G3 Megaprimer Generation

Did you run the PCR reaction before realizing that you were adding too much template? And if so, how much template did you add?

We should add 5-10 ng

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, August 1, 2023

Protocol:

1. Performed a 1:10 dilution of G2-D
2. Prepared a 25 µL reaction in a PCR tube with the following components:

Component	Volume
10 µM forward primer	1.25 µL
10 µM reverse primer	1.25 µL
10 ng template DNA (G2-D)	1 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	9 µL
Total	**25 µL**

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

Component	Volume
10 ng template DNA (G2-D)	1 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	11.5 µL
Total	**25 µL**

4. Prepared a 25 µL G3.1 reaction in a PCR tube with the following components:

Component	Volume
10 µM forward primer	1.25 µL
10 µM reverse primer	1.25 µL
10 ng template DNA (G2-D)	1 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	10.25 µL
Total	**25 µL**

5. Prepared a 25 µL G2.2 reaction in a PCR tube with the following components:

Component	Volume
10 µM forward primer	1.25 µL
10 µM reverse primer	1.25 µL

10 ng template DNA (G2-D)	1 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	10.25 µL
Total	**25 µL**

Are the G3.1 and G2.2 reactions also control reactions? Is one the forward primer and the other the reverse primer?

6. Amplified the DNA in each of the 25 µL reaction 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	60 °C	30 sec	30
Extension	72 °C	3 min 30 sec	30
Final extension	72 °C	2 min	1
???	**98 °C**	**30 sec**	**1**
???	**43 °C**	**15 sec**	**1**
???	**10 °C**	**Forever**	**-**

Did you do this for all of the reactions you prepared, and what are the last three temperatures for?

7. **Combined G3.1 and G2.2 reactions**
8. **Heated to 98 °C for 1 minute**
9. **Slowly cooled to 10°C at a rate of 0.1°C/s**

Did you do this in the thermocycler? Why did you do this?

Results: N/A

Conclusion: We need to make more G2-D.

Anaerobic jar plan

We will sterilize a glass jar and put our plates inside along with a burning candle to consume all of the oxygen. To seal the jar, we will first try to use Vaseline, but if that is not successful, we will 3D print a gasket. We will know that there is no oxygen in the chamber when the flame goes out

G3 Plasmid 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**

What volumes did you use for the reaction?

3. Incubated all reactions at 37 °C for 20 minutes

Did you have to inactivate the reaction?

Results: N/A

Conclusion: N/A

Tomorrow, we will transform E. coli