

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

Results: N/A

Conclusion: N/A

Measurement of G3 DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, May 9, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 μ L Elution Buffer
3. Cleaned NanoDrop before loading 2 μ L sample
4. Repeated step 3 for all G1 samples

Results:

Sample	DNA Concentration	260/280	260/230
G3NS	132.9 ng/ μ L	1.81	1.86
G3NM	71.2 ng/ μ L	1.87	1.15
G3NL	59.0 ng/ μ L	1.85	1.14
G3SS	102.0 ng/ μ L	1.77	1.22
G3SM	79.6 ng/ μ L	1.83	1.13
G3SL	99.4 ng/ μ L	1.82	1.81

Conclusion: N/A