Colony PCR

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 27, 2023

Protocol:

Find GoTaq protocol??

1. Prepared a 163.2 µL colony PCR reaction master mix with the following components:

Component	Volume
GoTaq **concentration?**	60 µL
Forward primer	1.6 µL
Reverse primer	1.6 µL
Nuclease-free water	80 µL
Total	**163.2µL**

2. Pipetted 20 µL master mix into 8 PCR tubes
3. Identified 8 colonies on the pRH plate circled each one with Sharpie
4. Swabbed each colony with a different sterile toothpick and swirled it in their respective PCR tube
5. Amplified the DNA in each 20 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: Tuesday, June 27, 2023

Protocol:

1. Prepared a 1% agarose running gel:
    a. Added 0.25 g of agarose to 25 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 **4 µL of 100 bp DNA ladder** into the first well
    b. Loaded **20** 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:

3 and 4 have faint bands

7& 8 have bright bands – means that the colony PCR was a success – the bands mean that our primers landed inside of the bacteria which means that the insert is in the bacteria somewhere – primers should only bind to the insert.

E. coli Inoculation

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 27, 2023

Protocol:

1. **Prepared 4 liquid cultures each with 3 µL (are you sure its micro?) LB-ampicillin media**
2. Swabbed colonies 3, 4, 7, and 8 with a different sterile toothpick and dropped it into their respective liquid culture
3. Incubated all 4 cultures at 37 °C and shook at 200 rpm overnight **(18-20 hours?)**

Results: N/A **If there's a control, there should be no bacteria in the culture tube. This is quality assurance of the culture. The culture was not contaminated? Was there growth in the liquid cultures & checked May 3**

Conclusion: N/A

Did you turn on a Bunsen burner while performing the inoculation?

Did you reflame the bottles and caps?

Supposed to reflame the culture tube and cap as well

Was there a negative control?