E. coli pRSET-iGluSnFR3 DNA Miniprep

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Date: Saturday, June 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 of each liquid culture into 8 different microfuge tubes
2. Centrifuged all 8 tubes at 13,000 rpm for 30 seconds and discarded supernatant
3. Resuspended pelleted cells in each tube 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 all 8 tubes at 13,000 rpm for 5 minutes
9. Inserted a spin column into 8 collection tubes and transferred the supernatant into their respective column
10. Centrifuged all 8 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 2 minutes
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: We will measure the DNA concentration of each miniprepped sample for downstream use.