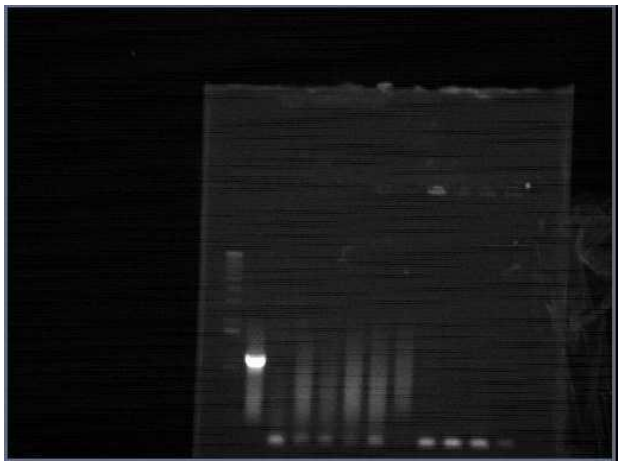
Gel Electrophoresis

Angelica

Table40

	A	B	C	D	E
1	Well 1	Well 2	Well 3	Wells 4-12	Well 13
2	Ladder	GFP	pSMART	TAX10 Plate 1B #1-9	Blank

Angelica62516.jpg

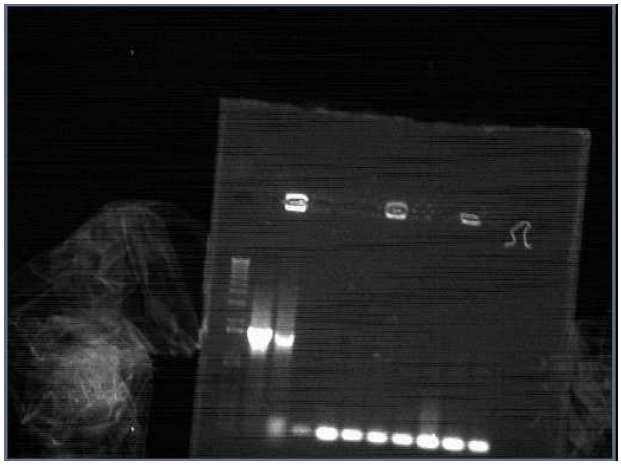


Brandon

Table41

	A	B	C	D	E
1	Well 1	Well 2	Well 3	Wells 4-11	Wells 12-13
2	Ladder	TBT Miniprep	TBT Colony	TAX10 Plate 1B #10-17	Blank

Brandon62516.jpg

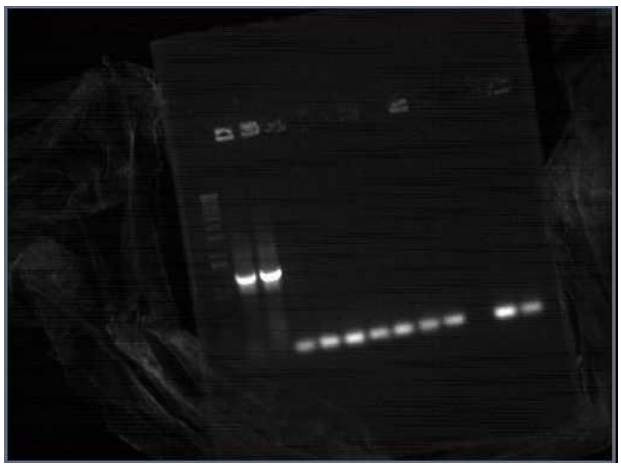


Suzie

Table46

	A	B	C	D	E
1	Well 1	Wells 2-3	Wells 4-10	Well 11	Wells 12-13
2	Ladder	ProD Colonies	TAX10 Plate 2B #1-7	Blank	TAX10 Plate 2B #5-6

Suzie62516.jpg

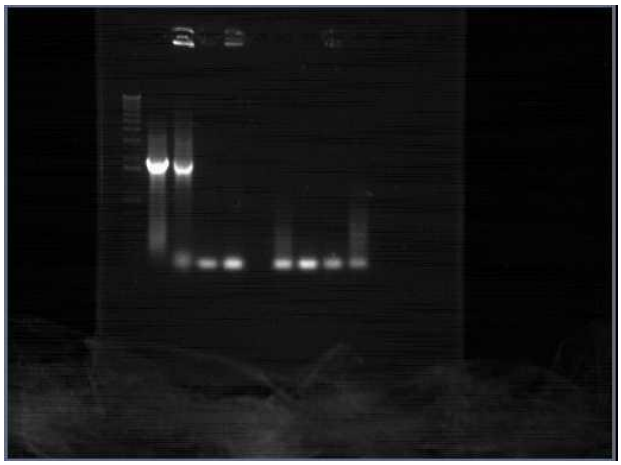


Mike

Table47

	A	B	C	D	E	F	G
1	Well 1	Well 2	Well 3	Wells 4-5	Well 6	Wells 7-10	Wells 11-13
2	Ladder	BadA Miniprep	BadA Colony	TAX10 Plate 2B #8-9	Blank	TAX10 Plate 2B #10-13	Blank

Mike62516.jpg



Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

TUESDAY, 6/28

Digest/Ligation/Transformation of TAX10+ pSB1C3 (Parth)

Protocols:

- Digest
  - Edits: 2uL pSB1C3+2uL dH2O, 1uL TAX10+3uLdH2O to make the amounts equimolar for the ligation
  - TAX10 taken was directly from gBlock
- Ligation
  - Two attempts at ligation (Samples: #1, #2)
- Transformation
  - Edits: Plated Sample #1 @ 75uL and 25uL, Plated Sample #2 once @ 75 uL

Result: Failure, no colonies grew

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

WEDNESDAY, 6/29

Autoclaved Epi Tubes and washed glassware.

