iGEM 2016: Pittsburgh
**Week 4 Lab Notebook**

*Monday, June 13*

Lab meeting

Kanamycin and ampicillin plates

250 mL each

1:1000 dilution of stock solution

Transformation (Claire and Maddie)

lacZ alpha fragment (part BBa_I732006) Plate 3, 3B

eGFP (part BBa_E0040) Plate 4, 13L

50 uL competent cells + 5 uL resuspended DNA

T7 -- GFP (from last year's team) + 150 uL competent cells

Incubation for 1 hour

lacZ and eGFP initial incubation on ice for closer to 1 hour waiting for T7 -- GFP

Liquid cultures overnight of T7 - RBS and terminator sequences from last week

Mini-prep liquid cultures of T7 - RBS -- amilCP (Maya)

| DNA | Concentration (ng/ul) |
|---|---|
| 5:1-1 | 94.2 |
| 5:1-2 | 114.2 |
| 5:1-3 | 72.3 |
| 5:1-4 | 67.0 |
| 5:1-5 | 99.7 |
| 3:1-1 | 130.1 |
| 3:1-2 | 130.4 |
| 3:1-3 | 93.3 |
| 3:1-4 | 93.4 |
| 3:1-5 | 122.0 |

Digest and gel electrophoresis check of ligation with SpeI and XbaI of 5:1 and 3:1 samples

Used 100ng DNA

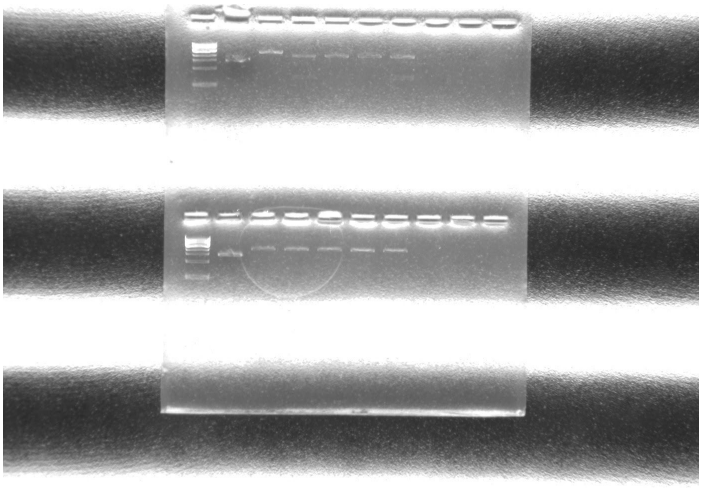
-3-2, 3-5 looked good; 3-3, 3-4 were weird; rest were incorrect

Control is T7-RBS uncut

Ladder is 1kb

Top lane is 3:1 (Ladder, control, 1, 2, 3, 4, 5)

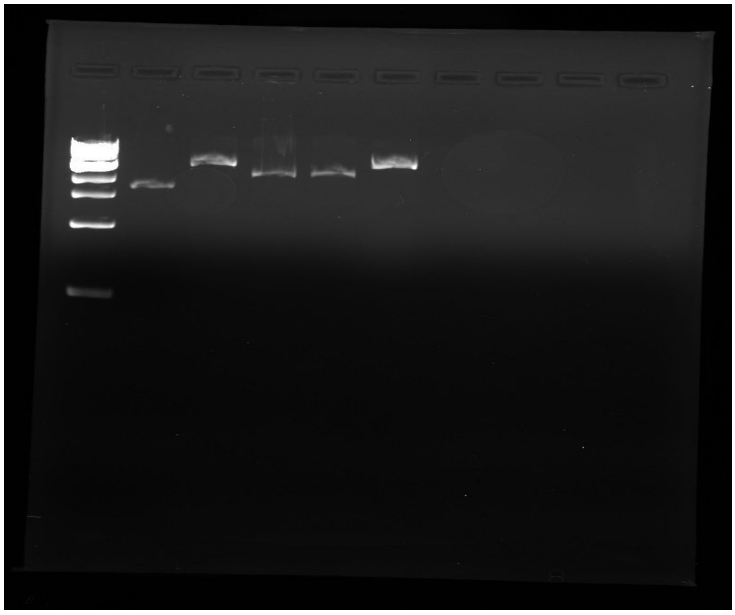
Bottom lane is 5:1 (Ladder, control, 1, 2, 3, 4, 5)



