LSCI 3000 Synthetic Biology Workshop
Lab Log 2

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1. Experiment 1

1.1. Objective

- To amplify GFP encoding gene via PCR

1.2. Precautions

- Wear gloves.
- Dispose of the pipette tips after each transfer.

1.3. Materials

1.3.1. Master mix solution

Note: The shown master mix material volumes are 125% of the necessary amount to allow for pipette errors.

- 62.5μL of *Taq* polymerase (TaKaRa) premix
- 2.5μL of 10μM forward GFP primer
- 2.5μL of 10μM reverse GFP primer
- 52.5μL of ddH_2O

1.3.2. DNA + negative control

- 3μL of GFP DNA template
- 1μL of ddH_2O

1.3.3. Miscellaneous

- Ice

1.4. Equipment

1.4.1. Machineries

- PCR thermocycler

1.4.2. Apparatus

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

1.4.3. Containers

- 10 200μL centrifuge tubes
- Ice box

1.4.4. Miscellaneous

- Marker pen

1.5. Preparations

- Transfer all materials into separate centrifuge tubes and pre-chill them with an ice box.
- Label each experimental centrifuge tube appropriately. (3 for PCR, 1 for negative control, 1 for the master mix)

1.6. Procedures

1. Pipette 62.5μL of Taq polymerase premix to the master mix centrifuge tube.
2. Pipette 2.5μL of each primer to the master mix. (The final concentrations should be 0.2μM.)
3. Pipette 52.5μL of ddH_2O to the master mix.
4. Pipette 24μL of the master mix to each centrifuge tube (3 positive PCR + 1 negative control).

5. Pipette 1μL of DNA template to each of the positive PCR centrifuge tubes.
6. Pipette 1μL of ddH_2O to the negative control centrifuge tube.
7. Mix all the tubes gently.
8. Put the tubes in the PCR thermocycler and set the parameters as follows.

1.7. PCR thermal cycle

Phase	Temperature	Duration	Cycles
Initial denaturation	98℃	5m	1
Denaturation	98℃	30s	34
Annealing	55℃	30s	
Extension	72℃	1m 30s	
Final extension	72℃	7m	1
Sample keeping	4℃	∞	1

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

2.1. Objective

- To create small cultures using the transformed colonies from before.

2.2. Precautions

- Wear gloves.
- Dispose of the pipette tips after each transfer.
- Complete procedures with a flame nearby to ensure aseptic conditions.
- Lightly flame the openings of the flacon tubes with each opening and closing.

2.3. Materials

- 20mL of LB cell culture broth
- 20μL of kanamycin (1000x)
- 1 petri dish of DH5α E. coli colonies transformed with pRSFDuet-1
- 1 petri dish of DH5α E. coli colonies transformed with pLadder6K

2.4. Equipment

2.4.1. Machineries

- 37℃ incubator
- 4℃ refrigerator
- Bunsen burner

2.4.2. Apparatus

- Pipette (P200) and appropriate pipette tips

2.4.3. Containers

- 4 falcon tubes

2.4.4. Miscellaneous

- Parafilm
- Marker pen
- Lighter
- 70% ethanol
- Paper towel

2.5. Preparations

- Label the falcon tubes appropriately.
- Light a flame to generate an aseptic convection current.

2.6. Procedures

1. Pipette 20μL of kanamycin to the LB cell culture broth.
2. Gently mix the LB broth.
3. Pour 4.5mL of the LB broth mixture into each falcon tube.
4. Circle and label two non-satellite colonies from each petri dish that will be extracted. Choose colonies that are far apart from each other.
5. Clean the pipette with 70% ethanol and paper towel.
6. Mount a suitable pipette tip to a P20 pipette and lightly flame the tip.
7. Using the tipped P20 pipette, gently scrape a labelled colony from a petri dish while the dish is still upside-down.
8. Eject the entire pipette tip into the respective flacon tube.
9. Repeat steps 5-8 until two colonies from each petri dish has been isolated and put into their separate flacon tubes.
10. Loosen the caps slightly.
11. Use tape to secure the caps such that they would not unscrew during incubation while allowing air flow.
12. Put the tubes in an incubator at 37℃ with 220rpm overnight (12-16 hours).
13. Wrap the petri dishes with parafilm.
14. Store the petri dishes at 4℃ for future use.