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 6/30

Splitting pSmart into Pieces before Gibson

PCR

- 2 reactions: 1) linear pSmart (17 ng/uL), 1/2 forward primer, 1/2 reverse primer; 2) linear pSmart (17 ng/uL), 2/2 forward primer, 2/2 reverse primer
- 63 degrees Celsius annealing temperature

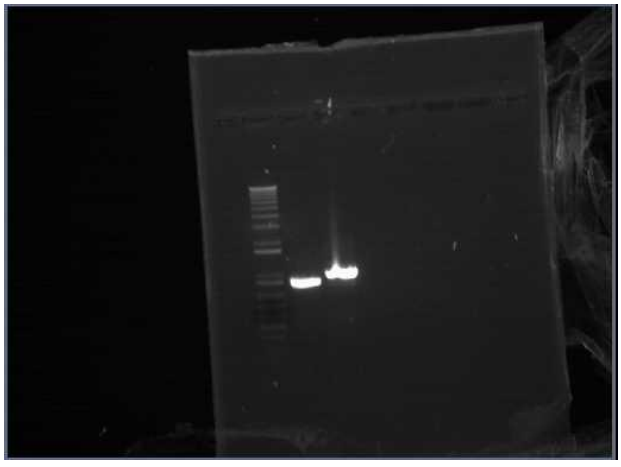
Table48

	A	B	C	D
1				
2	Thing	1 rxn	2.2	
3	Q5-2xMM	25	55	
4	Forward Primer	2.5	5.5	(not mixed)
5	Reverse Primer	2.5	5.5	(not mixed)
6	DNA	1	2.2	
7	H2O	19	41.8	
8	Total	50	110	

Master Mix

Gel Electrophoresis

PSmartChew063016.jpg



Ladder, Reaction 1, Reaction 2

Monarch PCR DNA Cleanup (see protocol)

- Concentration Check: Piece 1 @ 61 ng/uL; Piece 2 @ 91 ng/uL
- Conversions: Piece 1 @ 0.1098 picomoles/uL; Piece 2 @ 0.1452 picomoles/uL
- (Conversion from ng/uL to picomoles/uL: x1000/(bp length x 650))

10 uL Dilutions to 0.020 picomoles/uL for Gibson Reaction

- Concentration Check: Piece 1 @ 12 ng/uL; Piece 2 @ 14 ng/uL
- Conversions: Piece 1 @ 0.0216 picomoles/uL; Piece 2 @ 0.0223 picomoles/uL

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

THURSDAY, 6/30

Autoclaved glassware and biohazard trash.

Made Electrocompetent Cells (E cloni)

- 9 aliquots were made

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 7/1

Gibson Reaction with (TAX10+ pSMART 1/2+ pSMART 2/2)

Protocol:

- Gibson Protocol

Samples:

- TAX10
    - 1 µL TAX10 from gBlock
    - 1 µL pSMART 1/2 dilution
    - 1 µL pSmart 2/2 dilution
    - 2 µL dH_2O
    - 5 µL Gibson HiFi Master Mix
- Control
    - 1 µL pSMART 1/2 dilution
    - 1 µL pSmart 2/2 dilution
    - 3 µL dH_2O
    - 5 µL Gibson HiFi Master Mix

Electroporation

- TAX10 #1: 1620 V, 2.5 ms; TAX10 #2: 1620 V, 654 ms; TAX10 #3: 654 ms (voltage not recorded)
- Put for recovery in Lynch Lab shaker around 11:45 AM

Plating

- After 3 PM
- 2 plates of each TAX10, 80 uL each (some plates soft)
- Put in incubator at 37 degrees Celsius for 20 hours

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

FRIDAY, 7/1

Made and autoclaved 1 L LB broth: transferred to autoclaved jar.

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SATURDAY, 7/2

Colony PCR on TAX10 Plates 1B, 2B, 3A

3:00 elongation
60 Reactions: 16 colonies from 1B, 16 colonies from 2B, 16 colonies from 3A, 3 x 1 uL GFP, 3 x 1 uL pSMART, 3 x 1 uL TBT miniprep, 3 x 1 TBT colony

Table49

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	66
3	EconoTaq	12.5	825
4	SL1	0.25	16.5
5	SR2	0.25	16.5
6	H2O	12	792
7	Total	25	1650

Master Mix

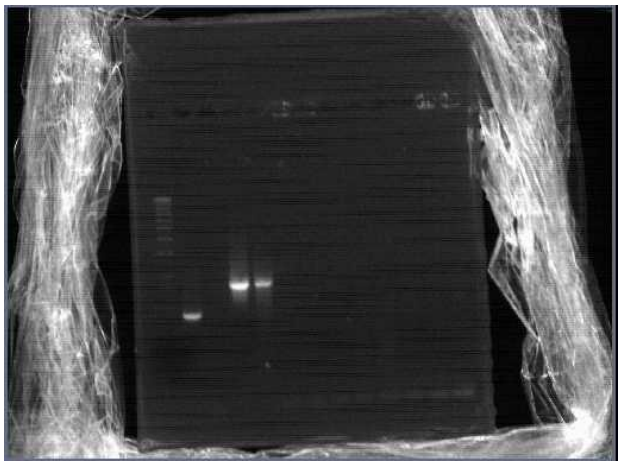
Gel Electrophoresis

Angelica

Table50

	A	B	C	D	E	F
1	Well 1	Well 2	Well 3	Well 4	Well 5	Wells 6-13
2	Ladder	GFP (JCS)	PSmart (JCS)	TBT-Miniprep (JCS)	TBT-Colony (JCS)	TAX10 Plate 1B #1-8

Angelica7216.jpg

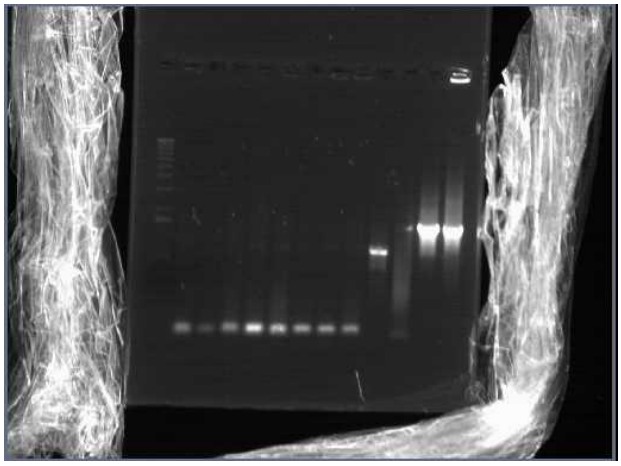


Brandon

Table51

	A	B	C	D	E	F
1	Well 1	Wells 2-9	Well 10	Well 11	Well 12	Well 13
2	Ladder	TAX10 Plate 1B #9-16	GFP (NV)	PSmart (NV)	TBT-Miniprep (NV)	TBT-Colony (NV)

