LSCI 3000 Synthetic Biology Workshop
Lab Log 1

Chan Cheuk Ka (1155174356)
24-02-2023

1. Experiment 1

1.1. Objective

- To prepare LB bacteria culture broth.

1.2. Materials

- 20g LB culture powder
- 1L ddH_2O

1.3. Equipment

1.3.1. Machineries

- Electronic balance
- Autoclave

1.3.2. Tools

- Spatula

1.3.3. Containers

- Glass jar

1.3.4. Miscellaneous

- Weighing paper
- Autoclave labels

1.4. Procedures

1. Weigh and add 20g of LB culture powder to a glass jar.
2. Add ddH_2O to the jar until there is 1L of culture broth.
3. Gently mix the culture solution.
4. Put an autoclave label onto the cap.
5. Loosen the jar cap slightly to allow pressure to equalise during the autoclave process.
6. Add the culture solution to an autoclave for 45 minutes at 120℃.

2. Experiment 2

2.1. Objective

- To transform E. coli (DH5α) with two different plasmids (pRSFDuet-1 and pLadder6K).

2.2. Precautions

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

2.3. Materials

- 300μL of competent DH5α E. coli cells
- 1 μL of pRSFDuet-1 plasmid
- 1 μL of pLadder6K plasmid
- 1200μL of LB culture broth
- LB agar solution with diluted kanamycin and ampicillin
- Ice

2.4. Equipment

2.4.1. Machineries

- 37℃ incubator
- 42℃ heat block
- 4℃ cooling chamber
- Bunsen burner

2.4.2. Tools

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

2.4.3. Containers

- 5 centrifuge tubes
- 3 petri dishes
- Ice bucket
- Beaker

2.4.4. Miscellaneous

- Marker pen
- Lighter
- 70% ethanol
- Aluminium foil
- Laboratory wrapping film

2.5. Preparations

- 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.
- Transfer 100μL of E. coli sample to each of the three separate centrifuge tubes.
- Transfer the two plasmids to two separate centrifuge tubes.
- Label the centrifuge tubes and petri dishes appropriately.
- Light a flame to generate an aseptic convection current.

2.6. Procedures

1. Pre-chill the samples of E. coli and plasmids.
2. Pipette 1μL of pRSFDuet-1 plasmid to E. coli sample 1.
3. Pipette 1μL of pLadder6K plasmid to E. coli sample 2.
4. Gently flick the tubes to mix.
5. Chill the samples with ice for 5 minutes.
6. Heat shock the samples at 42℃ for 90 seconds.
7. Chill the mixtures with ice for 1 minute.
8. Pipette 400μL of LB culture broth into each of the samples.
9. Incubate the samples at 37℃ with ~250rpm shaking for 45 minutes.
10. Sterilise the spreader with ethanol and a flame and allow it to cool slightly.
11. Pipette 50μL of a sample onto a warm agar plate.
12. Spread the liquid evenly with a sterilised spreader until the surface looks dry.
13. Repeat steps 10-12 for the other two samples.
14. Incubate the petri dishes upside-down at 37℃ for 12-16 hours.
15. Wrap the petri dishes with laboratory wrapping film and keep them chilled at 4℃ for future use.

2.7. Results

Colonies can be observed in both sample 1 and 2 while no colonies can be observed in sample 3 (negative control).

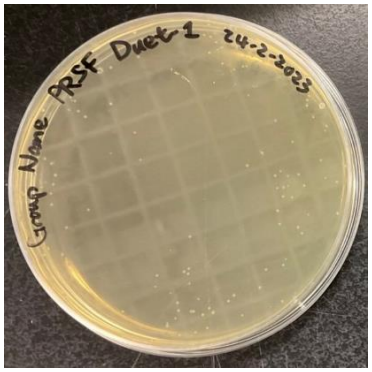

Figure 1 E. coli transformed with pRSFDuet-1 plasmid

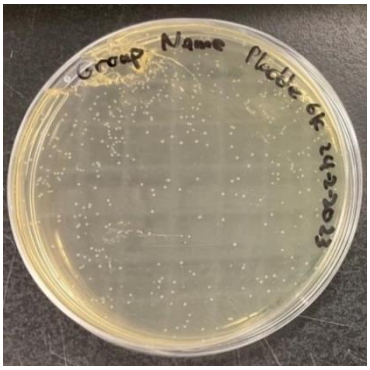

Figure 2 E. coli transformed with pLadder6K plasmid

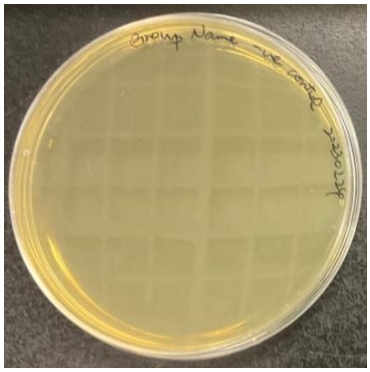

Figure 3 Untransformed E. coli (negative control)