NOTEBOOK

Converter Group（CBD+BirA）

CBD (Cellulose Binding Domain) Experiments

5/20/2019

1. Transferred the plasmid of dCBD-sfGFP (BBa_K1321348) into DH5α.
2. We found the chloramphenicol was invalid.

5/30/2019

Verified the validity of chloramphenicol and failed.

6/19/2019

Verified the validity of chloramphenicol and failed.

6/20/2019

Prepared for chloramphenicol and kanamycin solution again. Preserved and activated DH5α and BL21.

6/21/2019

Verified the validity of new compounded chloramphenicol and kanamycin. Faied.

6/24/2019

Changed the concentration of kanamycin and chloramphenicol in solution. Verified the validity. The result of kanamycin was successful, but chloramphenicol was failed.

6/25/2019

1. Verified the validity of chloramphenicol. Failed.
2. Activated the TOP10 strain containing pET28a-dCBM3a.

6/26/2019

1. Prepared for chloramphenicol solution again and verified its validity. Failed.
2. Reactivated TOP10 strain containing pET28a-dCBM3a.

6/27/2019

1. Verified the validity of chloramphenicol. Failed.
2. Inoculated a single colony of TOP10 strain containing pET28a-dCBM3a into 5mL liquid medium.

6/28/2019

Extracted the pET28a-dCBM3a plasmids.

6/29/20119

1. Verified the validity of chloramphenicol. Failed.
2. Double digestion analysis of the plasmids (BamHI and EcoRI).

6/30/2019

1. Verified the validity of chloramphenicol. Failed.
2. Analyzed the result of double digestion by electrophoresis. No bands appeared in the digested sample. We assumed the concentration of DNA was too low.
3. Double digestion of the plasmids in a larger system. Analyzed after 4h of digestion. 2 bands appeared in the digested sample. The plasmids sample showed three or four bands. We needed further single digestion analysis to make sure there was only one species of plasmids in the solution.

7/1/2019

1. Verified the validity of chloramphenicol. Failed.
2. Analysis the plasmids by single (EcoRI) and double digestion (EcoRI+BamHI). We affirmed the plasmid was pET28a-dCBM3a.

7/3/2019

Verified the validity of chloramphenicol. Failed.

7/4/2019

Verified the validity of chloramphenicol. Failed.

7/6/2019

1. Verified the validity of chloramphenicol. The verified chloramphenicol was from another lab. Failed.
2. Transferred pET28a-dCBM3a into BL21.

7/8/2019

Induced BL21 strain to produce dCBM3a protein.

Collected the bacteria and froze into -20℃.

7/10/2019

Tried to break the bacteria by lysozyme and ultrasonic.

7/11/2019

We spilled the green bacterial supernatant by incident. The induction experiment was failed.

7/15/2019

Re-induced the expression of dCBM3a protein.

7/16/2019

1. Verified the validity of chloramphenicol. We affirmed the DH5α and BL21 we used in the verification had no plasmids.
2. Purified dCBM3a protein. We found the function of Nickle column was bad.

7/17/2019

1. SAS-PAGE analysis of dCBM3a.
2. BC purification: boiled the marketed BC in NaOH for half an hour.

7/18/2019

Adjusted the purified BC to pH=7.

7/19/2019

1. Verified the validity of chloramphenicol. The chloramphenicol used was from another company.
2. Applied Bacteria Cellulose in each well on the 96-well plate. Mixed proteins

with BC in each well. Incubated refer to the method of 14 iGEM Imperial.

7/20/2019

1. Transferred dCBD-sfGFP (BBa_K1321348) into DH5α strain.
2. Measure the binding strength between dCBM3a and BC.

7/21/2019

1. Inoculated 5 tubes of dCBM3a bacteria.
2. Inoculated the transformant into LB medium.

7/22/2019

1. Extracted dCBM3a plasmids.
2. Purification of the expressed dCBM3a protein.
3. Extracted dCBD plasmids.

7/23/2019

1. SAS-PAGE analysis of purified dCBM3a protein.
2. Digested dCBD plasmids with EcoRI overnight.

7/24/2019

1. SDS-PAGE analysis of purified dCBM3a protein.
2. Inoculated bacteria containing dCBM3a.
3. Agarose gel electrophoresis analysis of digestion product.
4. Inoculate bacteria containing pET-28a.

