7/27/17 (Someone Deleted)

Tuesday, October 24, 2017 11:12 PM

7/27/17

Thursday, July 27, 2017

8:38 AM

Who's in Lab: Salma (9-11:30), Martin (9-11), Ayesha

SUMMARY:

Gel extraction of JOE/YCP gels (run on 7/26)

JOE –0.4 g (dissolved in 1.2 mL solubilization buffer)

Bands were evident at ~

YCP – 0.53 g (dissolved in ~1.5 mL solubilization buffer)

Bands were evident at ~

Plate Reader Results:

JOE

-Conc. 5.4 ng/uL

-Ratio 2.08

YCP

-Conc. 7.3 ng/uL

-Ratio 2.81

Because the DNA concentrations were very low, I started a DNA precipitation on both JOE and YCP. Needs to be continued tomorrow so that we can do another Gibson assembly.

For DNA Precipitation of YCP and JOE:

45 uL of YCP DNA, 5 uL Sodium Acetate, 125 uL Ethanol

50 uL of JOE DNA, 5.56 uL Sodium Acetate, 138.8 uL Ethanol

DNA precipitation of LC 1853 & LC 1539

Taken from -20oC freezer

After centrifuging at max speed for 30 min no pellet was evident but procedure was continued avoiding the pellet "area"

1853

- Conc. 329.1 ng/uL
- Ratio 1.

1539

- Conc. 138 ng/uL
- Ratio 1.9

Miniprep of LC 1853/1539 overnight cultures w/ Tyler

***For future reference we should not make overnight cultures in the 50mL falcon tubes- instead an erlenmeyer provides better aerations

- spin for 3 min at 3000g
- After discarding supernatant, Add 2mL of P1 to the falcon tubes and carefully resuspand the pellet
  - Pipet 250uL aliquots of the resuspension buffer and the cell mixture into 8 centrifuge tubes
- Follow MIniprep protocol as noted
- We eluted with Water because the sequencing center prefers this as some buffers have EDTA (a chelator) which skew sequencing results.

Plate Reader Results:

LC 1853

- Conc. 95.3 ng/uL
- Ratio 1.89

LC 1539

- Conc. 130.5 ng/uL
- Ratio 1.85

Gibson Assembly Trial # 1 Results:

The BL21 cells did not express the YCP and hence there were no visible yellow colonies even under UV light. The colonies with pUC19 are smaller than colonies with YCP+JOE.

Another Gibson Assembly must be done. This time the control would be doing a gibson assembly with vector but no insert (JOE W/O YCP) and the experimental group would be vector with insert (JOE+YCP) to make sure that JOE is being cut for insert to be added.

The plate with JOE W/O YCP should not have colonies on it because the mismatched ends (caused by the cutting during G.A.) will not reanneal. Some background may be possible but the plate with JOE+YCP should significantly more colonies.

COLONY TESTING OF THE GIBSON ASSEMBLY PRODUCTS

- Took a swab of 10 DH5a colonies from the CAM+IPTG petri dishes that were plated with G.A. Trial #1 products and boiled into 50 ul water.
- We then spun down the centrifuge tubes to let the cell membrane sit at the bottom and separate it from the YCP+JOE DNA.
- Made a grid consisting of 12 areas on a new plate. So that we could determine which colonies had the gibson assembly product as a result of transforming them.
- Plated the colonies that had been diluted in water to the corresponding grids.
- New plate labeled: BL21 YCP+JOE COLONY TEST 7/27/17
- We performed 12 PCR reactions:
  - PCR Reaction tubes labeled 1-10, corresponded to the colonies we swabbed form the original plate and replated onto a new plate.
    - 1.0 uL of Forward Primer for YCP
    - 1.0 uL of Reverse Primer for YCP
    - 12.5 uL Master Mix
    - 10.5 uL of the YCP+JOE DNA we extracted by boiling the cells.
    - Total Volume: 25 uL
  - PCR Reaction tube 11 contained the Gibson Assembly Trial #1 YCP+JOE plasmid that was saved in the 4 deg. Fridge.
  - PCR Reaction tube 12 contained only JOE plasmid.
  - For tubes 11 and 12:
    - 1 uL template plasmid
    - 1.0 uL of Forward Primer for YCP
    - 1.0 uL of Reverse Primer for YCP

    - 12.5 uL Master Mix
    - 9.5 uL DI H2O
    - Total Volume: 25 uL
- The annealing temperature used for all of these reactions was 75 deg C. This annealing temperature was determined based on results from 7/25/17

LABELED TUBES:

- 1839 super (supernatant)
- 1539 super (supernatant)
- 7/27 LC1539 Miniprep
- 7/27 LC1853 Miniprep
- 7/27 JOE DNA PRECIP (in –20 freezer in iGEM 2017 box)
- 7/27 YCP DNA PRECIP (in –20 freezer in iGEM 2017 box)

WHAT TO DO TOMORROW:

- Complete JOE and YCP precipitation
- Do Gibson assembly with precipitated YCP and JOE DNA.
- Do more PCR for YCP and JOE DNA. We need extra aliquots of DNA for future trials.

LC1853 and LC1539 have already been sent to the sequencing center. Order number 65739

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