11

Tuesday, February 11, 2014
3:16 PM

- Digested 2 pLac (up), 2 GFP (down), 2 destination
- 1st Gel (Melody's winning team):
    - Up
    - Up DNLP
    - Up
    - Down
    - Down DNLP
    - Ladder
    - Destination DNLP
    - Destination
- 2nd Gel (Andy's losing team):
    - Ladder
    - Up
    - Down
    - Destination

12

Thursday, February 13, 2014
2:31 PM

1. Julie Wong, Bethany Shieh

   Running gel purification n to purify the backbone for destination plasmid

2. Michael, Lily

   PCR purification on plac (upstream) and GFP (downstream)

13 (the better class)

Thursday, February 13, 2014
2:35 PM

NO UR NOT >:)

Melody, Rachel, and Andy:

Part 2 of 2: Growing up cell cultures

Put agar culture into liquid culture for Part A(Bba_J04500) and Part B (Bba_J04650)

Remember Mr. Clapper, please come in at 7am in the morning and seal the culture tube cap and store at4 degrees celsius

2. During FLEX
   Julie and Michael:

   Run Gel extraction on the destination cell from 12th February (#1)

14 (class with the 2400)

Friday, February 14, 2014
2:49 PM

1.

Gregory Huang
Christopher Yen
Edward Hsieh

-Gel Extraction of GFP and DP from 12th Feburary (#2)
-Finished Gel Extraction Protocol (up to step 4 DNA Elution)
Parts in the digested box (labeled as EG)

2.
Bethany Shieh
Lily Han
Julie Wong
Michael Pai

Plasmid DNA extraction on Part A(AK J04500) and Part B(AK J04650)
Parts in the pure plasmid box

17 best day

Monday, February 17, 2014
2:28 PM

Digestion

Finished digestion protocol

18 nom nom nom

Tuesday, February 18, 2014
2:10 PM

Ligation
-Lily Hahn

Momentous Days

Tuesday, February 11, 2014
3:22 PM

- 02/11/14 - iGEM Notebook Commences
    - Founders - Melody, Rachel, Andy, Brian, Edward, Rohan, Phillip

Julie was here >:D

Days that people did not clean up

Tuesday, February 11, 2014
3:22 PM

21

Friday, February 21, 2014
2:12 PM

Transformation Results:

- Failed
  - LacI
  - J23102+E0840
  - We have to retransform all of these, get new plasmids from NYMU
- Successes
  - Ampicillin:
    - pNhaA+E0840
    - K592016
    - pLac+RFP
  - Chlor.
    - I712074 + E0840
    - K592006
    - TermA
    - J23102
    - K592016
- 3 in 1 today:
  - 3 Colonies for each of the 8 successful transformations
  - 20 microL PCR mixes
    - Positive Control: E0840 GFP generator
    - 20 microL for each PCR tube
  - 2nd Time Streaks
    - Split 3 since we are taking 3 colonies from each plate
- PCR Mix Labeling of the Plasmids:
  - A- pNhaA+E0840 - amp
  - B- K592016 - amp
  - C- pLac+RFP - amp
  - D- I712074 + E0840 - chlor -
  - E- K592006 - chlor
  - F- TermA - chlor
  - G- J23102 - chlor
  - H- K592016 + E0840 - chlor
- Red color refers to the labeling key. Black refers to the name/resistance.
- PCR - took out C3 and put in the positive control

22

Saturday, February 22, 2014
8:40 AM

Julie, Beth, Edward, Rohan, Phillip

- Cell Culture results
  - A- pNhaA+E0840 - amp
  - B- K592016 - amp
    - FAIL - even though there are many cells on the plate, the cultures did not grow
    - Scraped cells off the plate and put it straight into LB centrifuge tubes for plasmid extraction
    - For comparison also scraped positive control off the plate and put it into centrifuge tubes for testing the plasmid extraction by directly scraping and putting into eppendorf tubes
  - C- pLac+RFP - amp
  - D- I712074 + E0840 - chlor
  - E- K592006 - chlor
  - F- TermA - chlor
  - G- J23102 - chlor
  - H- K592016 + E0840 - chlor
  - EVERYTHING ELSE GREW =)

- What we are doing today:
  - Run the PCR colony checks on:
    - A- pNhaA+E0840 - amp
    - B- K592016 - amp
      - FAIL - even though there are many cells on the plate, the cultures did not grow
    - C- pLac+RFP - amp
    - F- TermA - chlor
    - G- J23102 - chlor
    - H- K592016 + E0840 - chlor
  - Plasmid Extraction of:
    - A- pNhaA+E0840 - amp
    - C- pLac+RFP - amp
    - D- I712074 + E0840 - chlor
    - E- K592006 - chlor
    - F- TermA - chlor
    - G- J23102 - chlor
    - H- K592016 + E0840 - chlor
    - Not K592016 because it failed

- Things to do for next time:
  - Transform
  - LacI
  - J23102+E0840
  - B0015
    - We need to get these plasmids from NYMU
  - Do the 3 in 1 for K592016 again
  - Digest all these parts appropriately:
    - A- pNhaA+E0840 - amp