*Tuesday, June 14*

T7-GFP: many colonies
eGFP: a few colonies
lacZ: one colony in afternoon
Resuspended DNA placed in vial
Concentration = 15.9 ng/µL
Liquid cultures of T7-GFP (x2), eGFP (x2), and lacZ (x1) (Claire)
5 mL LB + 5 µL ampicillin
Miniprep liquid cultures of T7 RBS and terminator sequences
Concentrations: T7-RBS 1: 127.6 ng/uL
T7-RBS 2: 92.1 ng/uL
Terminator 1: 56.5 ng/uL
Terminator 2: 91.4 ng/uL
Restriction digests
T7-RBS: 1 uL SpeI
1 uL PstI
1 ug T7-RBS 1 = 7.84 uL DNA
2 uL 10x buffer
7.16 uL H2O
Terminator: 1 uL EcoRI
1 uL XbaI
1 ug Terminator 2 = 10.94 uL DNA
2 uL 10x buffer
5.06 uL H2O
Request full lacZ (BBa_K1444017) from iGEM, 2014 Submission Plates
*sequence not confirmed

Linearized 3:1 (2,3,4,5) with EcoRI using 1ul DNA (6/14 gel electrophoresis image)



1kb Ladder
T7-RBS uncut control
Ladder, control, 2, 3, 4, 5
2 and 5 migrated the least, these probably have T7-RBS-amilCP
3 and 4 migrated in between control and 2/5: Cut, but did not have insert
Contact TECBio and DiSCoBi0

*Wednesday, June 15*

Glycerol stocks: T7-GFP (MP), eGFP (MP), lacZ (Claire)
Mini-prep all liquid cultures (Claire)
Concentrations
T7-GFP MP: 120.8 ng/ul
T7-GFP CC: 110.9 ng/ul
eGFP MP: 160.6
eGFP CC: 188.5
lacZ: 103.8
restriction digests (Praneeth)
lacZ: 1 ul XbaI
1 uL PstI
1 ug DNA = 9.63 ul lacZ
2 uL 10x buffer
6.37 uL H2O
eGFP: 1 ul XbaI
1 uL PstI
1 ug DNA = 9.63 ul eGFP CC
2 uL 10x buffer

10.69 uL H2O

eGFP: 1 ul XbaI
1 uL PstI
1 ug DNA = 6.23 ul eGFP MP
2 uL 10x buffer
9.77 uL H2O

Incubate at 37 degC 30 min.

Incubate at 80 degC 20 min.

Gel electrophoresis

| | | |
|---|---|---|
| Lane | 1 | 2 ul ladder, 2 ul loading dye |
| | 2 | uncut T7, 2 ul dye |
| | 3 | 1 ul double digest T7 (SpeI/PstI) |
| | 4 | uncut terminator, 2 ul dye |
| | 5 | 1 ul double digest terminator (EcoRI/XbaI) |
| | 6 | 1 ul eGFP CC uncut, 2 ul loading dye |
| | 7 | all double digest eGFP CC (XbaI/PstI), 2 ul dye |
| | 8 | all double digest eGFP MP (XbaI/PstI), 2 ul dye |
| | 9 | 1 ul lacZ uncut, 2 ul dye |
| | 10 | all double digest lacZ (XbaI/PstI), 2 ul dye |

Purify T7 and terminator (Maya)

| | | |
|---|---|---|
| Concentrations: | T7: | 69.9 ng/uL |
| | Terminator: | 66.5 ng/uL |

Gel extraction of eGFP (2 lanes) and lacZ (Praneeth)

| | | |
|---|---|---|
| Concentrations: | eGFP CC: | 11.9 ng/ul |
| | eGFP MP: | 15.8 ng/ul |
| | lacZ: | 10 ng/ul |

Ligation: T7 – reporter (eGFP or lacZ) (Claire)

