Measurement of mScarlet Megaprimer DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, May 11, 2023

Protocol:

1. Combined all 4 samples in a microfuge tube
2. Cleaned NanoDrop spectrophotometer with DI water
3. Blanked NanoDrop with 2 µL Elution Buffer
4. Cleaned NanoDrop before loading 2 µL extracted mScarlet megaprimer

Results: The DNA concentration of the extracted mScarlet megaprimer sample was found to be 31.3 ng/µL

Conclusion: The concentration of DNA is too low, so we will further elute the spin columns that were used during gel extraction.

Elution of mScarlet Megaprimer

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, May 11, 2023

Protocol:

1. Added 30 µL Elution Buffer to each spin column
2. Waited 1 minute before centrifuging all of the tubes at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

Measurement of mScarlet Megaprimer DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, May 11, 2023

Protocol: see protocol on page 1

Results: The DNA concentration of the newly eluted purified mScarlet megaprimer sample was measured to be 17.3 ng/μL.

Conclusion: We gained a small but relatively insignificant amount of the mScarlet megaprimer. As such, we will extract more of the mScarlet megaprimer from our agarose gel so that we have enough for MEGAWHOP cloning.

Gel Extraction of mScarlet Megaprimer

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, May 11, 2023

Protocol:

Note: these steps follow those outlined in the Monarch® DNA Gel Extraction Kit Protocol (NEB #T1020) by New England Biolabs.

1. Excised mScarlet megaprimer DNA from the agarose gel and placed them in a microfuge tube. The weights of each agarose gel piece are listed in the table below.

Sample #	**1**	**2**	**3**	**4**
Mass	176.3 mg	201.9 mg	206.1 mg	206.8 mg
Volume of ethanol	705.2 µL	807.6 µL	824.4 µL	827.2. µL

2. Ethanol was added to each agarose gel piece in the volumes listed in the table above. The volume of ethanol was calculated to be four times the mass of the agarose piece.
3. Incubated agarose at 50 °C and shook at 400 rpm until the gel completely dissolved
4. Inserted a spin column into a 4 collection tubes and loaded each sample into their respective column
5. Centrifuged at 13,000 rpm for 1 minute and discarded flow-through
6. Added 200 µL DNA Wash Buffer 1 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
7. Added another 200 µL DNA Wash Buffer 1 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
8. Transferred each spin column to a clean microfuge tube
9. Added 30 µL DNA Elution Buffer
10. Waited 1 minute before centrifuging all of the tubes at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

Measurement of mScarlet Megaprimer DNA Concentration from Second Extraction

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, May 11, 2023

Protocol: see protocol on page 1

Results: The DNA concentration of the purified mScarlet megaprimer sample was found to be 25.9 ng/μL.

Conclusion: We now have enough purified mScarlet megaprimer, so we can proceed to inserting it into G1.

G1 with mScarlet Megaprimer Generation

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

Date: Thursday, May 11, 2023

Protocol:

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

Component	Volume
25 ng G1 plasmid	2.83 µL
100 ng mScarlet megaprimer	3.86 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free	5.81 µL
Total	**25 µL**

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

Component	Volume
25 ng G1	2.83 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	9.67 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 35 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

Results: N/A

Conclusion:

We did not run a gel to confirm the insertion of mScarlet megaprimer into G1 because we were assisted by our advisor when performing the PCR reactions, and we needed all of the DNA for a second MEGAWHOP reaction. Additionally, once we transform E. coli with this DNA, the red fluorescence of mScarlet can serve as sufficient evidence to confirm whether or not the mScarlet megaprimer insertion was successful.

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. Since G1 was extracted from E. coli, it is no longer synthetic DNA, so DpnI can break it down. However, because G1 with mScarlet megaprimer was generated through a MEGAWHOP reaction, it is synthetic DNA and cannot be digested by DpnI. G1 with mScarlet megaprimer 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.