- B- K592016 - amp
- C- pLac+RFP - amp
- D- I712074 + E0840 - chlor
- E- K592006 - chlor
- F- TermA - chlor
- G- J23102 - chlor
- H- K592016 + E0840 - chlor

24

Monday, February 24, 2014
4:48 PM

Michael & Lilee

Run gel on PCR check

PCR Check Results:

- A- pNhaA+E0840 - amp
  - Should be 1396 bp
  - PCR: 500
  - Wrong
- B- K592016 - amp
  - Should be 2034 bp
  - PCR: 2000
  - B2 is correct
- C- pLac+RFP - amp
  - C1, C2 Correct
  - PCR: 1400
  - C1, C2, Correct
- D- I712074 + E0840 - chlor
  - Should be 1238 bp
  - PCR: Over 2000
  - Wrong
- E- K592006 - chlor
  - Should be 564 bp
  - PCR: Around 600
  - All Correct, should be
- F- TermA - chlor
  - Should be 443 bp
  - PCR: Around 400
  - All correct
- G- J23102 - chlor
  - Should be 349
  - PCR: 1100
  - Wrong
- H- K592016 + E0840 - chlor
  - Should be 2988 bp
  - PCR: 1500
  - Wrong

- Good:
- B- K592016 - amp
  - Should be 2034 bp
  - PCR: 2000
  - B2 is correct
- C- pLac+RFP - amp
  - C1, C2 Correct
  - PCR: 1400
  - C1, C2, Correct
- K592006 - chlor
  - Should be 564 bp
  - PCR: Around 600
  - All Correct, should be
- TermA - chlor
  - Should be 443 bp
  - PCR: Around 400
  - All correct

Bad:

- pNhaA+E0840 - amp
  - Should be 1396 bp
  - PCR: 500
  - Wrong
- D- I712074 + E0840 - chlor
  - Should be 1238 bp
  - PCR: Over 2000
  - Wrong
- G- J23102 - chlor
  - Should be 349
  - PCR: 1100
  - Wrong
- H- K592016 + E0840 - chlor
  - Should be 2988 bp

Important note on labeling plates on the side:

- **Blue** line is Ampicillin
- **Red** line is Chloramphenicol

February

- PCR: 1500
- Wrong

- Gels:
  - A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3, LADDER
  - E1, E2, E3, F1, F2, F3, G1, G2, G3, H1, H2, H3, LADDER

25

Tuesday, February 25, 2014
2:42 PM

- Digest:
  - K592016 (ES), TermA (EX)
  - TermA (SP), pLac (XP)
  - Follow the same digestion protocol, just with different enzymes
- Run the gels on those cuts

- 3 extra gels in Fridge : 1 1.5% agarose gel, 2 1% agarose gel

- Things to do for tomorrow:

- Plasmids we need from NYMU
  - RBS (B0034)
  - J23102
  - LacI
  - Terminator
  - pTet
  - TetR

- PCR results order (16 wells)

1. Ladder
2. Ladder
3. TermA (SP) -1
4. TermA (SP) -2
5. TermA (SP) -3
6. pLac (XP) -1
7. pLac (XP) -2
8. pLac (XP) -3
9. TermA (EX) -1
10. TermA (EX) -2
11. TermA (EX) -3
12. YF1+FpcJ (ES) -1
13. YF1+FpcJ (ES) -2
14. YF1+FpcJ (ES) -3
15. Ladder
16. Blank

Note: There were two ladders to differentiate the order from the first well to the last one. In this case, the first well is one with the ladder present. It should also be the well on the left if the gel is oriented so that the well on the upper half.

26

Wednesday, February 26, 2014
2:28 PM

Culture Tubes

3 colonies picked from each of the following cultures

3-1 performed.

Labels

- A- pNhaA+E0840 - amp
  - Promoter plus
- B- J23100 - amp
  - 35 bp
  - Constitutive promoter
- C- K592006 - chlor
  - 250 bp
- D- K592016+Term - chlor
  - 1925 bp
- E- RBS - amp
  - 12 bp
- F- pLac + B0034 - chlor
  - 204 bp
- G- pLac + RBS - chlor

27

Friday, February 28, 2014
2:42 PM

Gel:

- Ladder, A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3, Ladder
- Ladder, blank, E2, E1, E3, F1, F2, F3, G1, G2, G3, negative control, positive control, ladder

GOOD

BAD

28

Friday, February 28, 2014
11:18 AM

Lily & Mike: When we run gels, place TWO LADDERS ON LEFT and ONE LADDER ON RIGHT to indicate which side is which. This is the established system for gel reading.

_ _ …… _
L L samples L
LEFT RIGHT

==========

L,L, E1, E2, E3, F1, F2, F3, G1, G2, G3, L

L, L, A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3, L

Results: The PCR results were smudged and there were no definitive lines on the PCR.

Additionally, the sample on the right, did't have the previous gel casing removed, and therefore didn't run within the time limit set.

The second sample was thereby set to rn for another half an hour.

Additionally, it was found that the voltage was set on 100 Volts so this may explain for the smudging.

41-42 C

Glossary

Tuesday, February 11, 2014
3:19 PM

- DNLP - DNA not loaded properly into the gel

Important note on labeling plates on the side:

- **Blue** line is Ampicillin
- **Red** line is Chloramphenicol