mScarlet Megaprimer Generation

Participants: Rori Hoover, Patrick Jiang

Date: Thursday, May 18, 2023

Protocol:

1. Prepared a template mix with the following components:

Component	Volume
100 µM forward primer	1 µL
100 µM reverse primer	1 µL
40 ng template DNA (R8)	0.25 µL
Nuclease-free water	8 µL

2. Prepared a 50 µL reaction in a PCR tube with the following components:

Component	Volume
Template mix	2.5 µL
Q5 High-Fidelity 2X Master Mix	25 µL
Nuclease-free water	22.5 µL
Total	**50 µL**

3. Amplified the DNA in the template mix 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	30 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

4. Prepared a 1% agarose running gel:
    a. Added 0.25 g of agarose to 25 mL 1X TBE in an Erlenmeyer flask
    b. Heated the flask in a microwave in 10 second increments until fully dissolved
    c. Cooled the flask until it was manageable to touch
    d. Added 2.5 µL SYBR Safe and swirled the flask to mix
    e. Poured the solution into a gel mold with a comb and left it to solidify for 20 minutes
    f. Removed the comb and covered the gel cast completely with 1X TBE
5. Loaded gel:
    a. Loaded 12 µL 1 kb DNA ladder into the first well
    b. Added 6 µL gel loading dye to the PCR product and incorrect G1 with mScarlet megaprimer from the last PCR

c. Loaded **12 µL** of each sample **into wells 2 & 3**

6. Placed the gel cast in a blueGel electrophoresis system and left the samples to run for 30 minutes

Results:

Conclusion: Our incorrect G1 with mScarlet megaprimer had two bands. The band at approximately 7 kb is the correct G2 DNA and the second band is leftover mScarlet Megaprimer. Inconclusive.

mScarlet is much clearer and the primer bands are present but not as overwhelmingly obvious as previously;

Megaprimer from most recent PCR in 2 & 3, megaprimer from 16th in last three – result of MEGAWHOP reaction not just megaprimer – ran to see what was wrong with the MEGAWHOP. Our PCR product was fine, but there wasn't enough for transformation

Gel Extraction of G1 with mScarlet Megaprimer

Participants: Rori Hoover, Patrick Jiang

Date: Thursday, May 18, 2023

Protocol:

1. Excised G1 with mScarlet megaprimer DNA from the agarose gel and placed it in a microfuge tube.
2. Added 779.6 µL Gel Dissolving Buffer to the agarose gel piece
3. Incubated the agarose piece at 50 °C while shaking at 400 rpm for 7 minutes
4. Inserted a spin column into a collection tube and loaded the sample into the column
5. Centrifuged the tube at 13,000 rpm for 1 minute and discarded the flow-through
6. Added 200 µL DNA Wash Buffer 1 to the column and centrifuged the column at 13,000 rpm for 1 minute
7. Added another 200 µL DNA Wash Buffer 1 to the column and centrifuged the column at 13,000 rpm for 1 minute
8. Transferred the spin column to a clean microfuge tube
9. Added 6 µL DNA Elution Buffer
10. Waited 1 minute before centrifuging the tube at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

Measurement of G1 with mScarlet Megaprimer DNA Concentration

Participants: Rori Hoover, Patrick Jiang

Date: Thursday, May 18, 2023

Protocol:

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

Results: The extracted G1 with mScarlet megaprimer DNA concentration was found to be 119.2 ng/µL.

Conclusion: N/A

PCR MEGAWHOP

Participants: Rori Hoover, Patrick Jiang

Date: Thursday, May 18, 2023

Protocol:

1. Prepared 50 µL MEGAWHOP reaction in a PCR tube with the following components:

Component	Volume
71.5 ng G1 plasmid	1.23 µL
715.2 ng mScarlet megaprimer	6 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	5.27 µL
Total	**25 µL**

There was a control??

2. Amplified the DNA 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: N/A

What is the point of this? Did you just not have enough G1 with mScarlet megaprimer?

DpnI Digestion

Participants: Rori Hoover, Patrick Jiang

Date: Thursday, May 18, 2023

Protocol:

1. Added 1 µL DpnI to the PCR product
2. Incubated the mixture for 1 hour at 37 °C

Results: N/A

Conclusion: N/A

Split control in half – one to be digested one to not

Transformation of E. coli with ???

Participants: Rori Hoover, Patrick Jiang

Date: Thursday, May 18, 2023

Protocol:

1. Thawed NEB Stable Competent E. coli cells on ice for 10 minutes
2. Added 25 µL E. coli cells to three microfuge tubes
3. Added 2 µL G1 (pDusk plasmid with pDusk Megaprimer) 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 shook at 400 rpm for 1 hour

Results: N/A

Conclusion: N/A

Plating G1 Transformed E. coli

Participants: Gabriella Cerna (mentor)

Date: Friday, April 21, 2023

Protocol:

1. Warmed 3 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: Colonies grew on the plate with E. coli that had G1 transformed into them, and there was no growth on the control plate.

Conclusion: Our results indicate that we were successful in generating G1.

Successfully transformed because undigested (positive control) and

Difference is G2 red – indicateing successful MEGAWHOP