5/30: PCR and Gel Extraction

Project: iGEM 2025
Author: Phuong Le
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Intro:

Today, we are coming into the lab to do PCR of the upstream and downstream flanks of the ADP1 genome. We will also run gel electrophoresis to confirm the quality of our PCR product and, if there is enough time, do a gel extraction. We used the master sheet to find the annealing temperature.

Procedure:

PCR:

PCR

Master Mix

- [ ] 50 µL 5x HF buffer
- [ ] 5 µL 10 mM dNTPs
- [ ] 10 µL 100% DMSO
- [ ] 10 µL 50 mM $MgCl_2$
- [ ] 155 H_2O
- [ ] 2.5 µL Phusion polymerase

Each reaction

- [ ] 1 µL DNA template
- [ ] 1.25 µL 10 µM forward primer
- [ ] 1.25 µL 10 µM reverse primer

Note

- 1: Type 1 5' flank ACIAD0611 (Annealing temp: 59.4°C)
- 3: Type 5 3' flank ACIAD0611 (Annealing temp: 58.8°C)
- 2 & 4: Negative controls with water

Gel electrophoresis:

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Gel:

- [ ] 50 mL of 1x TAE
- [ ] 0.5 g agarose powder
- [ ] 2 µL SYBR Safe

Sample:

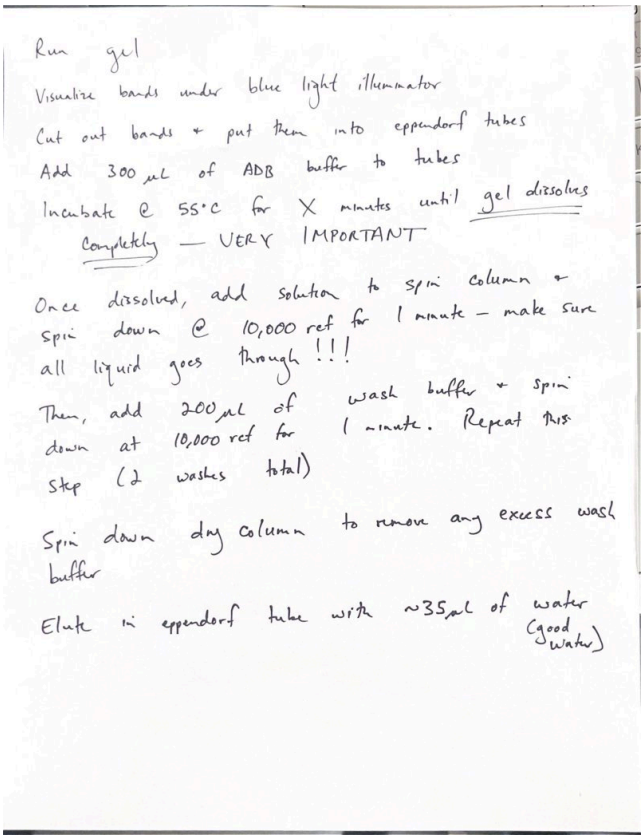
- [ ] 1 µL loading dye
- [ ] 5 µL sample

Loading

- [ ] 3 µL ladder
- [ ] 130 volts 15~20 minutesx

Gel Extraction:

IMG_6471.jpg



Results

Analysis of Results:

Conclusion/Future Steps:

We finished the PCR reaction and started on the gel electrophoresis. However, there was not enough time to run the gel, so we had to increase the voltage, which may be why the gel result was not very good. We do not have enough time for gel extraction, so we will communicate with the morning team to do it.

Agarose gel electrophoresis

Introduction

Agarose gel electrophoresis is a useful method of separating DNA molecules by size. Agarose, which is derived from dry seaweed, forms a gel with Tris-acetate-EDTA (TAE) buffer through which DNA molecules can move. Because the agarose gel has pores, the DNA is still able to move through it.

Materials

1x TAE

- This is the buffer that ensures that the electrical current moves through the gel correctly. Ensure that you use the same TAE to make and run your gel.

Agarose powder

- This is the material that causes the gel to solidify. Take care not to mix it up with Agar, which is used to make LB Agar.

SYBR Safe

- This is an interaclating dye, a type of dye that makes its way into molecules. In this case, SYBR Safe gets into DNA molecules and causes them to fluoresce under blue light. This is what allows us to visualize the DNA on the gel. Remember to add this.

