Name: Rehmat Babar, Shakera Thomas

Date: 6/14/19

Goal: mini preps for pCB302 plasmid

Materials

QIAprep Spin Miniprep Kit Lot 160021667

Protocol

a. Centrifuged the overnight culture of bacterial overnight culture at 8,000 rpm for 3 minutes at room temperature.
b. Discarded the supernatant and resuspended the pelleted bacterial cells in 500 µL Buffer P1 and transferred it to two different Eppendorf tube of 250 µL each.
c. Added 250 µL of Buffer P2 and inverted 5 times.
d. Added 350 µL of Buffer N3 and immediately mixed by inverting 5 times.
e. Centrifuged for 10 minutes at 13,000 rpm.
f. Micropipetted 800 µL of the clear supernatant into a spin column and centrifuged for 60 seconds and discarded the excess liquid.
g. Added 500 µL of PB and centrifuged the spin columns for 60 seconds. Discarded the flow through.
h. Added 750 µL of PE to the spin columns, centrifuged for 60 seconds, and discarded the flow through.
i. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discarded the flow through.
j. Transferred the spin columns to a clean eppendorf tube and added 50 µL of EB to the center of the spin column to elute the DNA.
k. Allowed the spin column to stand for one minute and then centrifuged for one minute.
l. Recorded the concentrations for each sample.

Results

Mini Prep A1 pCB302 plasmid	15 ng/µL
Mini Prep A1 pCB302 plasmid	5 ng/µL
Mini Prep A2 pCB302 plasmid	5 ng/µL
Mini Prep A2 pCB302 plasmid	7.5 ng/µL

Mini Prep A3 pCB302 plasmid	Too low
Mini Prep A3 pCB302 plasmid	7.5 ng/μL
Mini Prep A4 pCB302 plasmid	5 ng/μL
Mini Prep A4 pCB302 plasmid	2.5 ng/μL
Mini Prep A5 pCB302 plasmid	10 ng/μL
Mini Prep A5 pCB302 plasmid	5 ng/μL
Mini Prep B1 pCB302 plasmid	7.5 ng/μL
Mini Prep B1 pCB302 plasmid	7.5 ng/μL
Mini Prep B2 pCB302 plasmid	10 ng/μL
Mini Prep B2 pCB302 plasmid	10 ng/μL
Mini Prep B3 pCB302 plasmid	10 ng/μL
Mini Prep B3 pCB302 plasmid	10 ng/μL
Mini Prep B4 pCB302 plasmid	7.5 ng/μL
Mini Prep B4 pCB302 plasmid	12.15 ng/μL
Mini Prep B5 pCB302 plasmid	7.5 ng/μL
Mini Prep B5 pCB302 plasmid	10 ng/μL

Conclusion

The concentrations are too low, it is not likely the our desired plasmid was successfully transformed and/or isolated.

Name: Amirah Hurst

Date: 6/14/19

Goal: gel extract for Bluechromoprotien (K592009) and RFP promotor (J23102) from analytical digests performed on 6/13/19

Materials

Specific brand name and lot number

Protocol https://www.mcdb.ucla.edu/Research/Banerjee/protocols/gelextraction--Qiagen.pdf

1. Cut gel fragments from yesterday into 3 small pieces (1 per gel lane)
    a. Only used 1 lane. The rest were placed in the fridge.
2. Weighed 1.5 ml microcentrifuge tube, zeroed it, placed fragment in microcentrifuge tube to get weight of fragment in mg.
    a. K: 335.6 mg
    b. J: 291.2 mg
3. Multiplied that weight x3 to determine ul of Buffer QG to add to the 1.5 ml microcentrifuge tube
4. Added Buffer QG
    a. K: 1,006.8 ul
    b. J: 873.6 ul
5. Added 30 ul QIEX 2
6. Placed tubes in 50 degrees C water bath for 10 mins vortexing every 2-3 mins to completely dissolve
    a. Incubated for additional 5 mins because it was not completely dissolved
7. Added 1 gel volume of 2-propanol to sample, inverted to mix
    a. K: 335 ul
    b. J: 291.2 ul
8. Place samples in quick spin column
    a. Used 2 spin columns for each sample
9. Centrifuge for 1 min
    a. To bind DNA to column
10. Discarded flow through
11. Added 0.75 ml of Buffer PE to column and centrifuge for 1 min
12. Discard flow through and centrifuge for 1 min
13. Place column into a 1.5 ml microcentrifuge tube
14. Eluted DNA with 30 ul of Buffer EB, let sit for 2 mins then centrifuged for 1 min
15. Checked concentration on nanophotometer

Results:

Both samples had very low concentration

i. J looked like maybe some was there
ii. K was redone

Jessica and Christina did another gel extraction on blue chromoprotein (K592009) and promoter (J23102)