E. coli G3 DNA Miniprep

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, July 20, 2023

Protocol:

Note: these steps follow those outlined in the Monarch® Plasmid DNA Miniprep Kit Protocol (NEB #T1010) General Guidelines by New England Biolabs.

1. Transferred 5 mL of the G3 liquid culture into a microfuge tube
2. Centrifuged at 13,000 rpm for 30 seconds and discarded supernatant
3. Resuspended pelleted cells in 200 µL Plasmid Resuspension Buffer
4. Added 200 µL Plasmid Lysis Buffer to each tube and gently inverted the tubes until the solution turned dark pink
5. Incubated cells on the bench for 1 minute
6. Added 400 µL Neutralization Buffer to each tube and inverted the tubes until the solution turned yellow and a precipitate formed
7. Incubated cells on the bench for 2 minutes
8. Centrifuged tube at 13,000 rpm for 5 minutes
9. Inserted a spin column into a collection tube and transferred the supernatant into the column
10. Centrifuged the tube at 13,000 rpm for 1 minute and discarded flow-through
11. Added 200 µL Plasmid Wash Buffer 1 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
12. Added 400 µL Plasmid Wash Buffer 2 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
13. Transferred each column to a clean 1.5 mL microfuge tube
14. Added 30 µL Elution Buffer to each tube
15. Waited 1 minute before centrifuging all of the tubes at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

Sample Preparation for Sequencing

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1. **Added 3.28 µL 91.4 ng/µL plasmid (Colony D) to a microfuge tube**
2. **Diluted plasmid with 6.82 µL nuclease-free water**
3. **Shipped sample to Plasmidosaurus for sequencing**

Results: ??

Conclusion: The sequence of G3 shows that the His tags were not removed by our PCR so? We should do it again? We are going to colony PCr a colony from a plate that has hexanoic acid and glucose; we believe that the hexanoic acid concentration was too high, and therefore, too toxic. The colony PCR is to see if hexanoic acid is enough of a selective pressure for the bugs to keep the plasmid