LSCI 3000 Synthetic Biology Workshop
Case Study 2

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Preface

This section compiles the primary objective, initial timetable, and procedures to preface the case study report. However, the main purpose of this preface is to demonstrate how astray we have ended up from our initial plans in practice. More to be discussed in the reflection and discussion.

Primary Objective

To extract the GFP encoding gene from pRSFDuet-1-GFP and transfer it into pET-32a.

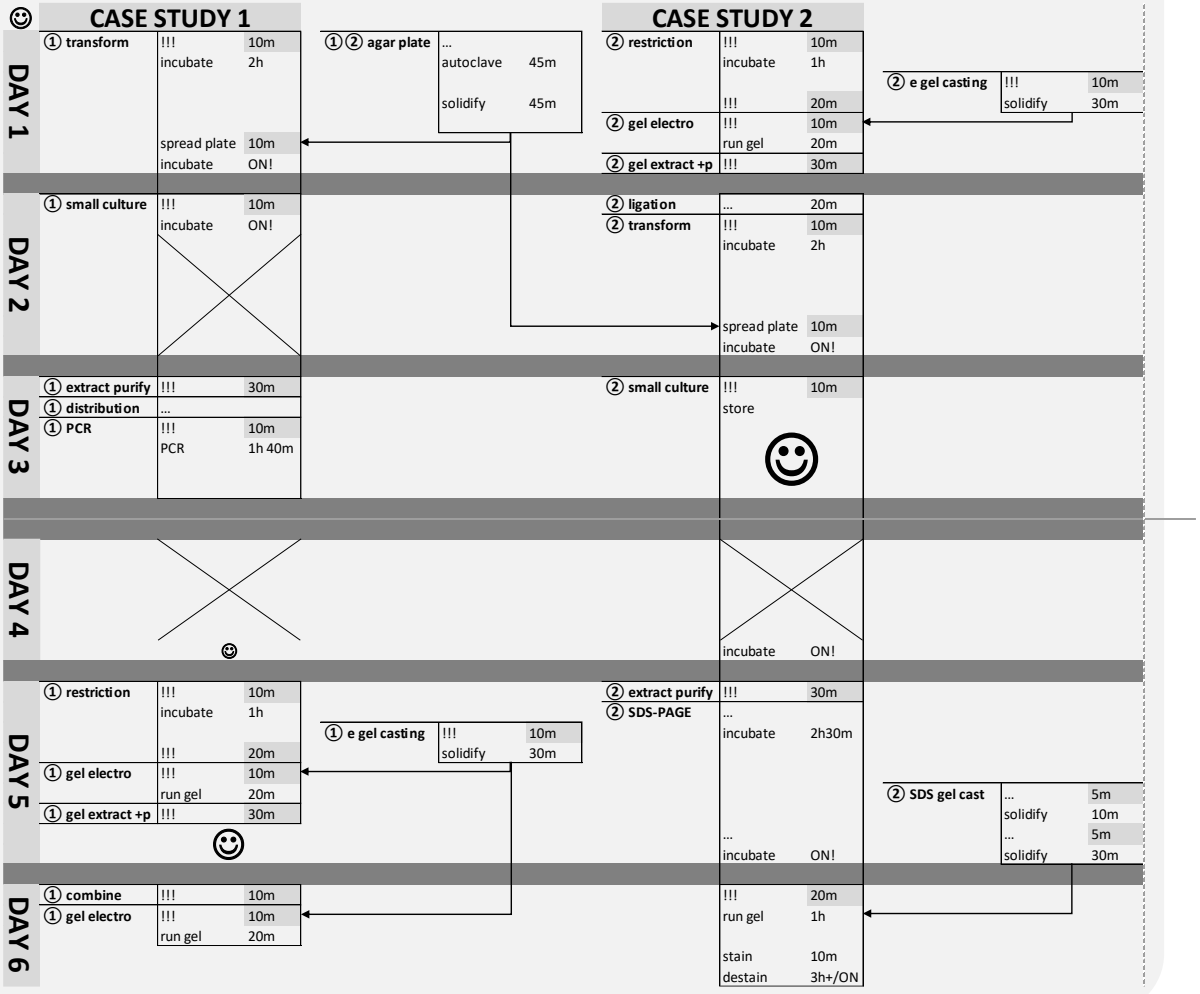
Initial Procedures Flow

Note that secondary steps (e.g., making agar plates and agarose gels) are omitted here.

