Measurement of G1 and G1 with mScarlet Megaprimer DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, May 15, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 µL Elution Buffer
3. Cleaned NanoDrop before loading 2 µL G1
4. Cleaned NanoDrop before loading 2 µL G1 with mScarlet megaprimer

Results: The G1 sample DNA concentration was 440.8 ng/µL. The G1 with mScarlet megaprimer sample DNA concentration was found to be 342.1 ng/µL and there was a phenol contamination.

Conclusion: The phenol contamination is likely due to an error from PCR. There was an excess of dNTPs from the Q5 High-Fidelity 2X Master Mix, but since this will not affect transformation, we decided to move on and not redo the PCR.

How did you get a G1 sample DNA concentration of 440.8? If you look at the May 9 entry, the average concentration was 48.4 ng/µL, so what did you do between these two dates that increased your DNA concentration so substantially?

DpnI Digestion

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, May 15, 2023

Protocol:

1. Prepared a 50 µL digestion reaction with the following components:

Component	Volume
1 µg G1 with mScarlet megaprimer	2.93 µL
10X rCutSmart buffer	5 µL
DpnI	1 µL
Nuclease-free water	41.07 µL
Total	**50 µL**

2. Prepared a 50 µL control reaction with the following components:

Component	Volume
1 µg G1	2.27 µL
10X rCutSmart buffer	5 µL
DpnI	1 µL
Nuclease-free water	41.73 µL
Total	**50 µL**

3. Incubated both reactions at 37 °C for 15 minutes
4. **Inactivated both reactions at 80 °C for 20 minutes**

How did you inactivate the reactions?

Results: N/A

Conclusion: N/A

G1 with mScarlet Megaprimer Purification

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, May 15, 2023

Protocol:

1. Diluted 50 µL of each sample in 100 µL DNA Cleanup Binding Buffer
2. Inserted a spin column into 2 collection tubes and transferred each sample into their respective column
3. Centrifuged both tubes at 13,000 rpm for 1 minute and discarded flow-through
4. Added 200 µL DNA Wash Buffer to each column and centrifuged both columns at 13,000 rpm for 1 minute
5. Added another 200 µL DNA Wash Buffer to each column and centrifuged both columns at 13,000 rpm for 1 minute
6. Transferred each column to a clean 1.5 mL microfuge tube
7. Added 30 µL Elution Buffer to each tube
8. Waited 1 minute before centrifuging all of the tubes at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

Transformation of E. coli with G1 with mScarlet Megaprimer and Control

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, May 15, 2023

Protocol:

1. Thawed NEB 10-beta Competent E. coli cells on ice for 10 minutes
2. Added 25 µL E. coli cells to two microfuge tubes
3. Added 2 µL purified G1 with mScarlet megaprimer DNA to one tube and 2 µL DNA from the control reaction to the other
4. Flicked tube to mix and briefly centrifuged the samples down
5. Placed tubes on ice for 30 minutes
6. Heat shocked E. coli in a 42 °C water bath for 30 seconds
7. Placed tubes on ice for 5 minutes
8. Added 475 µL outgrowth medium to each tube
9. Incubated E. coli at 37 °C and shaked at 400 rpm for 1 hour

Results: N/A

Conclusion: N/A

Plating G1 with mScarlet Megaprimer Transformed E. coli

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, May 15, 2023

Protocol:

1. Warmed 2 LB-Agar Kanamycin plates in a 37 °C incubator
2. Pipetted 50 µL E. coli cells from each microfuge tube onto their respective plate
3. Spread cells using 3 glass beads for each plate
4. Incubated at 37 °C overnight

Results: No colonies grew on either plate.

Conclusion:

Since no E. coli colonies managed to grow on the G1 with mScarlet megaprimer plate, transformation was unsuccessful as no E. coli were able to gain kanamycin resistance. We realized that the G1 with mScarlet megaprimer purification step was completely unnecessary and was actually likely the reason why transformation did not work. We already had a relatively low concentration of DNA and the purification process only lowered that concentration even more. As a result, there was likely not enough DNA for any of the E. coli to transform.

We now need to restart the process of generating G1 with mScarlet megaprimer. Looking back on our results, performing PCR with an annealing temperature of 61 °C yielded the best results, so we will just use that temperature for the annealing step instead of a gradient.