LSCI 3000 Synthetic Biology Workshop
Lab Log 4

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1. **Experiment 1** *(Prerequisite: Lab 3 Experiment 2 + Lab 3 Experiment 4)*

1.1. Objective

- To generate compatible sticky ends in the DNA samples (pRSFDuet-1 + GFP encoding gene) by double restriction digestion and purify them.

1.2. Precautions

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

1.3. Materials

1.3.1. Restriction digestion

- 32μL of extracted pRSFDuet-1
- 32μL of purified GFP encoding gene
- 8μL of Anza red buffer (10×)
- 4μL of EcoRI enzyme
- 4μL of PstI enzyme

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

- 400μL of BNL buffer
- 1500μL of washing buffer

1.3.3. Miscellaneous

- 60μL of ddH_2O
- Ice

1.4. Equipment

1.4.1. Machineries

- Centrifuge
- 37℃ incubator
- Vortex mixer
- -20℃ refrigerator

1.4.2. Apparatus

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

1.4.3. Containers

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

1.4.4. Miscellaneous

- Marker pen

1.5. Preparations

- Add ethanol to the washing buffer as per the instructions on the bottle.
- Pre-chill the DNA samples in ice.
- Pre-chill the enzymes in ice.
- Pre-chill the Anza red buffer in ice.

- Label all centrifuge tubes and columns appropriately.
- Mount the columns into the collection tubes as necessary before use.

1.6. Procedures

1. Pipette 16μL of pRSFDuet-1 into two separate centrifuge tubes.
2. Pipette 16μL of GFP DNA into two separate centrifuge tubes.
3. Pipette 2μL of Anza red buffer to each sample.
4. Pipette 1μL of EcoRI enzyme to each sample.
5. Pipette 1μL of PstI enzyme to each sample.
6. Centrifuge the samples at 1000rpm for 15 seconds to mix.
7. Incubate the samples at 37℃ for 1 hour.
8. Transfer one pRSFDuet-1 sample to the other with a pipette to combine both samples.
9. Transfer one GFP DNA sample to the other with a pipette to combine both samples.
10. Vortex the samples for 5 seconds or until mixed.
11. Pipette 200μL of BNL buffer to each sample.
12. Gently mix the samples by inverting the tubes 10 times.
13. Transfer the sample mixtures to separate columns with a pipette.
14. Centrifuge the samples at 11000×g for 30 seconds.
15. Discard the filtrate fluids in the collection tubes and remount the columns into the same collection tubes.
16. Pipette 750μL of washing buffer to each column.
17. Centrifuge the samples at 11000×g for 30 seconds.
18. Discard the filtrate fluids in the collection tubes and remount the columns into the same collection tubes.
19. Centrifuge the samples at 16000×g for 3 minute or until the filter membranes are dry.
20. Put the columns into separate sterile centrifuge tubes. Remove and discard the used collection tubes.
21. Pipette 30μL of ddH_2O directly onto the filter membrane of each column.
22. Let the samples stand for 2 minutes.
23. Centrifuge the samples at 16000×g for 1 minute.
24. Discard the columns and store the purified DNA at -20℃ for future use.