Name: Sijia Qin, Jiazi Tian, Chiara Brust, Justin Benton, Amirah, Kennex Lam, Asma Khimani
Date:7/24/19

Goals:

1. Mi**D**i prep
    a. K1357009
    b. Codon-optimized RFP
2. Make YM media

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Goals:

1. Make YM media

Protocol:

YM Media Recipe
Dissolve in 900 mL of diH2O
0.4 g Yeast extract
10 g Mannitol
0.1 g NaCl
0.2 g MgSO4ï 7H2O
0.38 g K2HPO4

Autoclaved for 45 minutes and brought to a pH of 7

Name: Asma Khimani, Justin Benton, Kennex, Amirah
Date:7/24/19

Goals:

1. Mi**D**i prep (5 samples)
    a. K1357009 150 ul LB-C #2
    b. K1357009 100 ul LB-C #2
    c. Codon-optmized RFP #3
    d. Codon-optimized RFP #10
    e. Condon-optimized RFP #7

Protocol:

QIAGEN Plasmid Midi Kit

1. Separated 100 mL of bacterial overnight culture into 5 separate 50 mL falcon tubes and centrifuged at **5,000 rpm** for 15 minutes at 4°C.
2. Poured out supernatant.
3. Added 4 mL of Buffer P1 to one tube, pipet mixed, and transferred to another tube. Mixed and transferred contents to the next tube with pelleted cells. Repeated until all tubes are combined.
4. Added 4 mL of Buffer P2 to the tube containing 4 mL of Buffer P1 and the combined resuspended pelleted cells. Inverted 6 times.
5. Incubated at room temperature for 3 minutes.
6. Added 4 mL of Buffer P3 and vigorously inverted 10 times.
7. Incubated on ice for 15 minutes.
8. Centrifuged at 20,000 x g at 4°C for 30 minutes.
9. After centrifuging, clear supernatant was transferred to another centrifuge tube while avoiding all of the flakes on the sides and in the solution. Used a syringe filter for this.
10. Centrifuged the tube again at 20,000 x g at 4°C for 15 minutes
11. While that ran, the QIAGEN-tip was equilibrated by adding 4 mL of QBT to the QIAGEN-tip.
12. Added the clear solution (from step 10) to the QIAGEN-tip and allowed it to enter the resin by gravity flow.
13. Next, 10 mL of Buffer QC was added to the QIAGEN-tip and allowed to gravity drip.
14. Once that passed through, 10 mL more of Buffer QC was added and allowed to flow through.
15. Then, 5 mL of Buffer QF was added and flowed through.

16. Added 3.5 mL of room temperature isopropanol to elute the DNA and mixed. Then centrifuged at 15,000 x g for 30 minutes at 4°C.
17. Carefully removed the supernatant making sure not to disrupt the clear pellet.
18. Added 2 mL of room-temperature 70% ethanol and centrifuged for 10 minutes at 15,000 x g at 4°C. Discarded the supernatant leaving as little liquid behind as possible, being careful not to disrupt the clear pellet.
19. Air-dried the pellet for 20 minutes in the vent hood and redissolved in 100 µL of Buffer EB.

Results:

Sample	**Concentration**	**Purity**
Codon rfp # 10	Too low	Too low
Codon rfp # 7	255	1.889
K1357009 100 ul	Too low	Too low
Rfp # 3	558	1.874
K1357009 150 ul	170	1.789

Conclusion:

We now have a high enough concentration of both codon-optimized-RFP and K1357009 to do gel extractions tomorrow.