Control: 1 ul T7
0 ul insert
2 ul buffer
16 ul H2O
1 ul T4 DNA ligase

3:1 eGFP: 1 ul T7
50 ng eGFP = 3.16 uL eGFP MP
2 ul buffer
12.84 ul H2O
1 ul T4 DNA ligase

7:1 eGFP: 1 ul T7
115 ng eGFP = 9.66 uL eGFP CC
2 ul buffer
10.34 ul H2O
1 ul T4 DNA ligase

3:1 lacZ: 1 ul T7

50 ng eGFP = 5 uL lacZ
2 ul buffer
11 ul H2O
1 ul T4 DNA ligase

Incubate at room temperature for 10 min
Incubate at 65 degC for 10 min

Transformation of ligation reactions (Aife)

50 uL Cheryl's competent cells
5 uL reaction
Ice 20 min
42 degC 2 min
Ice 5 min
Add 200 uL SOC medium
37 degC shaker for 1 hr 4 min
Plate 100 uL onto chloramphenicol plates

Sent out 3:1 (2), 3:1 (5), EGFP, and Terminator to GeneWiz for sequencing (Maya)

Used VF2 and VR both for 3:1 plasmids
For terminator and EGFP used VF2

*Thursday, June 16*

Liquid cultures of transformations (Aife)

3:1 lacZ (x2)
3:1 eGFP (x5)
7:1 eGFP (x1)

*Friday, June 17*

7:1 eGFP didn't actually contain a colony

Mini-prep yesterday's liquid cultures (Claire)

Concentrations:
lacZ 3-1: 193.3 ng/uL
lacZ 3-2: 170.8 ng/uL
eGFP 3-1: 144.3 ng/uL
eGFP 3-2: 151.1 ng/uL
eGFP 3-3: 198.6 ng/uL
eGFP 3-4: 182.8 ng/uL
eGFP 3-5: 208.7 ng/uL

Restriction digest: check for insert (Praneeth)

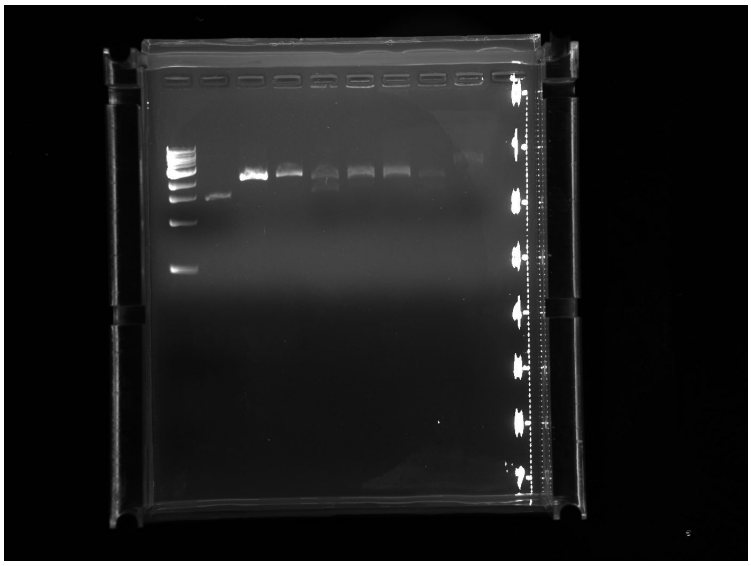
For all mini-preps,
1 uL EcoRI
0.5 uL DNA
1 uL buffer
7.5 uL H2O

Incubate at 37 degC for 30 min; 65 degC for 20 min

Gel electrophoresis

Lane 1 ladder

| | |
|---|---|
| 2 | uncut T7-RBS |
| 3 | T7 - lacZ 3-1 |
| 4 | T7 - lacZ 3-2 |
| 5 | T7 - eGFP 3-1 |
| 6 | T7 - eGFP 3-2 |
| 7 | T7 - eGFP 3-3 |
| 8 | T7 - eGFP 3-4 |
| 9 | T7 - eGFP 3-5 |



lacZ 1 and 2; eGFP 2, 3, and 4 good?