SSI Biology PCR Workshop

Written by **Cynthia Hao and Alan Tomusiak**

For the Stanford Student Space Initiative Biology Subteam

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1 Procedure Purpose

Basic polymerase chain reaction (PCR) and agarose gel electrophoresis workshop procedure.

2 Overview

Amplify the ampicillin resistance gene (about 1000bp) from a plasmid using Polymerase Chain Reaction, and image the results on a 1% agarose gel.

3 Safety Information

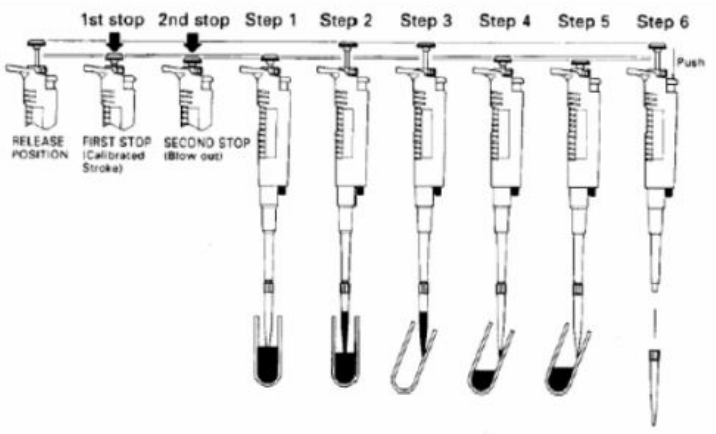
1. Working in a communal lab space is hazardous. Do not assume your fellow workers cleaned up sufficiently, and be sure to sign into and out of lab with a lifeguard or SSI safety officer.

4 Materials

1. precast 1% agarose gels
2. 6x Purple Loading Dye
3. .5x TBE Buffer
4. 1 uM forward primer
5. 1 uM reverse primer
6. .1 ng/µL pBR322 plasmid template DNA
7. .25 U/µL Taq polymerase
8. 5 mM dNTP Mix
9. 10x Taq PCR buffer
10. nuclease-free water
11. 1kb prestained DNA ladder working stock
12. Bio-Rad gel apparatus and power supply
13. Thermal Cycler
14. Gel Imager

5 Pipetting

We're about to get started, but first, a quick note about pipetting:



6 PCR Procedure

1. Obtain aliquots of Taq buffer, dNTP mix, forward primer, reverse primer, template DNA, and Taq polymerase for your group from the reagent ice bucket.
2. Label two PCR tubes with your initials.
3. For each person: Pipette 15 µL water, 5 µL 10x Taq buffer, 2 µL 5 mM dNTP mix, 10 µL each 1 uM reverse primer stock and forward primer stock, 3 µL (.3 ng) of plasmid template DNA, and 5 µL Taq polymerase stock into each PCR tube, in that order. The total reaction volume should be 50 µL.

Table 1: PCR reaction concentrations

Reagent	Stock Concentration	Desired Concentration	Volume (µL)
Water			15
Taq Buffer	10x	1x	5
dNTP Mix	5 mM	200 uM	2
Forward Primer	1 uM	.2 uM	10
Reverse Primer	1 uM	.2 uM	10
Template DNA	.1 ng/µL	.3 ng/50 µL reaction	3
Taq Polymerase	.25 U/µL	1.25 U/50 µL reaction	5
Total			50

4. Pipette up and down thoroughly in each tube to mix. Do not press down to the second pipette stop.
5. Place your PCR tubes in the thermal cycler, starting from the top left position and filling in column by column.
6. Get an SSI member to help you program in the following temperature protocol for a gradient PCR:

Table 2: Gradient PCR Temperature Protocol

Step	Temperature (C)	Time
Initial Denaturation	98	30 sec
Denaturation	98	30 sec
Annealing (depends on primer melting temperature)	46-60	30 sec
Extension (1 min per 1kb product)	72	1 min
GOTO Denaturation Step 29 times		
Final Extension	72	10 min
Hold	4	Infinity

7. This protocol will set up a temperature gradient from 46-60C, with a different temperature for each row of the thermal cycler for the annealing step.

7 Gel Electrophoresis Procedure

1. Obtain a pre-cast, SYBR Green pre-stained 1% agarose gel, 1kb ladder working stock for the group, and 1 PCR tube of DNA sample for gel electrophoresis per group.
2. Position the gel in the gel apparatus so that the wells are closer to the negative electrode, and fill the gel apparatus with .5x TBE buffer so that it completely covers the gel.
3. Obtain a piece of parafilm for your group. Draw a labeled grid on the parafilm with 9 boxes (3 for each person in the 3-person group). Keep track of where you are pipetting.
4. Each person in the group should pipette one 2 µL droplet of 6x Purple Loading Dye onto the parafilm into each of three boxes.
5. Pipette 5 µL of 0.5x TBE buffer into each of three loading dye droplets.
6. Pipette 5 µL of the appropriate sample into each of three loading dye droplets.
7. As you go, pipette up and down to mix thoroughly. Be sure not to press down to the second stop.
8. Load 5µL of the prestained ladder mix directly from the aliquot into the first well of the gel. Ensure pipette tip is in the well but does not puncture the bottom, and depress slowly and carefully to avoid bubbles.
9. Each person should load 5µL of their three PCR sample droplets into the next three wells of the gel.

Well number	Sample
1	1 kb Ladder (control)
2	Person 1 DNA Sample 1
3	Person 1 DNA Sample 2
4	Person 1 DNA Sample 3
5	Person 2 DNA Sample 1
6	Person 2 DNA Sample 2
7	Person 2 DNA Sample 3
8	Person 3 DNA Sample 1
9	Person 3 DNA Sample 2
10	Person 3 DNA Sample 3

Figure 1: Gel Layout

10. Cover the gel with the lid and plug the gel into the power supply.
11. Run the gel at 120V until the red portion (the first front) of the loading dye is at least 50% of the way to the other side of the gel.
12. Proceed to imaging. Have an SSI member help you position the gel in the gel imager, ensure correct gel orientation with the wells on the left side, and enter in the imaging protocol, optimizing for intense bands with SYBR Green pre-stain.
13. Note down how many bands you see, the length of the bands, and whether or not the PCR was successful.
14. Wipe down the gel imager with kimwipes and ethanol.
15. Clean up your lab station, replace reagents in freezer or room temperature storage, throw pipette tips in regular trash, put pipette boxes back in the drawers, rinse out gel boxes, and refill buffer stock with .5x TBE buffer.
16. Sign out of lab with the appropriate google form.