When did you prepare the solutions? Was it the 25th or the 26th?

Preparation of M? Salt Solution

Participants: Brianna Branson, Isabella (Bella) Lirtzman

Date: Wednesday, July 26, 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**
3. **Autoclaved solution for ____**

Results: N/A

Conclusion: N/A

Saponification of 20 mM Hexanoic Acid

Participants: Brianna Branson, Isabella (Bella) Lirtzman

Date: Wednesday, July 26, 2023

Protocol:

1. **Dissolved _____ KOH in 20 mM (is this a stock or did you make this?) hexanoic acid until the solution had a pH of 7.0**

Results: N/A

Conclusion: N/A

Preparation of Agar Solution

Participants: Brianna Branson, Isabella (Bella) Lirtzman

Date: Wednesday, July 26, 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**
4. **Added 150 mL M salt solution and trace mineral solution (when did you make this)**
5. **Gently mixed solution to avoid creating bubbles**
6. **Split solution evenly into two bottles so that each had 25 mL**
7. **Added saponified hexanoic acid into one of the bottles**
8. **Added 5 mM hexanoic acid to the other bottle**
9. **Left solutions to cool before adding hexanoic acid**

Results: N/A

Conclusion: N/A

Added to 25 mL of 20 mM hexanoic acid:

- **50 µL 0.1 M NaOH**
- **50 µL 6 M NaOH**
- **25 µL 6 M Na OH**
- **Added 1 mL m-salts to act as buffer**
- **Added 120 µL 0.1 M HCl to achieve final pH of 7.13**

Apparently, y'all plated stuff – idk

Plates from 7/26 demonstrated minimal growth (like micrometer-sized colonies). Plan is to repeat experiment, increasing the concentration of hexanoic acid

We will spread hexanoic acid on top of the plates instead of integrating it into the agar

E. coli Inoculation

Participants: Brianna Branson, Isabella (Bella) Lirtzman

Date: Wednesday, July 26, 2023

Protocol:

1. **Prepared 8 liquid cultures each with 3 mL kanamycin outgrowth media**
2. Identified 8 colonies on the G3 plate circled each one with Sharpie
3. Swabbed each colony with a different sterile toothpick and dropped it into their respective liquid culture
4. Incubated all 8 cultures at 30 °C and shook at **200 rpm** overnight

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?

G5 PCR

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, July 26, 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 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	53.5 °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: **We used the wrong forward and reverse primers. 1 # tube is empty because we tested the gradient with G5.1 therefore we already have the PCR product we needed. We ran a gel of our PCR products but used too little DNA so we will run the rest on a gel and the gel purify it.**

G5 PCR Gel Purification (what exactly are you purifying?)

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, July 26, 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 2 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 **5** µL gel loading dye to colony PCR product
    c. Loaded **28** 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:

Insert scanned photo (page 46)

Conclusion:

There was too little DNA, so we were only able to see faint bands in our gel. What did you do then?

Did you do two gels on this day?

You said there's no picture – what is the picture supposed to be from?

G5 PCR Gel Purification (what exactly are you purifying?)

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, July 26, 2023

Protocol:

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    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
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3. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes
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Results:

Insert scanned photo (page 46)

Conclusion:

There was too little DNA, so we were only able to see faint bands in our gel. What did you do then?

Did you do this gel extraction??

Gel Extraction of G5

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, May 10, 2023

Protocol:

Note: these steps follow those outlined in the Monarch® DNA Gel Extraction Kit Protocol (NEB #T1020) by New England Biolabs.

1. Excised mScarlet megaprimer DNA from the agarose gel and placed them in a microfuge tube. The weights of each agar gel piece are listed in the table below.

Test tube #	1	2	3	4
Mass	224.9 mg	177.4 mg	215 mg	214.7 mg
Volume of ethanol	899.6 µL	709.6 µL	860 µL	858.8 µL

2. Ethanol was added to each agar gel piece in the volumes listed in the table above. The volume of ethanol was calculated to be four times the mass of the agar piece.
3. Incubated agar at 50 °C and shook at 400 rpm until the gel completely dissolved
4. Inserted a spin column into a 4 collection tubes and loaded each sample into their respective column
5. Centrifuged at 13,000 rpm for 1 minute and discarded flow-through
6. Added 200 µL DNA Wash Buffer 1 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
7. Added another 200 µL DNA Wash Buffer 1 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
8. Transferred each spin column to a clean microfuge tube
9. Added 30 µL DNA Elution Buffer
10. Waited 1 minute before centrifuging all of the tubes at 13,000 rpm for 1 minute

Results: N/A

Conclusion: At this point, we realized that we had been using the wrong primers the entire will repeat the G5 PCR with the correct primers

G5 PCR (Rerun)

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, July 26, 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 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	57°C (I thought it was 53 from the gradient?)	30 sec	30
Extension	72 °C	30 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

Results: N/A

Conclusion: Tomorrow we will miniprep G3. digest, gel, know which one is good. Run G5.X on gel, gel purifying, nanodrop, plan GG