LSCI 3000 Synthetic Biology Workshop
Lab Log 3

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1. Experiment 1

1.1. Objective

- To prepare a welded agarose gel for gel electrophoresis.

1.2. Precautions

- Wear gloves.
- Handle the agarose gel with care to prevent breakage.

1.3. Materials

- 0.16g of agarose gel powder
- 20mL of TAE buffer (1×)
- 1μL of RedSafe DNA staining dye (20000×)

1.4. Equipment

1.4.1. Machineries

- Electronic balance
- Microwave

1.4.2. Apparatus

- Spatula
- Pipette (P10) and appropriate pipette tips

1.4.3. Containers

- Conical flask
- Gel casting tank (from the gel maker set)
- Gel tray (from the gel maker set)
- Gel comb (from the gel maker set)

1.5. Preparations

- Set up the gel maker set. Mount the gel tray into the tank.

1.6. Procedures

1. Weigh and add 0.16g of agarose gel powder into the conical flask.
2. Pour 20mL of TAE buffer into the flask.
3. Heat the solution in a microwave for 30 seconds or until all the powder is completely dissolved.
4. Cool the flask down to ~60℃ in the sink with running water.
5. Pipette 1μL of DNA staining dye into the flask.
6. Gently mix the sample by swirling.
7. Pour the mixture onto the gel tray. Remove air bubbles with a pipette tip if any are formed.
8. Insert the gel comb to create 25μL wells in the gel.
9. Wait 30 minutes or until the gel completely solidifies.
10. Remove the comb carefully.

2. Experiment 2 *(Prerequisite: Lab 2 Experiment 2)*

2.1. Objective

- To extract plasmids from the transformed E. coli (DH5-α) small cultures.

2.2. Precautions

- Wear gloves.
- Dispose of the pipette tips after each transfer.
- When placing tubes into the centrifuge, position the tubes such that their hinges face outwards to allow consistent pellet settling behaviour.

2.3. Materials

2.3.1. Small cultures

- 2 pRSFDuet-1 transformed DH5-α E. coli small cultures in LB broth in falcon tubes
- 2 pLadder6K transformed DH5-α E. coli small cultures in LB broth in falcon tubes

2.3.2. iNtRON easy-spin total RNA extraction kit

- 1000μL of resuspension buffer
- 1000μL of lysis buffer
- 1400μL of neutralisation buffer
- 2800μL of washing buffer B

2.3.3. Miscellaneous

- 200μL of ddH_2O
- Ice

2.4. Equipment

2.4.1. Machineries

- Centrifuge
- -20℃ refrigerator

2.4.2. Apparatus

- Pipettes (P200/P1000) and appropriate pipette tips

2.4.3. Containers

- 4 centrifuge tubes
- 4 spin columns (from the iNtRON kit)
- 4 collection tubes (from the iNtRON kit)
- Ice box

2.4.4. Miscellaneous

- Marker pen

2.5. Preparations

- Add RNase A solution to the resuspension buffer as per the instructions on the bottle, then chill the buffer with ice until use.
- Add ethanol to washing buffer B as per the instructions on the bottle.
- Centrifuge the E. coli small culture before use.
- Label all centrifuge tubes and columns appropriately.
- Mount the columns into the collection tubes as necessary before use.

2.6. Procedures

1. Pipette 250μL of resuspension buffer to each small culture falcon tube.
2. Gently mix the samples by vortexing until all bacteria clumps are resuspended.
3. Transfer all samples into separate centrifuge tubes with a pipette.
4. Pipette 250μL of lysis buffer to each tube.
5. Gently mix the samples by inverting the tubes 10 times.

