E. coli G1 DNA Miniprep

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, May 3, 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 **(what volume did you do, the notebook says 1-5 mL)** of each liquid culture into separate microfuge tubes
2. Centrifuged all 4 tubes at 13,000 rpm for 30 seconds and discarded supernatant
3. Resuspended pelleted cells in each tube in 200 µL Plasmid Resuspension Buffer and vortexed until fully resuspended
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 all 4 tubes at 13,000 rpm for 5 minutes **(how many minutes did you centrifuge it? In the notebook you just wrote 2-5 minutes)**
9. Inserted a spin column into 4 collection tubes and transferred the supernatant into their respective column
10. Centrifuged all 4 tubes 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. Centrifuged all of the tubes at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

Measurement of post-HiFi is G4 DNA Concentration

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Date: Monday, June 5, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 µL Elution Buffer
3. Cleaned NanoDrop before loading 2 µL sample
4. Repeated step 3 for all pRSET samples

Results:

Colony	DNA Concentration	260/280	260/230	Volume	Mass
3	243 ng/µL	1.83	1.72	27.3 µL	6633.9 ng
4	252.6 ng/µL	1.87	2.25	24.4 µL	6163.44 ng
7	234.3 ng/µL	1.82	1.85	26.4 µL	6185.52 ng
8	236.0 ng/µL	1.87	2.26	24.4 µL	5758.4 ng

Conclusion: N/A