iGEM 2016: Pittsburgh
Week 12 Lab Notebook

Monday, August 8

Modeling Meeting (Maya with Natasa)
Work on poster for H2O Day (Claire)
Digest prescreen of last year's common plasmids for LacZ (Maya)
10 ul reaction with EcoRI and pstI
1 ul DNA
Negative results

Tuesday, August 9

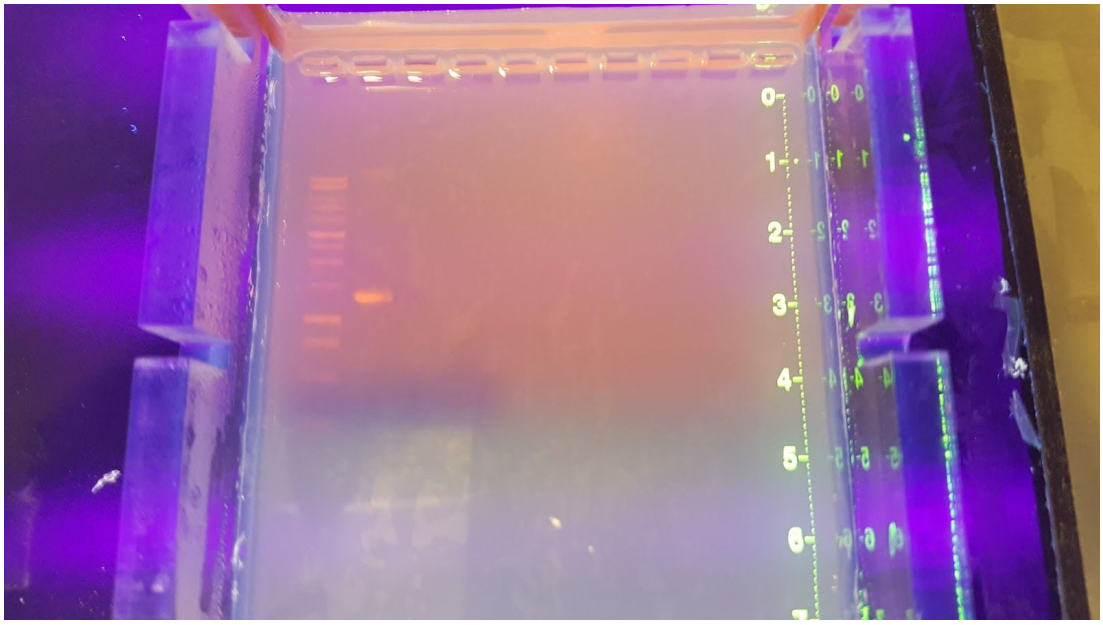
Work on poster for H2O Day (Claire)
Sequencing for PT3 and PT3-RBS came in (Maya/Maddie)
Confirmation of successful PT3 ligation
PT3-RBS not successful
Colonies looked bad on plate, so new ligation for colonies
Started construction PT3-RBS-T3 (Maddie)
Digested PT3 with pstI and SpeI
20 ul reaction
Details
However, put all into gel so will try to gel extract
If not good, will start over on Wednesday
Mutagenesis of LacZ (Maya)
Phosphorylation of forward and reverse primers with PNK T4
1 ul T4 PNK
5 ul 10X T4 Ligase buffer
2 ul DNA
42 ul Nuclease-free Water
Incubation 37°C for 30 minutes
0.5 µl forward primer 2.5 pmoles/µl
0.5 µl reverse primer, 2.5 pmoles/µl
0.25 µl 40 mM dNTP mix, (10 mM each)
1.25 µl Phusion Buffer Buffer
1 µl Template DNA, 2 ng/µl
0.25 µl Phusion
8.75 µl sterile H2O

Resuspend all of Lead DNAzymes and substrates (6 of them) (Praneeth)
Measured all of their concentrations and wrote it on the top.
Annealed 5 nm substrate:5 um dnazyme of the original DNAzyme in a 50 uL reaction, not the modified ones
Also did a 1:500, 1:250 and 1:100 ratios

Stored in the Roy -20 in the top left.
Labeled as ‘Pb 1000’ , ‘Pb 500’ etc
Did not have lead so couldn’t do much else

Wednesday, August 10

Work on poster for H2O Day (Claire/Maya)
Weekly meeting
Annealed 5 nm substrate:5 um dnazyme of the modified D and G Dnazymes (Praneeth)
Also did a 1:500, 1:250 and 1:100 ratios
Stored in the Roy -20 in the top left.
Labeled as ‘Pb G 1000’ , ‘Pb G 500’ etc
Did not have lead so couldn’t do much else
LacZ Mutagenesis Cont.
Gel check 2.5 ul
No lines detected against Collins 15 control



