- Transformation of pSB1C3 plasmid into DH5 alpha E. coli

- To multiply the amounts of pSB1C3 plasmids

- 5 LB plates with Chloramphenicol

- 500mL Liquid LB culture

- Set up LB culture with E.coli for interlab; transform E.coli for interlab

- Colony selection of E.coli which we transformed on LB plate yesterday and incubate into liquid LB culture

- We grew more culture for plasmid preparation.

pSB1C3:

- Extract and purify pSB1C3 plasmids from cultured DH5 alpha E.coli (liquid)

- We extracted PSB1C3 plasmids which would be used as vectors in the Gibson Assembly.

- Store extracted plasmids for preparation of liberalization of pSB1C3 vector

- Blend to obtain BG-11 for cyanobacteria culture (used in function test)

We prepared 5L BG-11 medium for cyanobacteria culture

- Make protein polyacrylamide gel (run gel to analyze expressed protein)

- Make 5 LB plates and 3L liquid LB culture

pSB1C3:

- Perform PCR to linearize the pSB1C3 backbone

- Make electrophoresis agarose gel

- Run electrophoresis on linearized vector

Observation and analysis: it showed clear bands between 2000 bp and 3000 bp, meaning that the linearization was successful

- Collect and purify DNA fragment from the gel

PET28A and EX-1:

- Perform PCR to obtain PET-28A and EX-1 plasmids with homogenous site (backbone)

- Transform cyanobacteria into the BG-11 culture

- Seal and store BG-11 culture with cyanobacteria

PET-28 and Ex-1

- Gel electrophoresis to examine the result of PCR (linearized PET-28A and EX-1 plasmids)

Observation and analysis: The microfuge tubes labelled "28a-2" and "ex-2-2" showed no bands, demonstrating that the PCR employing mix buffer didn't work. The tubes labeled with "1" exhibited clear bands, meaning that their PCR were successful.

- Gel purification and recollection of linearized vector

- Perform PCR to amplify our lysozyme gene with homogeneous site (inserted gene)

- Run electrophoresis to examine the result of amplified lysozyme gene

- Gibson Assembly to combine PET-28A or EX-1 backbone with lysozyme gene

- Transformation of plasmids into DH5-alpha E.coli

- Transfer transformed DH5-alpha onto K+ agar plates

6 plates were created: 28a-0, which should contain DH5-alpha with linearized PET-28A; 28A-1&28A-2, containing DH5-alpha with PET-28A plasmid; Ex-1-1 control, which was covered by DH5-alpha with linearized plasmid, while the EX-1-1 and EX-1-2 retained DH5-alpha with transformed plasmid.

pSB1C3

- Infusion of lineared pSB1C3 vector and amplified lysozyme gene through one-step cloning fusion

- Transformation of pSB1C3 into DH5-alpha E.coli on LB plate

PET-28A and Ex-1:

Observation and analysis for plating of PET-28A and EX-1 transformed bacteria:

The 28A-0 negative control had ~40 growth, 28A-2 had ~40 growth whereas 28A-1 had fewer growth, indicating that there could possibly be errors when plating the bacteria or tubes were mislabelled in the first place. The EX-1-1 control had 1 growth, while the EX-1-1 and EX-1-2 has 4-6 growth. The result indicates that there could possibly be empty plasmids in all transformed bacteria, and that was possibly because when we were using dpn-1 enzymes to cut methylated plasmids, the reaction time was not enough.

- Inoculate the transformed DH5 alpha 28A-1, 28A-2, EX-1-1 and EX-1-2 into liquid LB culture.

- Extract plasmids and Use restriction enzyme to test if the target gene is in the plasmid PET-28 and Ex-1.

- Perform PCR to amplify the T7 promoter and the possible targeting gene inserted within and verify if the target gene is correctly inserted.

observation and analysis:

The PCR results indicated that 28A-1 and 28A-2 were both lack of targeted gene; EX-1-1 possibly had the targeted gene whereas EX-1-2 did not. The EX-1-1 had a clear band at ~500bp, indicating that it had the targeted gene. Whereas 28a-1 and 28a-2 were both lack of the target gene.Therefore, we sent 28A-1, 28A-2, and EX-1-1 for sequencing and did further transformation.

- Run a gel to test if the targeted gene is on the plasmid extracts

- Send 6 uL of the EX-1 plasmid for sequencing and transform the rest of the plasmids into BL-21 E. coli for protein expression.

pSB1C3

Observation and analysis

We got no transformants on the LB plates. After looking through the instruction of infusion kit, we suspected we add too much DNA fragments of lysozyme as templates, or we added insufficient competent cell.

- Onestep Cloning: Fusion of vectors and lysozyme gene

- Transformation of fused plasmid with lysozyme gene

PET-28A and Ex-1:

- Result of sequencing of Ex-1 plasmid

The sample was missing 1 bp, which led to 2 presumptions: either the synthesized strand was originally wrong, or mistakes in PCR caused the deletion. The underlying cause could only be revealed by the PCR product later today. If all of them had the same deletion, then this would be a problem of the original strand, and could only be solved by designing new primers to cover the missing bp

- Inoculate 16 colonies and run PCR to examine the existence of lysozyme gene in the plasmids

- 10 from PET-28A-2, 6 from Ex-1&Ex-2 together

Observation and Analysis

All 16 colonies showed clear band at the site near 500bp, implying that all of the plasmids might be successfully transformed. Yet the band could be the result of errors in PCR

