G3 Megaprimer Generation

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Protocol:

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

Component	Volume
10 µM forward primer	2.5 µL
10 µM reverse primer	2.5 µL
10 ng template DNA (G2-D)	3 µL
Q5 High-Fidelity 2X Master Mix	25 µL
Nuclease-free water	17 µL
Total	**50 µL**

2. Amplified the DNA in 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
Hold	10 °C	Forever	-

Results: N/A

Conclusion: We need to make more G2-D. This is when we removed the His-tags