Brandon7216.jpg

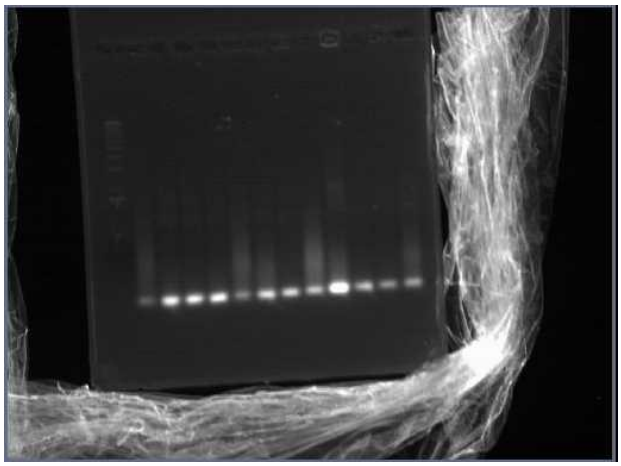


Suzie

Table52

	A	B
1	Well 1	Wells 2-13
2	Ladder	TAX10 Plate 2B #1-12

Suzie7216.jpg

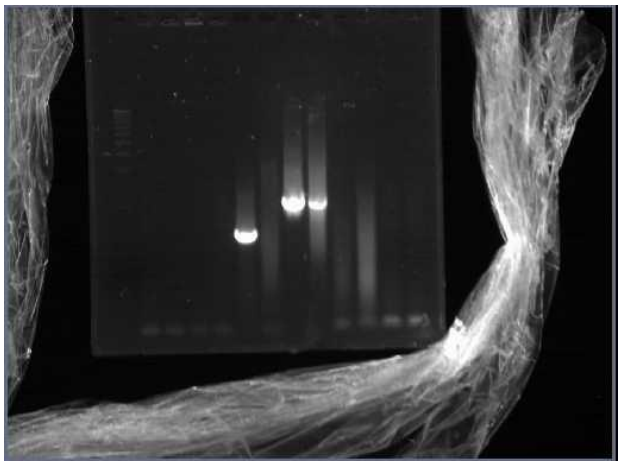


Mike

Table53

	A	B	C	D	E	F	G
1	Well 1	Wells 2-5	Well 6	Well 7	Well 8	Well 9	Wells 10-13
2	Ladder	TAX10 Plate 2B #13-16	GFP (PP)	PSmart (PP)	TBT-Miniprep (PP)	TBT-Colony (PP)	TAX10 Plate 3A #1-4

Mike7216.jpg

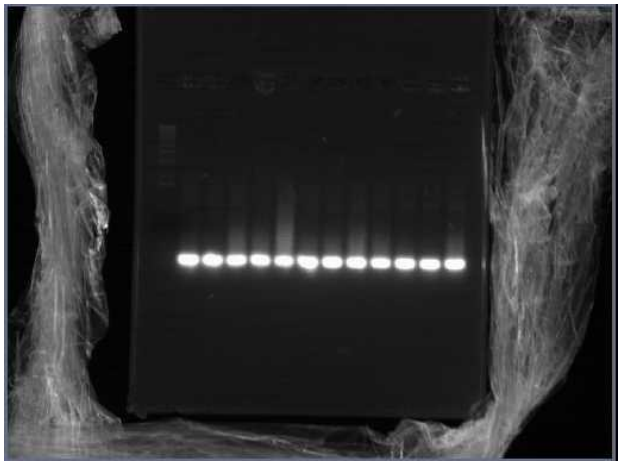


Ed-Ward

Table54

	A	B
1	Well 1	Wells 2-13
2	Ladder	TAX10 Plate 3A #5-16

Ed-Ward7216.jpg



Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

TUESDAY, 7/5

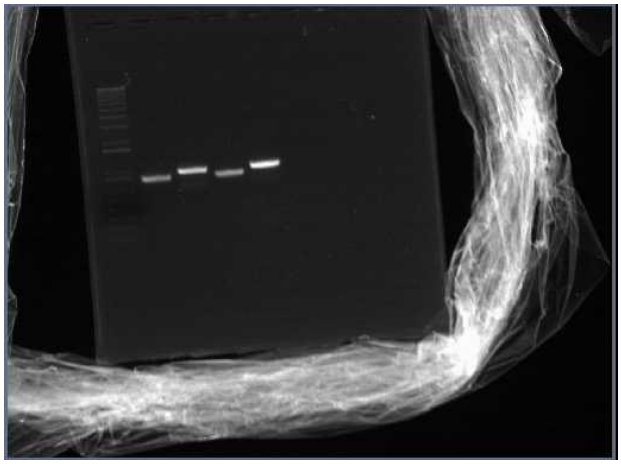
Running Controls for Cutting pSmart into Pieces before Gibson

PCR

- 4 reactions: 1) 1 uL linear pSmart (1:10 dilution of 17 ng/uL), 2.5 uL 1/2 forward primer, 2.5 uL 1/2 reverse primer, 19 uL PICO water, 25 uL Q5-2xMM; 2) 1 uL linear pSmart (1:10 dilution of 17 ng/uL), 2.5 uL 2/2 forward primer, 2.5 uL 2/2 reverse primer, 19 uL PICO water, 25 uL Q5-2xMM; 3) 1 uL linear pSmart (1:100 dilution of 17 ng/uL), 2.5 uL 1/2 forward primer, 2.5 uL 1/2 reverse primer, 19 uL PICO water, 25 uL Q5-2xMM; 4) 1 uL linear pSmart (1:100 dilution of 17 ng/uL), 2.5 uL 2/2 forward primer, 2.5 uL 2/2 reverse primer, 19 uL PICO water, 25 uL Q5-2xMM
- 63 degrees Celsius annealing temperature
- Q5 PCR protocol on old PCR machine

Gel Electrophoresis

PSmartSplit7516.jpg



Ladder, Reaction 1 (10 uL dilution), Reaction 2 (10 uL dilution), Reaction 1 (100 uL dilution), Reaction 2 (100 uL dilution)

Monarch PCR DNA Cleanup (see protocol)

- Concentration Check: 10 uL dilution Piece 1 @ 23 ng/uL; 10 uL dilution Piece 2 @ 33 ng/uL; 100 uL dilution Piece 1 @ 24 ng/uL; 100 uL dilution Piece 2 @ 38 ng/uL
- Conversions: Piece 1 @ 0. picomoles/uL; Piece 2 @ 0. picomoles/uL
- (Conversion from ng/uL to picomoles/uL: x1000/(bp length x 650))

10 uL Dilutions to 0.020 picomoles/uL for Gibson Reaction

- Concentration Check: Piece 1 @ 12 ng/uL; Piece 2 @ 15 ng/uL
- Conversions: Piece 1 @ 0.0 picomoles/uL; Piece 2 @ 0.0 picomoles/uL

Gibson Reactions

Protocol:

- Gibson Protocol

Samples:

- Control: Pieces
    - 1 µL pSmart 1/2 100 uL dilution
    - 1 µL pSmart 2/2 100 uL dilution
    - 3 µL dH_2O