Cut with DpnI
Transformed (But no kanamycin plates, so did not plate)

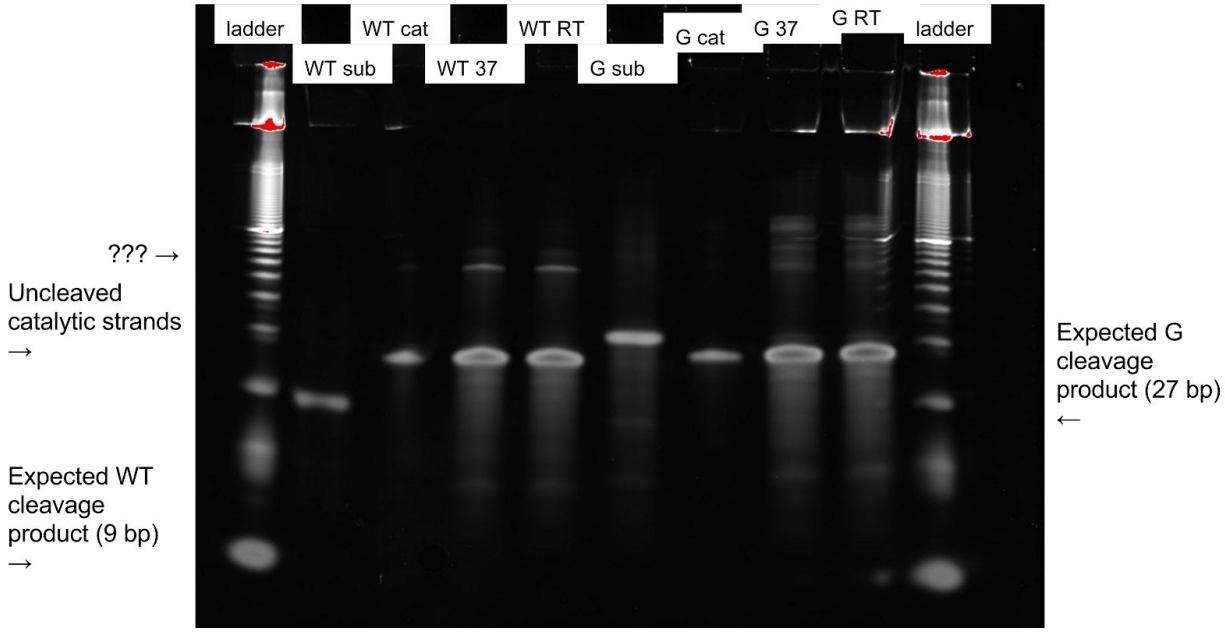
Thursday, August 11

Work on poster for H2O Day (Claire/Maya)
Work on video for fundraising (Maya)
Made kanamycin and chloramphenicol plates
Gel extraction of backbone and RBS-T3 with XbaI and PstI
5 ug DNA
40 ul reaction
2 ul XbaI
2 ul PstL

4 ul buffer
Details ul DNA
--- ul H2O
45 min incubation at 37, 20 min at 65
Ligated PT3-RBS-Backbone
Details
Transformed LacZ mutagenesis product

Friday, August 12

Poster and activity sheet for H2O Day finished
Work on video for fundraising (Praneeth/Maddie)
Abstract/Title/Track Selection done (Claire)
No LacZ on plates (Maddie)
Liquid cultures of PT7-RBS-T3 and PT3-RBS-T3 (Maddie)
Ligation and transformation of PT3-RBS-Backbone (Maddie)
Details
Annealed 50 nm substrate:50 um dnazyme of the unmodified and G Dnazymes (Praneeth)
Only did 1 ratio
30 uL annealing volume
Stored in the Roy -20 in the top left.
Labeled as 'Pb WT 1000', 'Pb G 1000'
Got lead from Dr. Bain
Made two dilutions of the lead so that it was easier to work with
Ran a reaction to see if the DNAzyme was working
50 uL reaction volume
2 um lead in the solution
Added 5 uL of the annealed product from today so that the final concentration was 5 nm substrate:5 um dnazyme
Each DNAzyme was looked at two different temperatures - 37 C and RT
1 hour incubation
Ran a denaturing gel to see if cleavage took place as expected
No observable cleavage product in WT or G
Mystery bands above reaction lanes



Saturday, August 13

Spun down liquid cultures, freeze

Took out PT3-RBS-Backbone Plates

H2O Day at Carnegie Science Center