1. Restrict pRSFDuet-1-GFP and pET-32a to form compatible sticky ends.
2. Extract and purify the GFP encoding gene with gel extraction.
3. Ligate the restricted GFP encoding gene and pET-32a.
4. Transform the ligated pET-32a-GFP into competent DH5-α E. coli.
5. Extract colonies of transformed E. coli to create a small culture.
6. Extract and purify the pET-32a-GFP from the E. coli.
7. Induce protein expression on the transformed E. coli.
8. Run SDS-PAGE for verification.

Initial Timetable

Procedures in the same row denotes concurrency. The timetable for case study 1 is also included.



Interjection

However, the parameters and objective of the case study was changed at the very last second on day 1. The TAs were unable to provide pRSFDuet-1-GFP; hence, we have to reformulate a procedure list. For the updated parameters, we were given the standalone GFP encoding gene, and instructed to transform it into pET-32a, finally to compare the protein expression between E. coli transformed by pET-32a-GFP and that by the provided pET-28a-GFP. This was a rather large shift in parameters and involved some major workflow changes, including an overhang PCRs.

But this was not the only issue for this change. Unbeknownst to us at the time, the GFP we were provided with was not a standalone DNA strand as we were led to believe, but instead recombined with a plasmid still unknown to us till this day. Even the TAs did not seem to know the precise details of the provided materials. This fact has led to some major confusion throughout the case study. This report would be written with regards to the knowledge we had at the time.

Modified Procedures Flow

Note that secondary steps (e.g., making agar plates and agarose gels) are omitted here.

1. Perform overhang PCR on the GFP encoding gene to generate restriction sites.
2. Restrict the PCR'd GFP and pET-32a to form compatible sticky ends.
3. Extract and purify the GFP encoding gene with gel extraction.
4. Ligate the restricted GFP encoding gene and pET-32a.
5. Transform the ligated pET-32a-GFP into competent DH5-α E. coli.
6. Transform pET-28a-GFP into competent DH5-α E. coli.
7. Extract colonies of transformed E. coli to create a small culture.
8. Extract and purify the pET-32a-GFP from the E. coli.
9. Induce protein expression on the transformed E. coli.
10. Run SDS-PAGE for verification and comparison.

Day 1 (05-04-2023)

Note

We were notified about the changes mentioned in the preface. No work was done on this case study on this day.

Day 2 (06-04-2023)

1. Experiment 1

Note

We were under the impression that the given GFP encoding gene was standalone DNA strand, and hence, calculated the annealing temperature using and online calculator as though it was one.

1.1. Objective

- To generate restriction sites around the GFP encoding gene with overhang PCR.

1.2. Precautions

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

1.3. Materials

- 62.5µL of Taq polymerase (TaKaRa) premix
- 2.5µL of *Eco*RI-GFP forward primer
- 2.5µL of *Xho*I-pET-32a-GFP reverse primer
- 4µL of GFP encoding gene
- 53.5µL of ddH_2O
- 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

- 5 PCR 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. (4 positive + 1 negative control)

1.6. Procedures

1. Pipette 10.5µL of ddH_2O to each tube.
2. Pipette 0.5µL of *Eco*RI-GFP forward primer to each tube.
3. Pipette 0.5µL of *Xho*I-pET-32a-GFP reverse primer to each tube.
4. Pipette 1µL of DNA to each positive tube.
5. Pipette 1µL of ddH_2O to the negative control tube.
6. Pipette 12.5µL of Taq polymerase premix to each tube. Wait until the PCR machine is set up before doing this step to minimise unwanted prior reaction.
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	10
Annealing	52℃	30s	
Extension	72℃	1m 30s	
Denaturation	98℃	30s	24
Annealing	59℃	30s	
Extension	72℃	1m 30s	
Final extension	72℃	7m	1
Sample keeping	4℃	∞	1

1.8. Products

- 4 PCR tubes each with 25µL of extended GFP encoding gene
- 1 PCR tube with 25µL of negative control

Day 3 (07-04-2023)

2. Experiment 2 *(Prerequisite: Case Study 2 Experiment 1)*

2.1. Objective

- To restrict the extended GFP encoding gene and pET-32a to generate compatible sticky ends.