7/25/2019

1. Induced the expression of dCBM3a protein.
2. Extract pET-28a plasmids.

7/26/2019

1. Purification of target dCBM3a protein.
2. PCR to amplify the target bands.

7/27/2019

Gel extraction of PCR product.

7/29/2019

Inoculated DH5α strain and dCBM3a strain.

7/30/2019

Induced the expression of target dCBM3a protein.

7/31/2019

Explored the condition needed for ultrasonic to break up dCBM3a bacterial cell.

8/1/2019

1. Purified target dCBM3a proteins and SDS-PAGE analysis.
2. Reverse PCR of pET-28a and extracted the product DNA to obtain pET-28a backbone.

8/2/2019

Gibson assembly of dCBD with pet28a and transformation to DH5a.

8/11/2019

1. Reverse PCR of pET-28a.
2. Tested the strength of the binding between dCBM3a and bacterial cellulose.

8/12/2019

1. Extraction the product DNA of reverse PCR.

2. Tested the strength of the binding between dCBM3a and bacterial cellulose.

8/13/2019

Added sfGFP fused dCBD to pET-28a via Gibson assembly.

8/14/2019

Assembled sfGFP fused dCBD to pET-28a again via Gibson assembly method.

8/15/2019

Extracted plasmids of sfGFP and dCBD. analysis by restriction digestion and PCR and sequencing.

8/28/2019

Transferred the sequenced sfGFP into BL21 strain.

8/29/2019

Induced the expression of sfGFP.

PCR: linearized pET28a, dCBD+sfGFP (BBa_K1321348)

8/31/2019

Gibson assembly of pET28a-dCBD+sfGFP.

Lysis of the bacteria containing sfGFP.

2019/9/1

SDS-PAGE analysis of sfGFP

Sequenced pET28a-dCBD+sfGFP. The result of identification was successful.

9/6/2019

Extracted the dCBD plasmids after Gibson assembly and made analysis by electrophoresis.

9/7/2019

Transferred the pET-28a containing dCBD into BL21(DE3) strain.

9/8/2019

1. Inoculated the dCBD transformant and induce expression.
2. Tested the binding strength between dCBM3a and biotin by biotinylated magnetic beads.

9/9/2019

1. Broke up dCBD bacterial cells to purify target proteins.
2. Observed the dCBM3a binding ability via SAS-PAGE.

9/10/2019

Inoculated dCBM3a bacteria.

9/12/2019

Induced the expression of target dCBM3a protein.

9/13/2019

Broke up dCBM3a bacterial suspension by ultrasonic to collect target protein.

9/14/2019

1. Tested the function of streptomycin module in dCBM3a via biotinylated magnetic beads.
2. Quantificationally compared the binding strength of dCBD with bacterial cellulose and dCBM3a with bacterial cellulose.

9/15/2019

Quantificationally compared the binding strength between dCBD with BC and

dCBM3a with BC.

9/16/2019

Quantificationally compared the binding strength between dCBD with BC and dCBM3a with BC.

9/17/2019

Tested the function of streptomycin module in dCBM3a via biotinylated magnetic beads.

9/24/2019

Quantificationally compared the binding strength between dCBD with BC and dCBM3a with BC.

9/26/2019

1. Amplified dCBM3a-sfGFP fragments by PCR.
2. Amplified CBM3a-sfGFP fragments by PCR.

9/27/2019

Amplified pET-28a fragments by PCR.

10/6/2019

1. Assembled dCBM3a-sfGFP fragments onto pET-28a by Gibson assembly and transfer into competent cells.
2. Assembled CBM3a-sfGFP fragments onto pET-28a by Gibson assembly and transfer into competent cells.

10/7/2019

Extracted the product plasmids of Gibson assembly.

10/8/2019

1. PCR analysis of Gibson assembly.
2. Test the function of streptomycin module in dCBM3a via biotinylated magnetic beads.
3. Gibson assemble again.

10/9/2019

PCR and sequencing analysis of CBM3a-sfGFP Gibson assembly.

10/10/2019

PCR and sequencing analysis of CBM3a-sfGFP Gibson assembly.

10/11/2019

PCR and sequencing analysis of CBM3a-sfGFP Gibson assembly.

10/13/2019

Transferred the right plasmids to BL21.

10/14/2019

Induced the expression of target dCBM3a protein and got the lysate supernatant.

10/15/2019

Quantificationally compared the binding strength between CBM3a with BC and dCBM3a with BC.

