pDusk Megaprimer Purification

Participants: Brianna Branson, Rori Hoover, Patrick Jiang, Niam LeStourgeon

Date: Wednesday, April 26, 2023

Protocol:

Note: these steps follow those outlined in the Protocol for DNA Cleanup and Concentration Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) by New England Biolabs.

1. Diluted 25 µL pDusk megaprimer in 125 µL DNA Cleanup Binding Buffer
2. Inserted a spin column into a collection tube and loaded the diluted sample into the column
3. Centrifuged the column at 13,000 rpm for 1 minute and discarded the flow-through
4. Added 200 µL DNA Wash Buffer to the column and centrifuged it at 13,000 rpm for 1 minute
5. Repeated step 4 two times
6. Transferred the column to a clean 1.5 mL microfuge tube
7. Added 12 µL Elution Buffer to the tube
8. Waited 1 minute before centrifuging the tube at 13,000 rpm for 1 minute

Results: N/A

Conclusion: Our pDusk megaprimer is now ready for MEGAWHOP.

Measurement of pDusk Megaprimer DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang, Niam LeStourgeon

Date: Wednesday, April 26, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 µL Elution Buffer
3. Cleaned NanoDrop before loading 2 µL purified pDusk megaprimer

Results: The DNA concentration of the purified pDusk megaprimer sample was measured to be 131.1 ng/µL.

Conclusion: N/A

G1 Generation

Participants: Brianna Branson, Rori Hoover, Patrick Jiang, Niam LeStourgeon

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Protocol:

1. Prepared a 25 µL MEGAWHOP reaction in a PCR tube with the following components:

Component	Volume
25 ng pDusk plasmid	3.4 µL
100 ng pDusk megaprimer	0.76 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	8.34 µL
Total	**25 µL**

2. Prepared a 25 µL control reaction in another PCR tube with the following components:

Component	Volume
25 ng pDusk plasmid	3.4 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	9.1 µL
Total	**25 µL**

3. Amplified the DNA in both 25 µL reactions with a thermocycler using the following conditions:

Step	Temperature	Time	Number of cycles
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	30
Annealing	62°C	30 sec	30
Extension	72°C	3 min 6 sec	30
Final extension	72°C	2 min	1
Hold	10°C	Forever	-

Results: N/A

Conclusion: To confirm that the MEGAWHOP reaction was successful, we will perform a DpnI digest, transform E. coli with the digestion mixture, and plate the cells on LB-agar kanamycin plates. DpnI will break down pDusk plasmid, but it cannot digest G1 as it is synthetic DNA. G1 confers resistance to kanamycin, so if the MEGAWHOP reaction did occur, the transformed E. coli will be able to survive on the LB-agar kanamycin plates.

DpnI Digestion

Participants: Brianna Branson, Rori Hoover, Patrick Jiang, Niam LeStourgeon

Date: Wednesday, April 26, 2023

Protocol:

1. Added 0.5 µL DpnI to the G1 and control PCR tubes
2. Incubated the mixtures overnight at room temperature

Results: N/A

Conclusion: Since the digestion mixtures need to incubate overnight, we will transform E. coli the next time we are in the lab.