2.2. Precautions

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

2.3. Materials

2.3.1. Restriction digestion

- 24µL of PCR'd GFP encoding gene
- 12µL of pET-32a
- 12µL of Anza red buffer (10×)
- 6µL of *Eco*RI enzyme
- 6µL of *Xho*I enzyme
- 60µL of ddH_2O

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

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

2.3.3. Miscellaneous

- 80µL of ddH_2O
- Ice

2.4. Equipment

2.4.1. Machineries

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

2.4.2. Apparatus

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

2.4.3. Containers

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

2.4.4. Miscellaneous

- Marker pen

2.5. 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.

2.6. Procedures

1. Pipett 10μL of ddH_2O to each centrifuge tube.
2. Pipette 6μL of extended GFP to each of four centrifuge tubes.
3. Pipette 6μL of pET-32a to each of two centrifuge tubes.
4. Pipette 2μL of Anza red buffer to each sample.
5. Pipette 1μL of *Eco*RI enzyme to each sample.
6. Pipette 1μL of *Xho*I enzyme to each sample.
7. Centrifuge the samples at 1000rpm for 15 seconds to mix.
8. Incubate the samples at 37℃ for 1 hour.
9. Combine and transfer all samples of the same DNA type into a sterile centrifuge tube for each DNA type.
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 40μL 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.

2.7. Products

- 1 centrifuge tube with 40μL of double-restricted GFP
- 1 centrifuge tube with 40μL of double-restricted pET-32a

Interlude

Here omitted, we have performed gel electrophoresis on the restriction products of case study 2 experiment 1.

Results

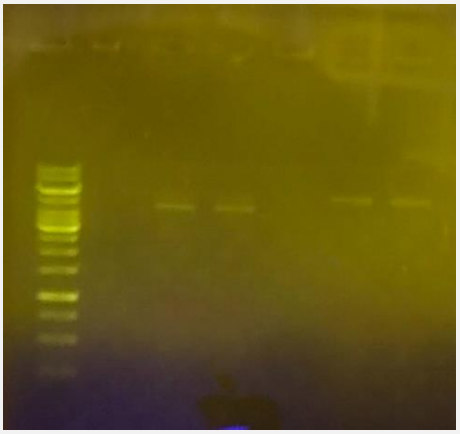


Figure 1 – Electrophoresed gel with restriction products under UV light
From left to right: DNA ladder, null, double-restricted GFP samples, null, double-restricted pET-32a samples

Band of ~6000bp can be observed for both sample types. For restricted pET-32a, a band size of ~6000bp is expected. However, for the GFP sample, its expected band size should be 723bp. Considering the initial GFP strand is only 714bp long, we are very baffled by this apparent growth in bp size. Although very sceptical, we concluded that there must have been some contamination or other strange occurrences with the PCR process. We then proceeded to redo the PCR for a second time this day.

3. Experiment 3 *(Prerequisite: Case Study 2 Experiment 2)*

3.1. Objective

- To extract and purify restricted pET-32a with gel extraction.

3.2. Precautions

- Wear gloves.
- Dispose of the pipette tips after each transfer.
- Do not look directly into the UV light without the protective shield.

3.3. Materials

3.3.1. Electrophoresed gel

- 1 electrophoresed gel

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

- 900μL of BNL buffer
- 750μL of washing buffer

3.3.3. Miscellaneous

- 50μL of ddH_2O

3.4. Equipment

3.4.1. Machineries

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

3.4.2.Apparatus

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

3.4.3.Containers

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

3.4.4.Miscellaneous

- Marker pen

3.5. Preparations

- Add ethanol to the washing buffer as per the instructions on the bottle.
- Put 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.

