?? E. coli Inoculation

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, July 17, 2023

Protocol:

1. **Prepared 1 liquid culture tag?** with 3 mL LB-kanamycin
2. Identified a colony on the G3 plate
3. Swabbed a colony with a sterile toothpick and dropped it into the liquid culture
4. Incubated the liquid cultures at 37 °C and shook at 300 rpm overnight **(18-20 rpm)**

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?

Troubleshooting M9 Plaates

Participants: Rori Hoover, Patrick Jiang

Date: Monday, July 17, 2023

Protocol:

1. **Take stock of G4 serieally streaked**
2. **Plate on M9**
3. **Added 270 µL 10 mM glucose to both new and old plates??? 270 uL**

Took out new M9 plate – plated new cells from glycerol stock and added 10 mM glucose to both plates

There was a plate that we tried to grow cells on an M9 palte (Pranav) – added glucose and new cells

Two hexanoic acid plates from pranav – both with glucose and one with new cells and one without

Third plate has no hexanoic acid and has glucose

Results: N/A **There was only growth on the G4 troubleshoot plate**

Testing to see if hexanoic acid killed cells – yes

Was there still active hexanoic acid – yes, killed cells

Compared to the plate with no hexanoic acid, there was less growth on the plate with glucose cells hexanoic acid – metabolic acid

Tells us heavy metals is not killing cells (proved by new plate with no hexanoic acid), hexanoic acid is killing cells (with glucose with new cells), and selective resistance for hexanoic acid (can bacteria proliferate still

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Conclusion: The M9 plates are functional, the initial concentration of hexanoic acid is too high, or G4 transformed cells that were plated had died from being in the fridge for too long. We will grow the plates further to see if there are any changes. Currently, the colonies are too small to pick well.

G5 PCR

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Friday, April 21, 2023

Protocol:

1. Prepared a **25/50??** µL reaction in a PCR tube with the following components:

Component	Volume
10 µM forward primer	1.25/2.5 µL
10 µM reverse primer	1.25/2.5 µL
10 ng template DNA (**??**)	??/1 µL
Q5 High-Fidelity 2X Master Mix	2.5/12.5 µL
Nuclease-free water	??/4 µL
Total	**25/50 µL**

Did 25 uL

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	57 °C	30 sec	30
Extension	72 °C	30 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

Results: N/A

Conclusion: N/A