- PCR to amplify lysozyme with linker primer

Preparation for nested PC

-Run Gel electrophoresis to verify the result

Observation and Analysis

The lysozyme gene was correctly amplified

- Gel Extraction of lysozyme gene with linker primer

- Gibson Assembly to fuse PET-28A backbone and lysozyme gene
- Restriction enzyme cutting of PET-28A to test the existence of lysozyme gene
- Run Gel electrophoresis of the cutting product

Observation and Analysis

PET-28A was missing the band at ~500bp, indicating that the plasmid wasn't successfully fused with the target gene

- Transform Ex-1&Ex-2 which contain lysozyme gene into BG21 for protein expression
- Send Ex-1&Ex-2 for sequencing

pSB1C3

Observation and Analysis

- The LB plate labeled with "small" grows several bacterial colonies

Colony selection of E.coli on "small plates" (lysoyzme gene)
- Send "small" for sequencing

PET-28A & EX-1:

Run PCR to amplify the chimeric protein gene

- Primer lyso-F&mlrA- R were used

Run PCR to amplify mlrA gene using the chimeric protein gene as a template

- Primer mlrA-F&mlrA-R were used

Run Gel electrophoresis to test the product of both PCR
-Gel Extraction of chimeric gene and mlrA gene
-Gibson Assembly of chimeric gene and backbone; mlrA gene and backbone
-Run Gel Electrophoresis to demonstrate successful infusion of backbone and gene

- The DNAs were arranged in the following sequence:
    - Empty PET-28A, chimeric, mlrA, lysozyme, marker, lysozyme, mlrA, chimeric, empty Ex-1

- Transformation of DH5-alpha onto K+ plates

- 4 plates were made:1 for pet28a & chimeric, 1 for PET-28A & mlrA, 1 for Ex-1 & chimeric, 1 for Ex-1& mlr A

- Transfer BL21(lysozyme) into bigger flask and induce expression
- Create 5L of LB

pSB1C3:

Perform PCR to amplify gene

- chimeric with histag
- chimeric without histag
- lysozyme with histag
- mlrA with histag

- mlrA without histag

- Run gel to analyze our gene

Observation and analysis: clear bands in all 5 micro tubes showed successful amplification of 5 types of gene

- Onestep Cloning: Fusion of vectors and five gene listed above

- Transformation of fused plasmids

- Send plasmids for sequencing

- Lyse Bacteria with high-pressure (from BL21 with lysozyme gene)

- Purification using nickel column (protein expressing lysozyme gene)

Observation and Analysis:

The fluid in Ex-1 column was murky, indicating that lysozyme deposits even with the Ex-1 tag. The precipitation would block the flow of fluid, hindering the purification process. While the fluid in PET-28A was clear, whether it contained the intended protein was unknown.

- Verification of protein (with Ex-1 tag)

Observation and Analysis

The 200mm Ex-1 tude(protein indicator) exhibited brighter blue, showing that the protein is more concentrated inside. Yet the concentration of intended protein needed to be verified by gel electrophoresis

- Perform the same procedure on PET28a

Observation and Analysis

The 20mm PET-28A is brighter than 200mm PET28a, which is not preferable. Result will be examined by gel electrophoresis

-Run Protein gel electrophoresis to verify if protein exists

Data:

1.170mg/ml 260/280: 0/65

- Inoculate DH5-alpha colonies (mlrA and chimeric gene)

- Inoculate 5 colonies from each plate made yesterday
- Put into tubes, label mlr-PET28a colonies from 1 to 5, label mlra- Ex-1 6-10, linker-28 11-15, linker-Ex-1, 16-20
- Culture

- Perform Bacteria fluid PCR to verify the existence of mlrA gene and chimeric gene

Run Gel electrophoresis

Only tubes 1,2,6,7, 14,15,18,20 exhibit clear bands at intended region

- Transformation of DH5-alpha

Observation and analysis result of dialysis

Both dialysis bags contain murky solution, indicating the protein precipitated once again.

- Filtration and further purification of proteins (get rid of tags)

- Pour the Tev+ lysozyme solution into the nickel column, wait for all fluid to pass through the column

- Add protein indicator to in FT solution and non-Tev solution to see protein concentration

Observation and analysis

The solution containing non-Tev exhibits blue-gray, meaning there was few protein inside. The tube was then disposed. While the solution containing Tev needed to flow through the nickel column again to get rid of the tags to see the existence of protein, the FT solution displayed a brighter blue color.

- Wash off the TEV and tags on the column

- Perform Protein Gel electrophoresis of purified proteins

- Concentrate lysozyme and dilute imidazole concentration

- Nanodrop to examine the concentration of lysozyme.

The lysozyme content after dilution of imidazole is 0.113mg/ml

Culture BG-21 (chimeric and mlrA gene)

Function test:

- In a microfuge tube, take 300uL cyanobacteria with a OD660nm absorptance of 1.5.
- Centrifuge and take out all the supernatant.
- Add 30uL bugbuster, 30uL of pH buffer(dependent on pH), 60uL of enzyme solution(made previously according to different protein concentration requirement), and 180uL of water.
- React different tubes in shaker under different temperatures(according to different temperature requirement) for a 0.5,1,1.5 or 2 hours(depends on different reaction time requirement.
- Take out the microfuge tube, centrifuge for 10 minutes at 14500 rpm.
- Take out 270uL of the supernatant to test the OD660nm light absorptance.