3.6. Procedures

1. Place the gel on a UV transilluminator.
2. Isolate all the desired bands with a sterile scalpel.
3. Transfer each gel slice into a separate centrifuge tube.
4. Pipette 450μL of BNL buffer to each tube.
5. Gently mix the sample by vortexing.
6. Place the tubes in a heat block at 55℃ for 7 minutes or until the gel slices are completely dissolved.
7. Allow the sample to cool to room temperature.
8. Transfer one sample mixture to separate columns with a pipette.
9. Centrifuge the samples at 11000×g for 30 seconds.
10. Discard the filtrate fluids in the collection tubes and remount the columns into the same collection tubes.
11. Transfer the remaining sample mixture to the column with a pipette.
12. Repeat steps 9-10.
13. Pipette 750μL of washing buffer to the column.
14. Centrifuge the sample at 11000×g for 30 seconds.
15. Discard the filtrate fluid in the collection tube and remount the column into the same collection tube.
16. Centrifuge the sample at 16000×g for 3 minute or until the filter membrane is dry.
17. Put the column into a sterile centrifuge tube. Remove and discard the used collection tube.
18. Pipette 50μL of ddH_2O directly onto the filter membrane of the column.
19. Let the sample stand for 2 minute.
20. Centrifuge the sample at 16000×g for 1 minute.
21. Discard the column and store the purified DNA at -20℃ for future use.

3.7. Products

- 1 centrifuge tube with 50μL of restricted pET-32a

4. Experiment 4

Note

Considering the PCR failure in case study 2 experiment 1, we were not able to conclusively determine the cause of failure and the apparent illogical increase in DNA size. We opted to decrease the annealing temperature to 57℃ for the post-overhang phases.

4.1. Objective

- To generate restriction sites around the GFP encoding gene with overhang PCR.

4.2. Precautions

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

4.3. Materials

- 62.5µL of Taq polymerase (TaKaRa) premix
- 2.5µL of *Eco*RI-GFP forward primer
- 2.5µL of *Xho*I-pET-32a-GFP reverse primer
- 4µL of GFP encoding gene
- 53.5µL of ddH_2O
- Ice

4.4. Equipment

4.4.1. Machineries

- PCR thermocycler

4.4.2. Apparatus

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

4.4.3. Containers

- 5 PCR tubes
- Ice box

4.4.4. Miscellaneous

- Marker pen

4.5. Preparations

- Transfer all materials into separate centrifuge tubes and pre-chill them with an ice box.
- Label each experimental centrifuge tube appropriately. (4 positive + 1 negative control)

4.6. Procedures

1. Pipette 10.5µL of ddH_2O to each tube.
2. Pipette 0.5µL of *Eco*RI-GFP forward primer to each tube.
3. Pipette 0.5µL of *Xho*I-pET-32a-GFP reverse primer to each tube.
4. Pipette 1µL of DNA to each positive tube.
5. Pipette 1µL of ddH_2O to the negative control tube.
6. Pipette 12.5µL of Taq polymerase premix to each tube. Wait until the PCR machine is set up before doing this step to minimise unwanted prior reaction.
7. Mix all the tubes gently.
8. Put the tubes in the PCR thermocycler and set the parameters as follows.

4.7. PCR thermal cycle

Phase	Temperature	Duration	Cycles
Initial denaturation	98℃	5m	1

Denaturation	98℃	30s	10
Annealing	52℃	30s	
Extension	72℃	1m 30s	
Denaturation	98℃	30s	24
Annealing	57℃	30s	
Extension	72℃	1m 30s	
Final extension	72℃	7m	1
Sample keeping	4℃	∞	1

Day 4 (12-04-2023)

Interlude

Here omitted, we have performed gel electrophoresis on the restriction products of case study 2 experiment 4.

A similar result as case study 2 experiment 1 can be observed. We were still unable to determine to cause of the illogical DNA size growth. We then proceeded to redo the PCR for a third time this day.

5. Experiment 5

Note

Considering the PCR failure in case study 2 experiment 4, we were not able to conclusively determine the cause of failure and the apparent illogical increase in DNA size. We opted to use the same annealing temperature for overhang and post-overhang phases to eliminate the two-phase thermocycle and decrease the annealing temperature to 55℃.

5.1. Objective

- To generate restriction sites around the GFP encoding gene with overhang PCR.