6. Let the samples sit at room temperature for 3 minutes.
7. Pipette 350μL of neutralisation buffer to each tube.
8. Gently mix the samples by inverting the tubes 10 times.
9. Let the samples sit in ice for 5 minutes.
10. Centrifuge the samples at 13000rpm for 10 minutes.
11. Pipette the supernatant fluids into separate columns. Avoid transferring the pellets.
12. Centrifuge the samples at 13000rpm for 1 minute.
13. Discard the filtrate fluids in the collection tubes and remount the columns into the same collection tubes.
14. Pipette 700μL of washing buffer B to each column.
15. Centrifuge the samples at 13000rpm for 1 minute.
16. Discard the filtrate fluids in the collection tubes and remount the columns into the same collection tubes.
17. Centrifuge the samples at 13000rpm for 1 minute or until the filter membranes are dry.
18. Put the columns into separate centrifuge tubes. Remove and discard the used collection tubes.
19. Pipette 50μL of ddH_2O to each column.
20. Let the samples stand for 1 minute.
21. Centrifuge the samples at 13000rpm for 1 minute.
22. Discard the columns and store the plasmids at -20℃ for future use.

3. Experiment 3 *(Prerequisite: Lab 2 Experiment 1 + Lab 3 Experiment 1)*

3.1. Objective

- To isolate the desired DNA (GFP encoding gene) from the PCR products with gel electrophoresis.

3.2. Precautions

- Wear gloves.
- Dispose of the pipette tips after each transfer.
- Handle the agarose gel with care to prevent breakage.
- Do not run gel electrophoresis with >140V or the gel could melt.
- Ensure the electrodes of the gel electrophoresis machine are connected correctly before starting the machine.
- Slightly lift the pipette tip while loading samples into the gel wells to minimise spillage.
- Do not look directly into the UV light without the protective shield.

3.3. Materials

- 4 centrifuge tubes each with 25μL of PCR products (3 positive + 1 negative control)
- 25μL of dyed 1kb Plus DNA ladder
- 10μL of DNA loading dye (10×)
- 1 agarose gel with 25μL wells in a gel tray
- Ample TAE buffer (1×)

3.4. Equipment

3.4.1. Machineries

- 120V constant voltage supply
- UV transilluminator

3.4.2. Apparatus

- Pipettes (P10/P200) and appropriate pipette tips

3.4.3.Containers

- Gel electrophoresis tank
- Gel tray (from the gel maker set)

3.4.4.Miscellaneous

- Paper towels

3.5. Preparations

- Pre-chill the PCR products in ice.
- Put a plastic wrap on the UV transilluminator sample surface before use.

3.6. Procedures

1. Put the gel with its gel tray into the gel electrophoresis tank and orient it such that the wells are closer to the black negative electrode.
2. Slowly pour TAE buffer into the tank until the gel is completely submerged.
3. Carefully pipette 25µL of DNA ladder into the leftmost well of the gel.
4. Pipette 2.5µL of DNA loading dye into each PCR product mixture.
5. Gently mix the samples.
6. Carefully pipette 25µL of each mixture into separate wells of the gel.
7. Connect the electrodes of the tank to the constant voltage supply.
8. Electrophorese at 120V for 20 minutes or before the samples run off the gel. Check that bubbles are formed at the negative electrode.
9. Remove the gel from the tray and transfer it onto a paper towel to dry slightly.
10. Place the gel on a UV transilluminator to observe the results.

3.7. Results

The most prominent bands can be observed at ~750bp, which highly correlates to the desired 730bp GFP encoding gene.

