G5.1 PCR Gradient (why a gradient?)

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, July 25, 2023

Protocol:

1. 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 (??)	1 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	4 µL
Total	**25 µL**

2. Amplified the DNA in the ?? 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	**50-57 °C (when did you decide to do the gradient?)**	30 sec	30
Extension	72 °C	30 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

Results:

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Conclusion: The best temperature is in the 53.1-53.8°C range because. For the next G5 PCR, we will use an annealing temperature of 53.5°C.

Preparation of Salt Solution

Participants: Brianna Branson, Isabella (Bella) Lirtzman

Date: Tuesday, July 25, 2023

Protocol:

1. **Combined 13.8 g Na_2HPO_4, 3 g KH_2PO_4, 1 g NH_4Cl, 0.5 g Na_2SO_4 in a 500 mL volumetric flask??**
2. **Filled the flask to the 500 mL mark with DI water**

Results: N/A

Conclusion: N/A

Preparation of Agar Solution

Participants: Brianna Branson, Isabella (Bella) Lirtzman

Date: Tuesday, July 25, 2023

Protocol:

1. **Added 2 g agar to a 50 mL volumetric flask??**
2. **Filled the flask to the 50 mL mark with DI water**
3. **Autoclaved solution for 15 minutes**

Results: N/A

Conclusion: N/A

Preparation of Hexanoic Avid Solution

Participants: Brianna Branson, Isabella (Bella) Lirtzman

Date: Tuesday, July 25, 2023

Protocol:

1. **Added 0.6265 mL hexanoic acid to a 1 L volumetric flask**
2. **Filled the flask to the 1 L mark with DI water**

Results: N/A

Conclusion: N/A

Transformation of E. coli with G3

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, July 25, 2023

Protocol:

1. Thawed **what kind?** E. coli cells on ice for 10 minutes
2. Added 25 µL E. coli cells to a microfuge tube
3. Added 2 µL G3 to the tube
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 250 rpm for 1 hour

Results: N/A

Conclusion: N/A

Was there a control? Idk if this protocol is correct. If it's BL21, it was done differently by Bella and Brianna

Plating G3 Transformed E. coli

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, July 25, 2023

Protocol:

1. Warmed an LB-agar kanamycin plate in a 37 °C incubator
2. Mixed cells by gently inverting the tube
3. Serially streaked **50-100 µL** G3 transformed E. coli onto the plate using a sterilized streaking tool
4. Incubated at 37 °C overnight

Results: ??

Conclusion: ??

We had accidentally were transforming G3 with electrocompetent cells but???