    - 5 µL Gibson HiFi Master Mix
- Control: Full
    - 1 µL pSmart 17 ng/uL dilution
    - 4 µL dH_2O
    - 5 µL Gibson HiFi Master Mix
- Control: Fake Pieces
    - 1 µL pSmart 1/2 100 uL dilution
    - 1 µL pSmart 2/2 100 uL dilution
    - 8 µL dH_2O
- Control: Fake Full
    - 1 µL pSmart 17 ng/uL dilution
    - 9 µL dH_2O

Electroporation

- Gibson Pieces, Fake Pieces, Fake Full (Gibson Full did not electroporate)
- 1 uL Gibson, 35 uL electrocompetent cells, electroporate, 250 uL LB Broth
- Put for recovery in original shaker at 37 degrees Celsius around 6 PM

Plating

- After 9 PM
- 2 plates of each, 35 and 80 uL
- Put in incubator at 37 degrees Celsius

Restriction Digest

Protocol:

- Digest

Edits:

- Digested pSB1C3 (45 ng/uL) and DBAT (40ng/uL) with EcoRI-HF and PstI-HF
- Added DpnI in the Master Mix for pSB1C3

Ligation

Protocol:

- Ligation

Edits:

- Ligated EcoRI/PstI digested pSB1C3 with EcoRI/PstI digested TAT9
- 2 uL of each were used

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

WEDNESDAY, 7/6

Made Electrocompetent Cells (E cloni)

- 25 aliquots were made

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 7/7

Transformed water, new dilution linear DNA, and gibson on new dilution linear DNA into E Cloni
Plated 35 uL and 80 uL of each
After 20 hours, only growth on fake 35 uL plate > 2 small colonies

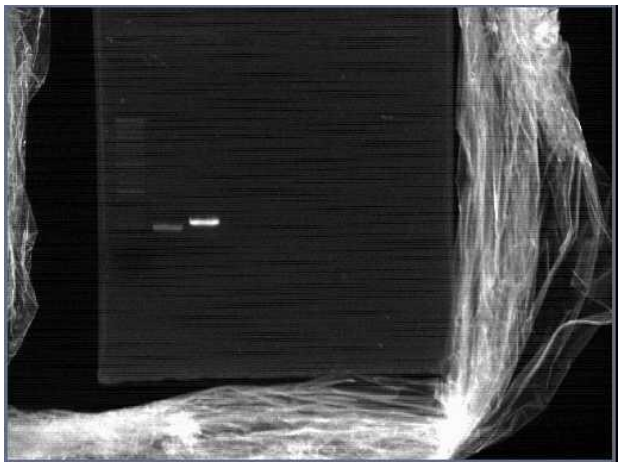
Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 7/8

Chewed up 1:100 dilution of new psmart dilution into pieces with Q5 PCR

PSmartChew7816.jpg



Piece 1 @ 18 ng/uL, Piece 2 @ 40 ng/uL >> Diluted: Piece 1 @ 12 ng/uL, Piece 2 @ 15 ng/ul
2 Gibsons: control with no insert and TBT insert
Transformed Gibsons at 2:30 PM: TBT @ 1660 V, 3.2 ms; control @ 1660 V, 3.1 ms
Plated 35 uL TBT, 80 uL TBT, 50 uL control around 9:30 PM

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SUNDAY, 7/10

Colony PCR on DBAT pSB1C3

3:00 elongation, ran initially with normal protocol (50°C annealing) for about 30 cycles, then ran on another PCR with 59°C annealing

10 Reactions: 4 colonies from 2A, 4 colonies from 2B, 2 from 1

Table55

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	12
3	EconoTaq	12.5	150
4	Amp1	0.25	3
5	Amp2	0.25	3
6	H2O	12	144
7	Total	25	300

Master Mix

Colony PCR on Thomas' Plates (Nisa)

3:00 elongation

7 Reactions: GFPA, GFPB, badA-B, TAT-9, DBAT

Table56

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	7
3	EconoTaq	12.5	87.5
4	SL1	0.25	1.75
5	SR2	0.25	1.75
6	H2O	12	84
7	Total	25	175

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

MONDAY, 7/11

2-piece PSmart Gibson Attempt w/TBT

- 1.5 uL 1/2 PSM, 1.5 uL 2/2 PSM, 2 uL TBT, 5uL Hi-Fi: 1660 V, 3.1 ms
- 2 uL Full PSM, 3 uL PICO water, 5 uL Hi-Fi: 1670 V, 3.2 ms
- 1.5 uL 1/2 PSM, 1.5 uL 2/2 PSM, 2 uL PICO water, 5 uL Hi-Fi: 1660 V, 3.0 ms

All recovered for 3 hours and plated 2 x 80 uL of each at 2:30 PM

Q5 Mutagenesis

Hydrated and suspended the new oligos in 100uM aliquots. Diluted to 10uM for the reaction. Protocol:

Q5 Hot Start Hi-Fi 2X Master Mix - 12.5 uL
10uM Forward - 1.25 uM
10uM Reverse Primer - 1.25 um
Template (BadA at 2 ng/uL. TBT at 3 ng/uL) - 1 uL
Water to 25uL toatl volume - 9 uL

PCR machine:
Saved as "BadA Mut Q5" under "CT001243 Root"

Gel for 35min > single band in each well > PCR products can be used directly for KLD reaction
1 uL of kinase, ligase, and DpnI each mixed together for KLD mix
1 uL KLD mix, 1 uL ligase buffer, 2 uL PCR product mixed in each reaction tube
Tubes incubated at room temperature for an hour
BadA and TBT from each tube transformed: BadA @ 1650 V, 3.1 ms; TBT @ 1630 V, 2.8 ms
Recovered for 1.5 hours
Plated 10 uL, 100 uL of each at 8:10 PM

Colony PCR of DBAT pSB1C3 (Nisa)

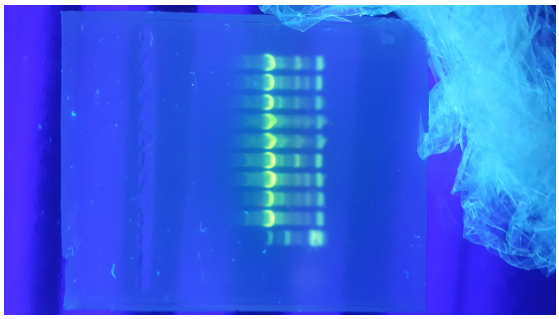
3:00 elongation

Table73

	A	B	C
1			(# col +3 control)
2	Thing	1 rxn	12
3	EconoTaq	12.5	150
4	Amp1	0.25	3
5	Amp2	0.25	3
6	H2O	12	144
7	Total	25	300

Ladder (too faint), pSB1C3+RFP (too faint), DBATxpSB1C3 75-2 (EV), DBATxpSB1C3 2A 1-4. 2B 1-4, 1-1

DBATpSB1C3 7.11.16



Inoculations of DBAT pSB1C3 (Nisa)

