Colony PCR

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, July 24, 2023

Protocol:

Did you follow a company's protocol?

1. Prepared 2 29.5 µL colony PCR reactions with the following components:

Component	Volume
GoTaq **concentration?**	20 µL
Forward primer	0.25 µL
Reverse primer	0.25 µL
Nuclease-free water	9 µL
Total	**29.5 µL**

2. Amplified the DNA in each 29.5 µL reaction with a thermocycler using the following conditions:

Step	Temperature	Time	Number of cycles
Initial denaturation	98 °C	2 min	1
Denaturation	98 °C	30 sec	30
Annealing	57 °C	30 sec	30
Extension	72 °C	39 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

Results: N/A

Conclusion: N/A

Colony PCR Gel Purification (what exactly are you purifying?)

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, July 24, 2023

Protocol:

1. Prepared a 1% agarose running gel:
    a. Added 0.2 g of agarose to 20 mL 1X TBE in an Erlenmeyer flask
    b. Heated flask in a microwave in 10 second increments until fully dissolved
    c. Cooled flask until it was manageable to touch
    d. Added 3 µL SYBR Safe and swirled the flask to mix
    e. Poured solution into a gel mold with a comb and left to solidify for 20 minutes
    f. Removed comb and covered gel cast completely with 1X TBE
2. Loaded gel:
    a. Loaded **# µL of 100 bp DNA ladder** into the first well
    b. Added # µL gel loading dye to colony PCR product
    c. Loaded # µL colony PCR product into the second well
3. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes
4. **15 minutes after loading the 100 bp DNA ladder, a 1kb ladder was loaded into the same well**

Results:

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Conclusion:

Colony PCR success on small colony – insert was unclear? – what is your conclusion?

G3 Assembly

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, July 24, 2023

Protocol:

5 µL of PCR (add DpnI)

Taq

PCR: 5 µL

Master mix: 5 µL

10 µL total

This is a test if the annealing temperature was an issue

Results: ??

Conclusion: ??

Transformation of E. coli with G3

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, July 24, 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: Monday, July 24, 2023

Protocol:

1. Warmed an **LB-agar kanamycin** plate in a 37 °C incubator
2. Serially streaked G3 transformed E. coli onto the plate using a sterilized streaking tool
3. Incubated at 37 °C over the weekend
4. **Plate 50 µL??**

Results: Nothing grew on the G3 plate

Conclusion: To troubleshoot, we will spin down the unplated transformed cells, discard the supernatant, resuspend with 100 µL outgrowth media, and plate 100 µL

Why did you decide to do this to troubleshoot