5.2. Precautions

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

5.3. Materials

- 62.5μL of Taq polymerase (TaKaRa) premix
- 2.5μL of *Eco*RI-GFP forward primer
- 2.5μL of *Xho*I-pET-32a-GFP reverse primer
- 4μL of GFP encoding gene
- 53.5μL of ddH_2O
- Ice

5.4. Equipment

5.4.1. Machineries

- PCR thermocycler

5.4.2. Apparatus

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

5.4.3. Containers

- 5 PCR tubes
- Ice box

5.4.4. Miscellaneous

- Marker pen

5.5. Preparations

- Transfer all materials into separate centrifuge tubes and pre-chill them with an ice box.
- Label each experimental centrifuge tube appropriately. (4 positive + 1 negative control)

5.6. Procedures

1. Pipette 10.5μL of ddH_2O to each tube.
2. Pipette 0.5μL of *Eco*RI-GFP forward primer to each tube.
3. Pipette 0.5μL of *Xho*I-pET-32a-GFP reverse primer to each tube.

4. Pipette 1µL of DNA to each positive tube.
5. Pipette 1µL of ddH_2O to the negative control tube.
6. Pipette 12.5µL of Taq polymerase premix to each tube. Wait until the PCR machine is set up before doing this step to minimise unwanted prior reaction.
7. Mix all the tubes gently.
8. Put the tubes in the PCR thermocycler and set the parameters as follows.

5.7. 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

Day 5 (13-04-2023)

Interlude

Here omitted, we have performed gel electrophoresis on the restriction products of case study 2 experiment 5.

A similar result as case study 2 experiment 1 can be observed. We were still unable to determine to cause of the illogical DNA size growth. We then proceeded to redo the PCR for a fourth time on a later date.

Note

Due to logistic issues with the PCR machines, we could not perform PCR and no work was done on this case study on this day.

Day 6 (14-04-2023)

6. Experiment 6

Note

Considering the PCR failure in case study 2 experiment 5, we were not able to conclusively determine the cause of failure and the apparent illogical increase in DNA size. We opted to perform gradient PCR at several different temperatures, increase the concentration of the primers and DNA template used, and increase the annealing time to 45s.

6.1. Objective

- To generate restriction sites around the GFP encoding gene with overhang PCR.

6.2. Precautions

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

6.3. Materials

- 200µL of Taq polymerase (TaKaRa) premix
- 16µL of *Eco*RI-GFP forward primer
- 16µL of *Xho*I-pET-32a-GFP reverse primer
- 24µL of GFP encoding gene
- 144µL of ddH_2O
- Ice

6.4. Equipment

6.4.1.Machineries

- PCR thermocycler

6.4.2.Apparatus

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

6.4.3.Containers

- 16 PCR tubes
- Ice box

6.4.4.Miscellaneous

- Marker pen

6.5. Preparations

- Transfer all materials into separate centrifuge tubes and pre-chill them with an ice box.
- Label each experimental centrifuge tube appropriately. (4 samples for each temperature)

6.6. Procedures

1. Pipette 9µL of ddH_2O to each tube.
2. Pipette 1µL of *Eco*RI-GFP forward primer to each tube.
3. Pipette 1µL of *Xho*I-pET-32a-GFP reverse primer to each tube.
4. Pipette 1.5µL of DNA to each tube.
5. Pipette 12.5µL of Taq polymerase premix to each tube. Wait until the PCR machine is set up before doing this step to minimise unwanted prior reaction.
6. Mix all the tubes gently.
7. Put the tubes in the PCR thermocycler and set the parameters as follows.

6.7. PCR thermal cycle

Phase	Temperature	Duration	Cycles
Initial denaturation	98℃	5m	1
Denaturation	98℃	30s	34
Annealing	55℃ 54℃ 51.9℃ 50.3℃	45s	
Extension	72℃	1m 30s	
Final extension	72℃	7m	1
Sample keeping	4℃	∞	1

Day 7 (19-04-2023)

Interlude

Here omitted, we have performed gel electrophoresis on the restriction products of case study 2 experiment 6.