Inoculated 2A3-4, 2B1, 1-1

Transformation of pSB1C3xE. cloni (Nisa)

Took 2 ug of pSB1C3+RFP (from iGEM kit) and transformed 35 uL E. cloni
Let recover for 1.5 hr outside and 1 hr in incubator
Plated on CM plate diluted to 5:95 at 25 uL and 75 uL

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

TUESDAY, 7/12

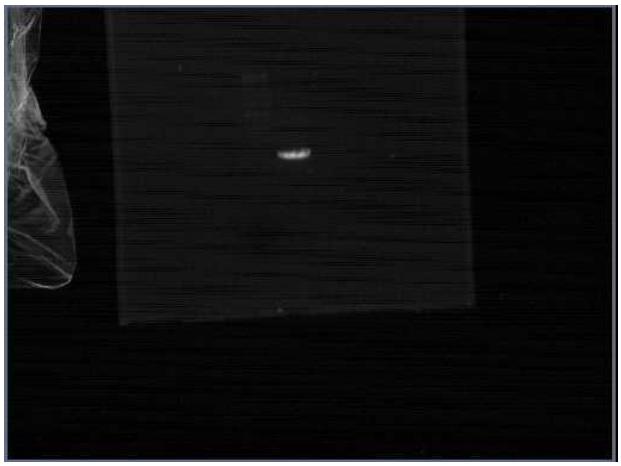
Q5 PCR to Cut Mismatched Base Pairs of Homology from PSmart

Table57

	A	B	C
1		1 rxn	3.3
2	Q5-2xMM	25	82.5
3	1/2 PSM Forward Primer	2.5	8.25
4	2/2 PSM Reverse Primer	2.5	8.25
5	PICO Water	19	62.7
6		49	161.7

3 reactions: 15 ng/uL PSM, 1:10 dilution, 1:100 dilution
Ladder and three reactions run in that order on gel

PSmartFix71216.jpg



Colony PCR to Assess Success of 2-Piece PSmart Gibson with TBT

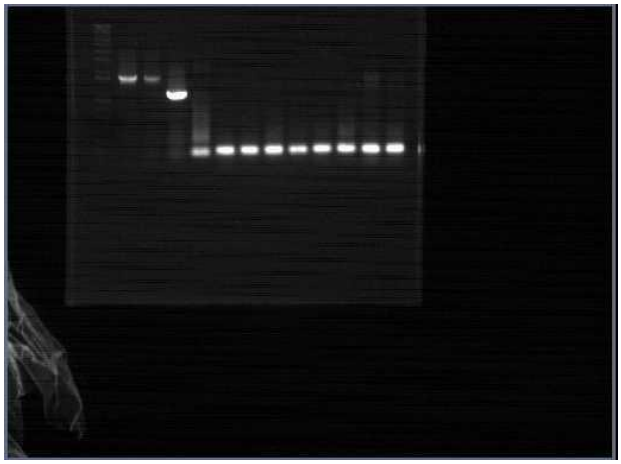
Table58

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	24.2
3	EconoTaq	12.5	302.5
4	SL1	0.25	6.05
5	SR2	0.25	6.05
6	H2O	12	290.4
7	Total	25	605

22 rxns: Plate A colonies #1-18, GFP, PSM, TBT-C, TBT-M

Suzie: Ladder, TBT-M, TBT-C, Colonies #1-10 - No positive screens
Suzie 2: Ladder, TBT-M, TBT-C, GFP, PSM, Colonies #11-18 - see below

Suzie2071216.jpg



Colony 17 incoluated at 6:15 PM (faint band at TBT level in image)

Miniprep/Concentration Check/Sequencing of DBAT pSB1C3 (Parth, Nisa)

Samples:

- DBATxpSB1C3 2A3-4, 2B1, 1-1

Protocols:

- Miniprep Protocol Zyppy
- Testing Miniprep Concentrations
- Preparation for Sequencing

Miniprep Edit: Used a 50 uL elution

Table61

	A	B	C	D	E	F
1	Sample:	DBATxpSB1C3 2A3	2A4	2B1	1-1	
2	Concentration (ng/uL)	99	60	61	48	

Results of Testing DNA Concentration

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

WEDNESDAY, 7/13

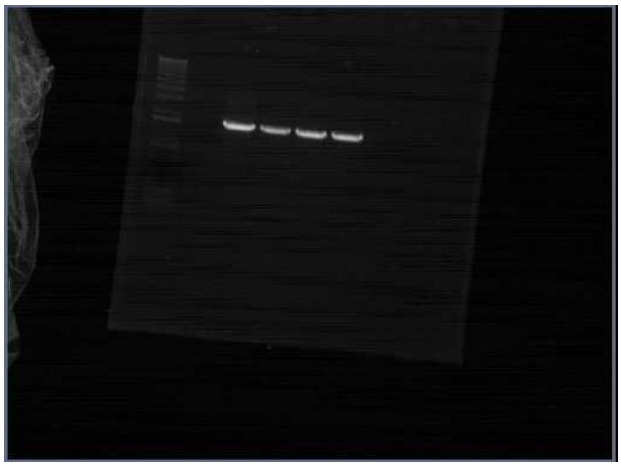
Q5 PCR to Cut Mismatched Base Pairs of Homology from PSmart

Table59

	A	B	C
1		1 rxn	4.4
2	Q5-2xMM	25	110
3	1/2 PSM Forward Primer	2.5	11
4	2/2 PSM Reverse Primer	2.5	11
5	PICO Water	19	83.6
6		49	215.6

4 reactions: 15 ng/uL PSM, 1:5 dilution, 1:10 dilution, 1:100 dilution
Ladder, 3 ng original psmart control, four reactions run in that order on gel

PSMMismatchCut071316.jpg



Inoculations of DBAT pSB1C3 (Nisa)

Inoculated 2A3-4, 2B1, 1-1

Inoculations of pSB1C3xE. cloni (Nisa)

Inoculated 3 colonies from 75 uL plate

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 7/14

Testing Full PSmart w/o Mismatched Tail for Gibsons

Decided to use 1:100 dilution PCR products for Gibsons > PCR Cleanup done, product @ 41 ng/uL, diluted to .016 picomoles/uL (tailless psm)
15 ng/uL actually 19 ng/uL Psmart, diluted to .016 picomoles/uL
TBT at 48 ng/uL, diluted to .032 picomoles/uL

3 Gibson reactions

- Tailless psm, TBT > transformation 1: 1660 V, 654 ms; transformation 4: 1680 V, 3.6 ms
- Tailless psm > transformation 2: 1680 V, 3.5 ms
- 19ng/uL psm, TBT > transformation 3: 1690 V, 654 ms

