G1 Plasmid Miniprep

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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 8 liquid cultures at 13,000 rpm for 30 seconds and discarded the supernatant
2. Resuspended the pelleted cells in each tube in 200 µL Plasmid Resuspension Buffer
3. Added 200 µL Plasmid Lysis Buffer to each tube and gently inverted the tubes until the solution turned dark pink
4. Incubated the cells on the bench for 1 minute
5. Added 400 µL Neutralization Buffer to each tube and inverted the tubes until the solution turned yellow and a precipitate formed
6. Incubated the cells on the bench for 2 minutes
7. Centrifuged all 8 tubes at 13,000 rpm for 5 minutes
8. Inserted a spin column into 8 collection tubes and transferred the supernatant into their respective column
9. Centrifuged all 8 tubes at 13,000 rpm for 1 minute and discarded the flow-through
10. Added 200 µL Plasmid Wash Buffer 1 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
11. Added 400 µL 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 microfuge tube
13. Added 30 µL 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: To further confirm that G1 was synthesized correctly, we will perform a BbsI digest of our miniprepped samples. For the generation of the pDusk megaprimer via PCR, we used forward and reverse primers that had been deliberately designed to have a base pair change at the BbsI recognition sites. This was done intentionally because in the future, we plan to use BbsI as the restriction enzyme for a Golden Gate assembly to insert genes that code for defluorinating enzymes into our domesticated pDusk plasmid. Thus, G1 should not have any sites to which BbsI can bind to for digestion.