

Name: Justin Benton, Shakera Thomas

Date: 26 July 2019

Goals:

1. MiDi prep DinIII-GFP P2
2. Finish MiDi prep pcb302 in E. Coli from papers 1 & 2 from glycerol stocks made on 7/3/19

Date: 7/26/19

Goal:

1. MiDi prep DinIII-GFP P2

Protocol:

MiDi prep for DinIII-GFP P2

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 were 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 5 minutes.
6. Added 4 mL of Buffer P3 and vigorously inverted 6 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 into a new tube.
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.

Results:

Samples	DNA Concentrations
P2 (4) 150 uL	32.5 ng/uL
P2 (10) 100 uL	85.0 ng/uL

Name: Sijia Qin, Jiazi Tian

Date: 7/26/19

Goals:

1. Finish MiDi prep pcb302 in E. Coli from papers 1 & 2 from glycerol stocks made on 7/3/19

Protocol:

1. The samples did not dry overnight.
2. Added 200 ml 70% ethanol and centrifuge at 15000 xg for 10 minutes.
3. Carefully removed the supernatant.
4. Incubated for a time.
5. Added 100ul of buffer EB.

Results:

Sample	Concentration
Pcb 302 1	Too low
Pcb 302 2	Too low
Pcb 302 3	Too low
Pcb 302 4	Too low
Pcb 302 5	Too low

Conclusions:

The concentrations were too low. We realized that we did not correctly follow the step 3. We also did not combine them together. We need to make pcb302 overnight cultures and do the midiprep again.