3 hour recovery, plated 2 x 80 uL of each reaction at 6:50 PM

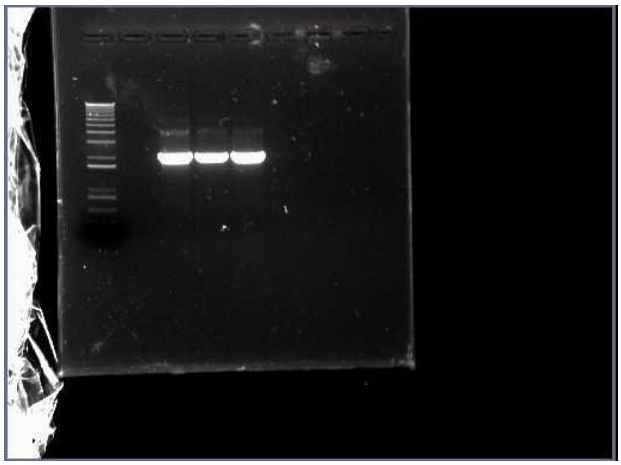
Q5 PCR to Cut Mismatched Base Pairs of Homology from LCKan PSmart

Table60

	A	B	C
1		1 rxn	3.3
2	Q5-2xMM	25	82.5
3	1/2 PSM Forward Primer	2.5	8.25
4	2/2 PSM Reverse Primer	2.5	8.25
5	PICO Water	19	62.7
6		49	161.7

3 reactions: 50 ng/uL LCKan PSM, 1:10 dilution, 1:100 dilution
Ladder, 10 ng original psmart control, three reactions run in that order on gel

LCKanMismatchCut071416.jpg



Miniprep/Concentration Check of pSB1C3 (Nisa)

Samples:

- pSB1C3xE. cloni 1, 2

Protocols:

- Miniprep Protocol Zyppy
- Testing Miniprep Concentrations
- Preparation for Sequencing

Miniprep Edit: Used a 30 uL elution

Table69

	A	B	C	D
1	Sample:	1	2	
2	Concentration (ng/uL)	138	185	

Results of Testing DNA Concentration

Restriction Digest of pSB1C3

Protocol:

- Digest

Edits:

- Digested pSB1C3 (samples from above) with EcoRI-HF and PstI-HF

Inoculations of pSB1C3xE. cloni (Nisa)

Inoculated 3 colonies from 75 uL plate

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 7/15

LCAmp Transformations

- TAX10: 1660 v, 3.1 ms; plated 2 x 70 uL and 1 x 20 uL plates
- Real control: 1650 V, 654 ms; plated 2 x 70 uL
- (Not LCAmp but done with same batch) Fake tailless PSM control: 1670 V, 3.5 ms; plated 2 x 70 uL
- Plated at around 8 PM

Miniprep/Concentration Check of pSB1C3 (Nisa)

Samples:

- pSB1C3xE. cloni 2, 3

Protocols:

- Miniprep Protocol Zyppy
- Testing Miniprep Concentrations
- Preparation for Sequencing

Miniprep Edit: Used a 30 uL elution

Table70

	A	B	C	D
1	Sample:	2	3	
2	Concentration (ng/uL)	151	142	

Results of Testing DNA Concentration

Colony PCR on DBAT pSB1C3 (Nisa)

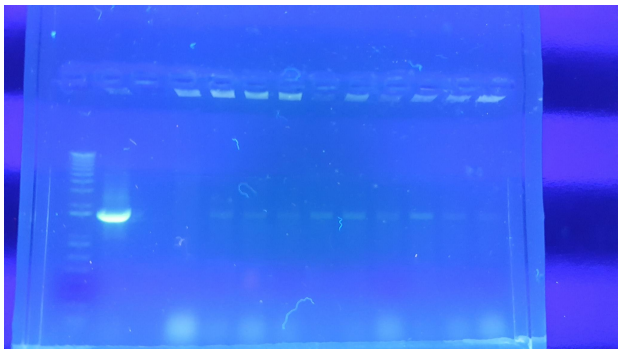
3:00 elongation

Table68

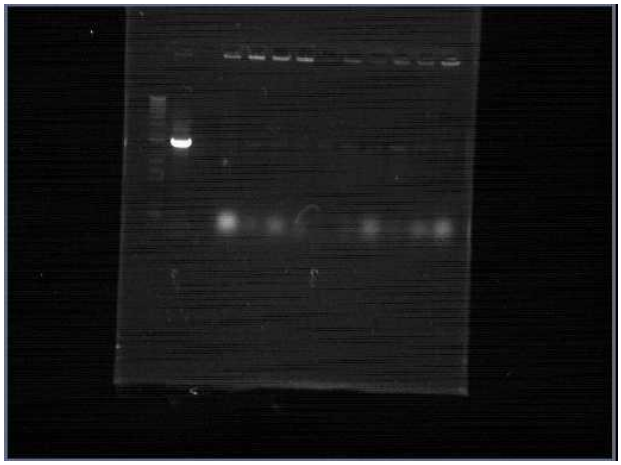
	A	B	C
1			(# col +2 control)
2	Thing	1 rxn	12
3	EconoTaq	12.5	150
4	Amp1	0.25	3
5	Amp2	0.25	3
6	H2O	12	144
7	Total	25	300

Master Mix for 12 reactions: Sequence confirmed DBAT-pSB1C3 (75-2), Seq. confirmed EV DBAT-pSB1C3 miniprep, 2A 1-4, 2B 1-4, 1- 1,2

7-15 DBAT-pSB1C3.jpg



Nisa071516-1.jpg



Ran extra positive control

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SATURDAY, 7/16

Colony PCR on LCAmp 20 uL Plate Colonies

Table62

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	13.2
3	EconoTaq	12.5	165
4	SL1	0.25	3.3
5	SR2	0.25	3.3
6	H2O	12	158.4
7	Total	25	330

Master Mix for 12 reactions: PSM, GFP, TBT-M, TBT-C, 8 colonies

Run at 8 PM and left overnight

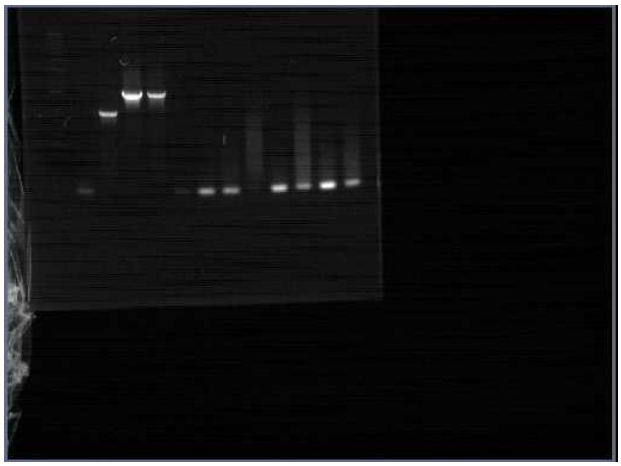
Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

