LSCI 3000 Synthetic Biology Workshop
Lab Log 5

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1. **Experiment 1** *(Prerequisite: Lab 4 Experiment 1)*
   1.1. **Objective**
   - To insert GFP encoding gene into the pSRFDuet-1 plasmid and transform it into E. coli cells.

   1.2. **Precautions**
   - Wear gloves.
   - Dispose of the pipette tips after each transfer.
   - Complete procedures with a flame nearby to ensure aseptic conditions.

   1.3. **Materials**

   1.3.1. Gene insertion
   - 24.7μL of purified pRSFDuet-1 with compatible sticky ends
   - 4.65μL of purified GFP encoding gene with compatible sticky ends
   - 4.65μL ddH_2O
   - 4μL of T4 DNA ligase buffer (10×)
   - 2μL of T4 DNA ligase

   1.3.2. Transformation
   - 200μL of competent DH5α E. coli cells
   - 1100μL of LB broth
   - 2 petri dishes of LB agar with diluted kanamycin (1000×)

   1.3.3. Miscellaneous
   - Ice

   1.4. **Equipment**

   1.4.1. Machineries
   - 42℃ heat block
   - 37℃ incubator
   - Centrifuge

   1.4.2. Apparatus
   - Pipettes (P10/P20/P200/P1000) and appropriate pipette tips
   - Spreader

   1.4.3. Containers
   - 4 centrifuge tubes
   - Ice box
   - Beaker

   1.4.4. Miscellaneous
   - Marker pen
   - Lighter
   - 70% ethanol

1.5. Preparations

- Measure the DNA samples concentration and calculate their appropriate amount necessary.
- Pour enough 70% ethanol into the beaker to submerge the spreading end of the spreader and cover the beaker with aluminium foil until use.
- Pour LB agar solution with antibiotics into the petri dishes. Allow them to set and keep them warm.
- Pre-chill the DNA samples in ice.
- Pre-chill the enzyme in ice.
- Transfer 100μL of E. coli sample each to two separate centrifuge tubes.
- Label all centrifuge tubes appropriately.
- Light a flame to generate an aseptic convection current.

1.6. Procedures

1. Pipette 12.35μL of pRSFDuet-1 into two separate centrifuge tube.
2. Pipette 4.65μL of purified GFP encoding gene to one sample.
3. Pipette 4.65μL of ddH_2O to the other sample as a negative control.
4. Pipette 2μL of ligase buffer to each sample.
5. Pipette 1μL of ligase to each sample.
6. Let the samples sit at room temperature for 10 minutes.
7. Resuspend the bacteria by pipetting up and down.
8. Pipette 10μL of the ligation product to each bacteria sample.
9. Mix the samples by pipetting up and down.
10. Chill the samples in ice for 5 minutes.
11. Heat shock the samples at 42℃ for 90 seconds.
12. Chill the samples in ice for 1 minute.
13. Pipette 450μL of LB culture broth to each sample.
14. Incubate the samples at 37℃ with ~250rpm shaking for 45 minutes.
15. Centrifuge the samples at 5000rpm for 3 minutes.
16. Remove and discard all the supernatant fluids. Avoid disturbing the pellets.
17. Pipette 100μL of LB broth to each sample.
18. Resuspend the bacteria by pipetting up and down.
19. Sterilise the spreader with ethanol and a flame and allow it to cool slightly.
20. Pipette 50μL of a sample onto a warm agar plate.
21. Spread the liquid evenly with a sterilised spreader until the surface looks dry.
22. Repeat steps 19-21 for the other sample.
23. Incubate the petri dishes upside-down at 37℃ for 12-16 hours.

1.7. Results

It appears that no colonies can be found growing in the agars. It is unlikely that procedural errors are the sole reason for failure, seeing that all other groups but one have also failed to produce any colonies. The bacteria seem to simply not have survived the transformation process. *(For future experiments that require successful colonies, we borrowed them from another group.)*

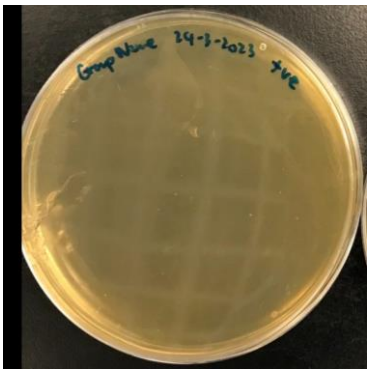

Figure 1 - E. coli transformed with pRSFDuet-1 with GFP insert

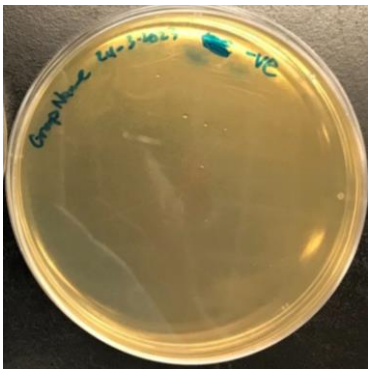

Figure 2 - E. coli transformed with pRSFDuet-1 without GFP insert

2. Experiment 2 *(Prerequisite: Lab 5 Experiment 1)*

2.1. Objective

- To screen the clones with colony PCR and gel electrophoresis.