Results

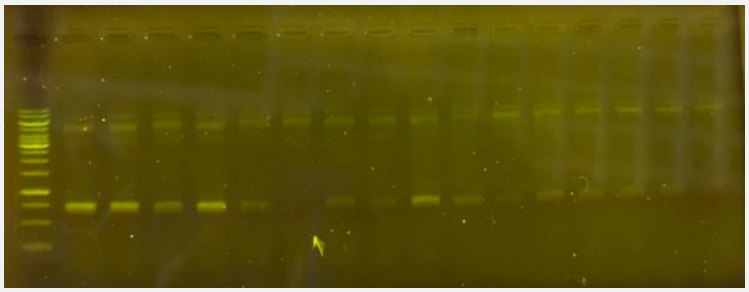


Figure 2 – Electrophoresed gel with PCR products under UV light
From left to right: DNA ladder, PCR'd GFP samples at various temperatures

Although there are by-products (6000bp), we can clearly observe the desired bands of successfully extended GFP (733bp).

7. Experiment 7 *(Prerequisite: Case Study 2 Experiment 6)*

7.1. Objective

- To extract and purify restricted extended GFP with gel extraction.

7.2. Precautions

- Wear gloves.
- Dispose of the pipette tips after each transfer.
- Do not look directly into the UV light without the protective shield.

7.3. Materials

7.3.1. Electrophoresed gel

- 1 electrophoresed gel

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

- 7200µL of BNL buffer
- 3000µL of washing buffer

7.3.3. Miscellaneous

- 120µL of ddH_2O

7.4. Equipment

7.4.1. Machineries

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

7.4.2. Apparatus

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

7.4.3. Containers

- 20 centrifuge tubes
- 4 spin columns (from the iNtRON kit)

- 4 collection tubes (from the iNtRON kit)

7.4.4.Miscellaneous

- Marker pen

7.5. Preparations

- Add ethanol to the washing buffer as per the instructions on the bottle.
- Put 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.

7.6. Procedures

1. Place the gel on a UV transilluminator.
2. Isolate all the desired bands with a sterile scalpel.
3. Transfer each gel slice into a separate centrifuge tube.
4. Pipette 450μL of BNL buffer to each tube.
5. Gently mix the sample by vortexing.
6. Place the tubes in a heat block at 55℃ for 7 minutes or until the gel slices are completely dissolved.
7. Allow the sample to cool to room temperature.
8. Transfer one sample mixture of each bp size to separate columns with a pipette.
9. Centrifuge the samples at 11000×g for 30 seconds.
10. Discard the filtrate fluids in the collection tubes and remount the columns into the same collection tubes.
11. Transfer and combine four sample mixtures to one column with a pipette.
12. Repeat steps 9-10.
13. Pipette 750μL of washing buffer to each column.
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. Centrifuge the samples at 16000×g for 3 minute or until the filter membranes are dry.
17. Put the columns into sterile centrifuge tubes. Remove and discard the used collection tubes.
18. Pipette 40μL of ddH_2O directly onto the filter membranes of each column.
19. Let the samples stand for 2 minute.
20. Centrifuge the samples at 16000×g for 1 minute.
21. Discard the columns and store the purified DNA at -20℃ for future use.

7.7. Products

- 4 centrifuge tubes each with 40μL of restricted GFP

Day 8 (20-04-2023)

8. Experiment 8 *(Prerequisite: Case Study 2 Experiment 3 + Case Study 2 Experiment 7)*

8.1. Objective

- To insert restricted GFP encoding gene into restricted pET-32a.

8.2. Precautions

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

8.3. Materials

8.3.1. Gene insertion

- 49.05μL of restricted pET-32a
- 1.95μL of restricted GFP encoding gene
- 6μL of T4 DNA ligase buffer (10×)
- 3μL of T4 DNA ligase

8.3.2. Miscellaneous

- Ice

8.4. Equipment

8.4.1. Machineries

- 42℃ heat block
- 37℃ incubator
- Centrifuge

8.4.2. Apparatus

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

8.4.3. Containers

- 4 centrifuge tubes
- Ice box
- Beaker

8.4.4. Miscellaneous

- Marker pen
- Lighter
- 70% ethanol

8.5. Preparations

- Measure the DNA samples concentration and calculate their appropriate amount necessary.
- Pre-chill the DNA samples in ice.
- Pre-chill the enzyme in ice.
- Label all centrifuge tubes appropriately.