SUNDAY, 7/17

Gel of PCR products run at 6 PM

LCKANTry071716.jpg



Ladder, PSM, GFP, TBT-M, TBT-C, LCAmp + TAX10 Colonies 1-8 (All negative)

Inoculations of DBAT pSB1C3 (Nisa)

Inoculated 4 colonies DBAT pSB1C3 1-2. 2A3, 2A4, 2B2

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

MONDAY, 7/18

Endura Competent Cells Transformations

- LC Amp + TAX10 in Broth: 1710 V, 4.3 ms - plated 2 x 50 uL + 2 x 10 uL
- LC Amp + TAX10 in Endura Special Recovery (875 uL): 1680 V, 3.5 ms - plated 2 x 50 uL + 2 x 10 uL
- LC Amp Control: 1690 V, 3.8 ms - plated 50 uL, 10 uL
- FAKE LC Amp Control: 1670 V, 3.2 ms - plated 50 uL, 10 uL

> 2 hour recovery, plated around 6 PM

Miniprep/Concentration Check of DBAT pSB1C3 (Nisa)

Samples:

- DBAT pSB1C3 1-2
- 2A3
- 2A4
- 2B2

Protocols:

- Miniprep Protocol Zyppy
- Testing Miniprep Concentrations
- Preparation for Sequencing

Miniprep Edit: Used a 40 uL elution

Table71

	A	B	C	D	E
1	Sample:	DBAT pSB1C3 1-2	2A3	2A4	2B2
2	Concentration (ng/uL)	69	65	85	27

pSB1C3 Purification and Concentration Check (Nisa)

Protocol:

- Gel Purification

Edits:

- Started with 1,500 ng of pSB1C3
- Used 6 uL elution buffer

Results:

Combined from 5 samples into 3 samples of pSB1C3

Table75

	A	B	C	D
1	Sample:	1	2	3
2	Concentration (ng/uL)	12	10	9

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

TUESDAY, 7/19

Colony PCR of 10 uL LCAmp Endura Plates

Table63

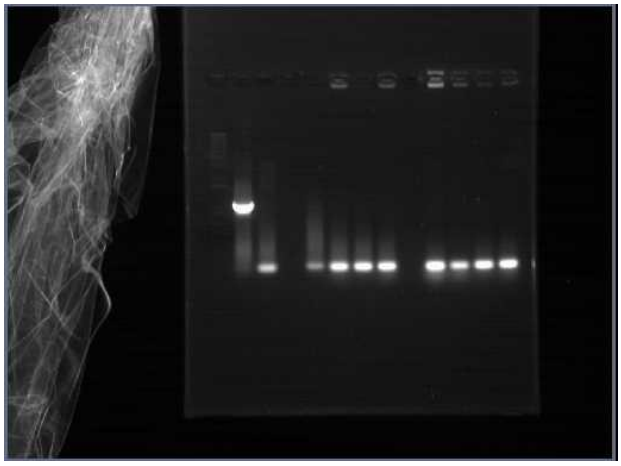
	A	B	C
1			(# reactions x 1.1)
2	Thing	1 rxn	33
3	MM	12.5	412.5
4	SL1 (3.2 uM)	7.8125	257.8125
5	SR2 (100 uM)	0.25	8.25
6	H2O	4.4375	146.4375

Master Mix

2:45 Elongation

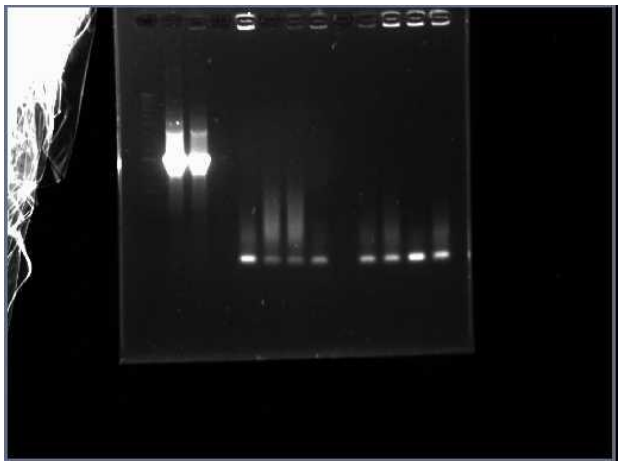
Brandon
Ladder, GFP, PSM, Blank, 1, 2, 3, 4, Blank, 10, 38, 39, 40

BrandonEndura071916.jpg



Suzie
Ladder, TBT-M, TBT-C, Blank, 17, 18, 19 20, Blank, 2, 3, 4, 5

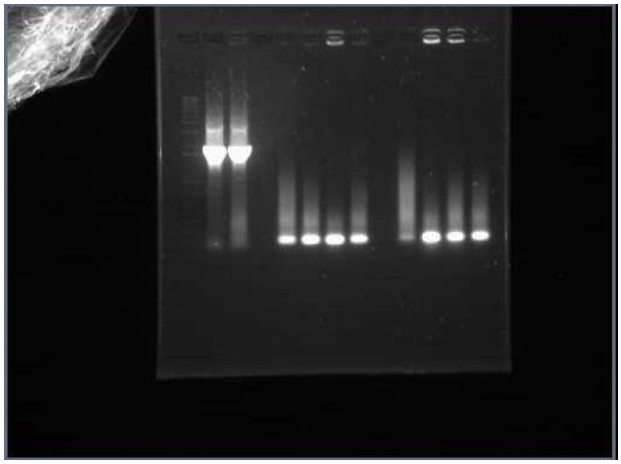
SuzieEndura071916.jpg



Mike

Ladder, BadA-M, BadA-C, Blank, 16, 17, 18, 19, Blank, 28, 31, 32, 39

MikeEndura071916.jpg



GBlock Amplification PCR

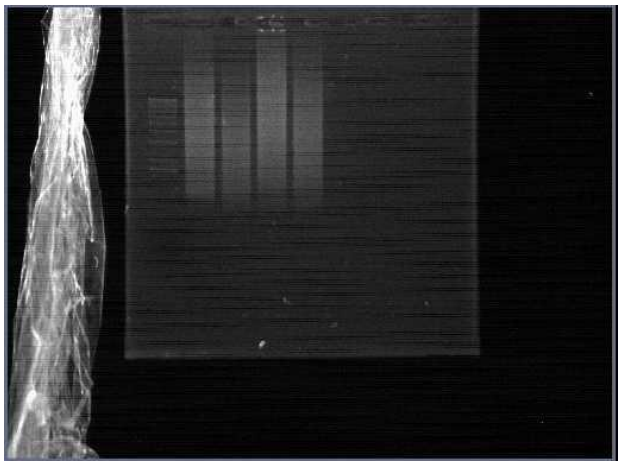
Table64

	A	B	C
1			(# reactions x 1.1)
2	Thing	1 rxn	4.4
3	Q5-2xMM	25	110
4	Forward Primer (10 uM)	2.5	11
5	Reverse Primer (10 uM)	2.5	11
6	PICO H2O	19	83.6
7	**Total**	**49**	**215.6**