2.2. Precautions

- Wear gloves.
- Dispose of the pipette tips after each transfer.
- Handle the agarose gel with care to prevent breakage.
- Complete procedures with a flame nearby to ensure aseptic conditions.
- Lightly flame the openings of the snap cap tube with each opening and closing.
- 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.

2.3. Materials

2.3.1. Gel casting

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

2.3.2. Colony picking + Colony PCR + Small culture

- 5mL of LB cell culture broth
- 5μL of kanamycin (1000x)
- 12.5μL of *Taq* polymerase (TaKaRa) premix
- 1μL of forward primer
- 1μL of reverse primer
- 10.5μL of ddH_2O
- 1 petri dish of DH5α E. coli colonies transformed with pRSFDuet-1 without insert

2.3.3.Gel electrophoresis

- 6μL of dyed 1kb Plus DNA ladder
- 2.5μL of DNA loading dye (10×)
- Ample TAE buffer (1×)

2.3.4.Miscellaneous

- Ice

2.4. Equipment

2.4.1.Machineries

- Electronic balance
- Microwave
- Bunsen burner
- 37℃ incubator
- PCR thermocycler
- 120V constant voltage supply
- UV transilluminator

2.4.2.Apparatus

- Spatula
- Pipettes (P10/P20/P200/P1000) and appropriate pipette tips

2.4.3.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 snap cap tube
- 1 centrifuge tube
- Gel electrophoresis tank
- Ice box

2.4.4.Miscellaneous

- Marker pen
- Lighter
- 70% ethanol
- Paper towels
- Plastic wrap

2.5. Preparations

- Set up the gel maker set. Mount the gel tray into the tank.
- Pre-chill the DNA samples in ice.
- Pre-chill the enzymes in ice.
- Label all centrifuge tubes and the snap cap tube appropriately.
- Light a flame to generate an aseptic convection current.
- Put a plastic wrap on the UV transilluminator sample surface before use.

2.6. Procedures

2.6.1.Gel casting

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.

2.6.2. *Colony picking + Colony PCR + Small culture*

1. Pour 5mL of LB broth into the snap cap tube.
2. Pipette 5μL of kanamycin to the broth.
3. Gently mix the broth.
4. Pipette 10.5μL of ddH_2O into a centrifuge tube.
5. Pipette 1μL of each primer to the PCR mixture.
6. Pipette 12.5μL of Taq polymerase premix to the PCR mixture.
7. Circle and label one non-satellite colony from the petri dish that will be extracted.
8. Clean the pipette with 70% ethanol and a paper towel.
9. Mount a suitable pipette tip to a P20 pipette and lightly flame the tip.
10. Using the tipped P20 pipette, gently scrape the labelled colony from the petri dish while the dish is still upside-down.
11. Lightly dip the pipette tip into the PCR mixture such that some bacteria are transferred to the mixture.
12. Eject the entire pipette tip into the snap cap tube.
13. Put the snap cap tube in an incubator at 37℃ with 220rpm overnight (12-16 hours).
14. Put the centrifuge tube in the PCR thermocycler and set the parameters as below.

2.6.3. *Gel electrophoresis*

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 6μL of DNA ladder into the leftmost well of the gel.
4. Pipette 2.5μL of DNA loading dye into the PCR product mixture.
5. Gently mix the sample.
6. Carefully pipette 20μL of the sample into a well 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.

2.7. PCR thermal cycle

Phase	Temperature	Duration	Cycles
Initial denaturation	98℃	5m	1
Denaturation	98℃	10s	29
Annealing	55℃	30s	
Extension	72℃	1m 30s	
Final extension	72℃	7m	1
Sample keeping	10℃	∞	1

2.8. Results

It appears that no clear DNA bands can be seen, but a faint florescence can be detected in the lanes of the wells in which the samples were loaded.

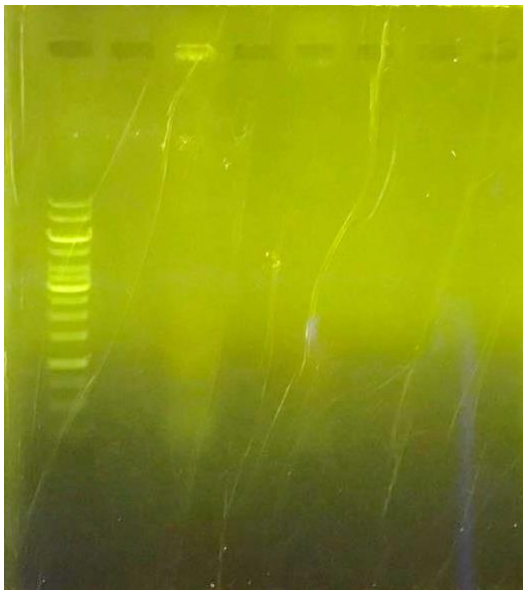

Figure 3 - Electrophoresed gel under UV light
From left to right: DNA ladder, null, sample 1, null, sample 2