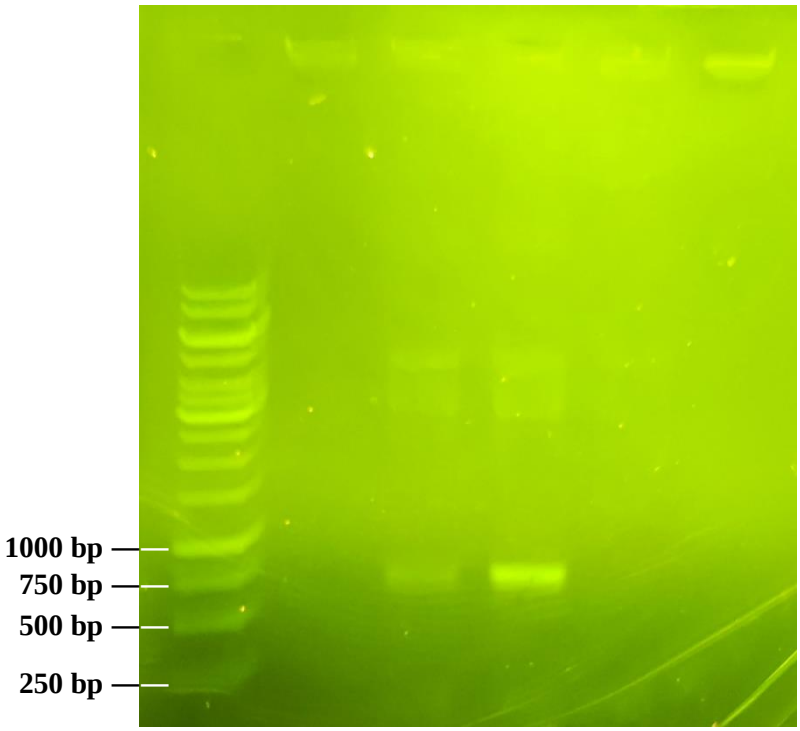

Figure 1 - Electrophoresed gel under UV light
From left to right: DNA ladder, null, samples 1-3, negative control

4. Experiment 4 *(Prerequisite: Lab 3 Experiment 3)*

4.1. Objective

- To extract and purify the desired DNA (GFP encoding gene) with gel extraction.

4.2. Precautions

- Wear gloves.
- Dispose of the pipette tips after each transfer.
- Do not look directly into the UV light without the protective shield.
- When placing tubes into the centrifuge, position the tubes such that their hinges face outwards to allow consistent pellet settling behaviour.

4.3. Materials

4.3.1. Electrophoresed gel

- 1 electrophoresed gel

4.3.2. iNtRON MEGAquick-spin plus total fragment DNA purification kit

- <600μL of BNL buffer
- 750μL of washing buffer

4.3.3. Miscellaneous

- 40μL of ddH_2O

4.4. Equipment

4.4.1. Machineries

- UV transilluminator
- 55℃ heat block
- Centrifuge
- -20℃ refrigerator

4.4.2. Apparatus

- Pipettes (P200/P1000) and appropriate pipette tips
- Scalpel

4.4.3. Containers

- 2 centrifuge tubes
- 1 spin column (from the iNtRON kit)
- 1 collection tube (from the iNtRON kit)

4.4.4. Miscellaneous

- Marker pen

4.5. Preparations

- Add ethanol to the washing buffer as per the instructions on the bottle.
- Put a plastic wrap on the UV transilluminator sample surface before use.
- Label the centrifuge tubes and columns appropriately.
- Mount the column into the collection tube as necessary before use.

4.6. Procedures

1. Place the gel on a UV transilluminator.
2. Locate a band of the desired length (730bp) with the most prominence and isolate the entire band with a sterile scalpel.
3. Weigh the gel slice. Trim until it is ≤300mg if necessary. *(We had 240mg in practice)*
4. Transfer the gel slice into a centrifuge tube.
5. Pipette $(\text{weight in mg} \times 2)$μL of BNL buffer to the tube. *(We pipetted 420μL in practice)*
6. Gently mix the sample by vortexing.

7. Place the tube in a heat block at 55℃ for 7 minutes or until the gel slice is completely dissolved. Vortex the tube every 2-3 minutes.
8. Allow the sample to cool to room temperature.
9. Transfer all of the sample mixture to a column with a pipette.
10. Centrifuge the sample at 11000×g for 30 seconds.
11. Discard the filtrate fluid in the collection tube and remount the column into the same collection tube.
12. Pipette 750μL of washing buffer to the column.
13. Centrifuge the sample at 11000×g for 30 seconds.
14. Discard the filtrate fluid in the collection tube and remount the column into the same collection tube.
15. Centrifuge the sample at 16000×g for 3 minute or until the filter membrane is dry.
16. Put the column into a sterile centrifuge tube. Remove and discard the used collection tube.
17. Pipette 40μL of ddH_2O directly onto the filter membrane of the column.
18. Let the sample stand for 1 minute.
19. Centrifuge the sample at 16000×g for 1 minute.
20. Discard the column and store the purified DNA at -20℃ for future use.