Master Mix: 49 uL added to 1 uL of DNA for each reaction

0:55 Elongation

GBlockAmp071916.jpg



Ladder, BAPT (5 ng/uL), 1:10 BAPT, TAX10 (9 ng/uL), 1:10 TAX10

Colony + Miniprep PCR on DBAT pSB1C3 (Nisa)

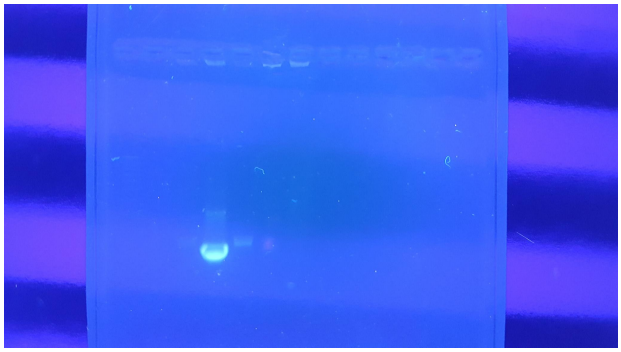
3:00 elongation

Table72

	A	B	C
1			(# col +3 control)
2	Thing	1 rxn	10
3	EconoTaq	12.5	125
4	Amp1	0.25	2.5
5	Amp2	0.25	2.5
6	H2O	12	120
7	Total	25	250

Master Mix for 12 reactions: Sequence confirmed DBAT-pSB1C3 (75-2) colony, Seq. confirmed EV DBAT-pSB1C3 miniprep, Seq. confirmed EV DBAT-pSB1C3 colony, Miniprep: 1-2, 2A3, 2A4, 2B2, Colony: 1-2, 2A4, 2B2

7-19 Colony Miniprep PCR of DBAT-pSB1C3.jpg



Only had bands on colonies, including negative control

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

WEDNESDAY, 7/20

Temperature Gradient PCR for GBlock Amplification

Table65

	A	B	C
1			(# reactions x 1.1)
2	Thing	1 rxn	4.4
3	Q5-2xMM	25	110
4	Forward Primer (10 uM)	2.5	11
5	Reverse Primer (10 uM)	2.5	11
6	PICO H2O	19	83.6
7	**Total**	**49**	**215.6**

Master Mix: 49 uL added to 1 uL of DNA for each reaction

1:05 Elongation

GBlockAmplification72016.jpg

Ladder, BAPT @ 63°C Annealing, BAPT @ 60.8°C Annealing, BAPT @ 58.4°C Annealing, BAPT @ 57°C Annealing, Blank, BAPT @ 60.8°C Annealing (redone)

Inoculations

From Endura 10 uL plates: Red #38 & #1 + Blue #3 & #28 > Inoculated in LB + Amp @ 4 PM, left in shaker

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 7/21

Troubleshooting GBlock Amplification, feat. Adim

Table66

	A	B	C
1			(# reactions x 1.1)
2	Thing	1 rxn	4.4
3	Q5-2xMM	12.5	55
4	Forward Primer (10 uM)	1.25	5.5
5	Reverse Primer (10 uM)	1.25	5.5
6	PICO H2O	4	17.6
7	GC Enhancer	5	22
8	**Total**	**24**	**105.6**

Master Mix: 24 uL added to 1 uL of DNA for each reaction

3:00 Elongation

Suzie

GBlock1Amp72116.jpg

Ladder, BAPT @ 63°C Annealing, BAPT @ 60.8°C Annealing, BAPT @ 58.4°C Annealing, BAPT @ 57°C Annealing

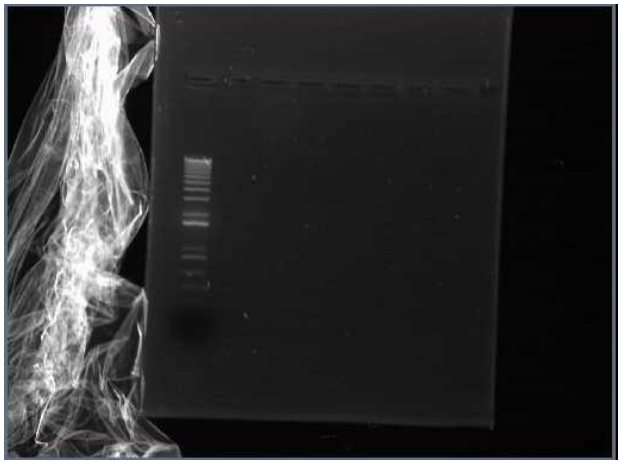
Table67

	A	B
1		
2	Thing	1 rxn
3	Q5-2xMM	12.5
4	Forward Primer (10 uM)	1.25
5	Reverse Primer (10 uM)	1.25
6	PICO H2O	8
7	**Total**	**23**

Added to 2 uL of DNA for reaction

3:00 Elongation

GBlock2Amplify072116.jpg



Ladder, BAPT @ 63°C Annealing

Note: 15 uL of 5 ng/uL BAPT left

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

FRIDAY, 7/22

Transformations of Q5 Mutagenesis S.C. Products into DLF-00286

- TBT: 1630 V, 654 ms
- BadA: 1670 V, 3.0 ms

> Plated each of 75 uL and 5 uL recovery:95 uL LB broth mixtures on LB+KAN plates for both transformations at 8 PM

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

MONDAY, 7/25

Creating Parts

Project: Duke iGEM 2016
Authors: Jaydeep Sambangi
Dates: 2016-05-18 to 2016-07-28

THURSDAY, 7/28

Table74

	A	B	C
1			(# col +1 control)
2	Thing	1 rxn	2
3	MM	12.5	25
4	Amp2_F	0.25	0.5
5	SR2	0.25	0.5
6	H2O	12	24

Lab Maintenance

Project: Duke iGEM 2016
Authors: Nisakorn Valyasevi
Dates: 2016-05-18 to 2016-08-27

SATURDAY, 8/27

Made Electrocompetent Cells (286 - J, R)

- 36 aliquots were made