Procedure

Making the Gel

1. Mix 50 mL of 1x TAE and 0.5 g agarose powder in a 250 mL flask.
2. Heat the mixture for 50 seconds in the microwave and then in successive 8-second intervals until all agarose is dissolved.

   Take care to swirl the mixture in order to visualize any undissolved agarose.
3. After heating add 2 µl SYBR Safe (5µl SYBR Safe for every 100mL gel) and swirl to mix.
4. Pour the liquid from the flask into the mold and wait about 30 minutes for it to solidify.

   Ensure that the comb is in the mold before leaving it to set.
   If running the gel with the intention to do a gel extraction, use thick wells. Otherwise, use thin wells.
5. Once the gel has solidified, remove the comb, loosen the gel tray, and place the gel into a rig. Pour 1x TAE buffer until the gel is thoroughly submerged.

   Make sure that the gel is fully solid in the center before removing the comb. Otherwise, the wells might close in on themselves.
   Make sure to use the same 1x TAE buffer that was used to create the gel.
   Make sure that the wells are on the black side (-) and that they **RUN TO RED (+).**

Loading the Gel

- 6. Mix your loading dye with your DNA sample.

  If using 5x loading dye, add 4 uL of DNA to 1 uL of loading dye.
  If using 6x loading dye, add 5 uL of DNA to 1 uL of loading dye.

- 7. Using an L10 micropipette, carefully add the samples with the loading dye into the wells.

  If continuing to gel extraction, skip a well.

- 8. Turn the rig on to 130 V and run for around 30 minutes before checking. Increase time as needed.

  Before leaving the rig to run, ensure that bubbles are forming in the buffer. If they are not forming, then the gel is not running correctly.

Protocol for PCR

Introduction

This is a general protocol for PCR that was adapted from 2018 Lab 4: PCR and agarose electrophoresis. Specific steps, concentrations, reagents, parameters, etc. may be necessary for certain PCRs.

Materials

- DNA template
- 5x HF buffer
- 10 mM dNTPs
- 10 µM forward primer
- 10 µM reverse primer
- 100% DMSO
- 50 mM $MgCl_2$
- diH_2O
- Phusion polymerase
- PCR tubes

Procedure

- [x] 1. To produce a master mix for four reactions follow the recipe below. This will yield enough volume for four reactions with an extra reaction, totaling five reactions all together. Add water and buffer first followed by the rest of the reagents

    0 µL DNA template
    50 µL 5x HF buffer
    5 µL 10 mM dNTPs
    0 µL 10 µM forward primer
    0 µL 10 µM reverse primer
    10 µL 100% DMSO
    10 µL 50 mM $MgCl_2$
    155 H_2O
    2.5 µL Phusion polymerase

- [x] 2. Gently mix the solution by tapping the tube with a finger. Spin down the mixture using a bench top microfuge

- [x] 3. Transfer 46.5 µL of master mix to each PCR tube

- [x] 4. For each PCR reaction add the following volumes of DNA and primers. Phusion polymerase should be added last

    1 DNA template
    1.25 µL 10 µM forward primer
    1.25 µL 10 µM reverse primer

- [x] 5. Gently mix the solution with the index and spin down the solutions.

- [x] 6. The PCR reaction parameters should be 94°C, XYZ, 72°C

Protocol for General Gel Electrophoresis

Introduction

This is a general protocol for gel electrophoresis that was adapted from 2018 Lab 4: PCR and agarose electrophoresis.

Materials

- 1X TAE
- 250 mL flask
- Agarose
- Sybr Safe
- Casting Tray
- Well comb
- Purple loading dye
- DNA ladder
- PCR products

Procedure

Steps

- 1. Add 50 mL of 1X TAE to 250 mL flask.
- 2. Weigh out 0.5 g of agarose to make 50 mL solution of 1% agarose. Add agarose to TAE in flask.
- 3. Heat solution in microwave until agarose dissolves. Start with 50 seconds and add 8 seconds as needed.
- 4. Add 2 µL of Sybr Safe to gel solution.
- 5. Set up gel tray.
- 6. Pour solution into casting tray. Then add well comb.
- 7. Let solidify (20-30 minutes).
- 8. Combine 1 µL of loading dye with 5 µL of each PCR reaction.
- 9. Place gel tray into agarose gel runner. ("Run to red"). Cover gel in 1X TAE buffer.

- [x] 10. Load 3 µL of ladder and 5 µL of samples into wells.
- [x] 11. Connect gel tank. Turn on power to 130 volts. Run for ~20 minutes. Check every 10 minutes until resolved.
- [x] 12. Confirm band size of products.