8.6. Procedures

1. Pipette 16.35μL of pET-32a to each centrifuge tube.
2. Pipette 0.65μL of GFP to each sample.
3. Pipette 2μL of ligase buffer to each sample.
4. Pipette 1μL of ligase to each sample.
5. Let the samples sit at room temperature for 10 minutes.

8.7. Products

- 3 centrifuge tubes each with 20μL of ligated pET-32a-GFP

9. Experiment 9 *(Prerequisite: Case Study 2 Experiment 8)*

9.1. Objective

- To transform E. coli (DH5α) with pET-28a-GFP and ligated pET-32a-GFP.

9.2. Precautions

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

9.3. Materials

- 250μL of competent DH5α E. coli cells
- 10 μL of pET-28a-GFP
- 60 μL of pET-32a-GFP
- 2000μL of LB culture broth
- 3 LB agar plates with diluted kanamycin
- 2 LB agar plates with diluted ampicillin
- Ice

9.4. Equipment

9.4.1.Machineries

- 37℃ incubator
- 42℃ heat block
- 4℃ refrigerator
- Bunsen burner

9.4.2.Apparatus

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

9.4.3.Containers

- 5 centrifuge tubes
- 5 petri dishes
- Ice box
- Beaker

9.4.4.Miscellaneous

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

9.5. 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.
- Label the centrifuge tubes and petri dishes appropriately.
- Light a flame to generate an aseptic convection current.

9.6. Procedures

1. Pre-chill the samples of E. coli and plasmids.
2. Transfer 50μL of E. coli to each centrifuge tube.
3. Pipette 5μL of pET-28a-GFP to each of three tubes.
4. Pipette 20μL of pET-32a-GFP to each of three tubes.
5. Gently flick the tubes to mix.
6. Chill the samples with ice for 5 minutes.
7. Heat shock the samples at 42℃ for 90 seconds.
8. Chill the mixtures with ice for 1 minute.
9. Pipette 400μL of LB culture broth into each of the samples.
10. Incubate the samples at 37℃ with ~250rpm shaking for 45 minutes.
11. Sterilise the spreader with ethanol and a flame and allow it to cool slightly.
12. Pipette 150μL of pET-28a-GFP-tranformed E. coli onto a warm ampicillin agar plate.
13. Spread the liquid evenly with a sterilised spreader until the surface looks dry.
14. Repeat steps 11-13 for the other two pET-28a-GFP.
15. Repeat steps 11-14 but for pET-32a-GFP and kanamycin agar plates.
16. Incubate the petri dishes upside-down at 37℃ for 12-16 hours.
17. Wrap the petri dishes with laboratory wrapping film and keep them chilled at 4℃ for future use.

9.7. Products

- 2 petri dishes each with pET-28a-GFP-transformed E. coli
- 3 petri dishes each with pET-32a-GFP-transformed E. coli

Day 9 (21-04-2023)

Finale

Figure 3 – Colony plates of pET-32a-GFP-transformed E. coli

Figure 4 - Colony plates of pET-28a-GFP-transformed E. coli

No colonies can be seen growing in the plates. It seems that the bacteria have not survived the transformation process.

Discussion

The case study was not successful. The first factor would be the last-minute change to the parameters, although I would name this to be the least contributing factor. However, the fact that we were not told clearly that the GFP was not actually a standalone DNA strand but instead embedded in a plasmid caused us a lot of confusion throughout the process, in conjunction with the lack of PCR progress we were all making in case study 1.

Logistics aside however, the main reason the transformation ended up not being successful should largely be due to the low concentration of the extracted pET-32a. According to the nanodrop measurement, the density was only 1.7ng/μL. It was nowhere near the optimal transformation concentration.

In terms of time management, case study 2 initially already demands a very tight schedule with little wiggle room since it has a lot of overnight incubations and PCRs to be done, and the parameter change definitely did not help our situation since one precious overnight time was already wasted in the very beginning. As clearly demonstrated by our lack of final results, we did not have sufficient time to conclude the case. Even if the final transformation had succeeded, we would still require at least three days for the incubation, small culture, protein expression induction, and SDS-PAGE staining and de-staining.

Given the circumstances, I doubt we could have done too much better, although I do believe that we could have tried to perform more trials on the PCR and restriction since they tend to be very error prone.