10/16/2019

Quantificationally compared the binding strength between CBM3a with BC and dCBM3a with BC.

10/17/2019
Quantificationally compared the binding strength between CBM3a with BC and dCBM3a with BC.
10/18/2019
Quantificationally compared the binding strength between CBM3a with BC and dCBM3a with BC.

BirA Enzyme Experiments

8/11/2019
Recovery of TOP10 strain containing pET28a-BirA plasmids.
8/12/2019
Inoculated a single colony into LB liquid medium containing Kanamycin.
8/13/2019
Extracted plasmids.
8/14/2019
Double digestion analysis (EcoRI+BamHI).
8/15/2019
Transferred the plasmids into BL21 strain.
9/1/2019
Extracted pET28a and pET28a-birA plasmids.
9/3/2019
PCR: amplified sfGFP-avitag fragments and linearized pET28a.
9/4/2019
Purification the product of PCR and test the result of purification.
9/5/2019
1. Gibson assembly of sfGFP-avitag to pET28a.
2. Transferred the recombinant plasmids into DH5α.
9/7/2019
Verified the result of Gibson assembly and transformation by colony PCR.
9/8/2019
1. Digested pET28a-birA plasmids (SphI) to acquire linearized vectors.
2. Extracted pET28a-sfGFP+avitag plasmids.
9/14/2019
PCR: amplified the fragments of T7-lacO+sfGFP-avitag+T7-terminator.
9/15/2019
1. Purified the linearized pET28a-birA but failed.
2. Purified PCR product of T7-lacO+sfGFP-avitag+T7-terminator fragments, but failed.

3. PCR: re-amplified the fragments of T7-lacO+sfGFP-avitag+T7-terminator; failed.
4. Re-digested pET28a-birA (SphI).

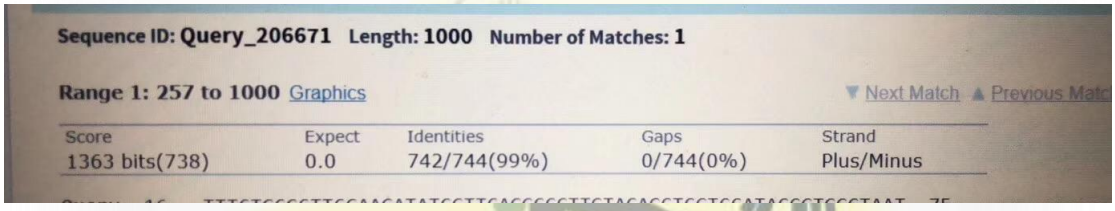
9/16/2019

Re-amplified fragments by PCR.
Purified the product of restriction enzyme digestion and PCR.

9/17/2019

Constructed the recombinant plasmids pET28a-T7-lacO+sfGFP-avitag+T7-terminator by Gibson assembly and transferred the plasmids into DH5α.

9/18/2019-9/28/2019



PCR amplified and sequenced the recombinant plasmids. Select the successful recombinants.

9/29/2019

Induced the expression of pet28a-sfGFP-Avitag**(pGa)** and pet28a-BirA-T7-lacO+sfGFP-avitag+T7-terminator **(pBGa)**.

9/30/2019

Collected the bacteria and chilled at -20℃.

10/4/2019

Purified target proteins and analyzed by SDS_PAGE

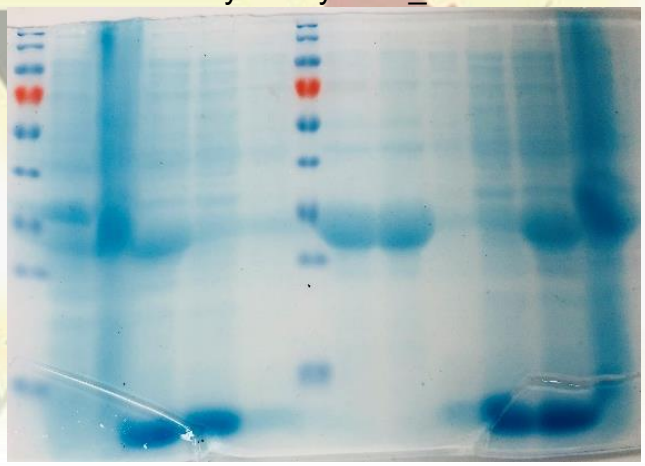


Figure 1 sfGFP-Avitag

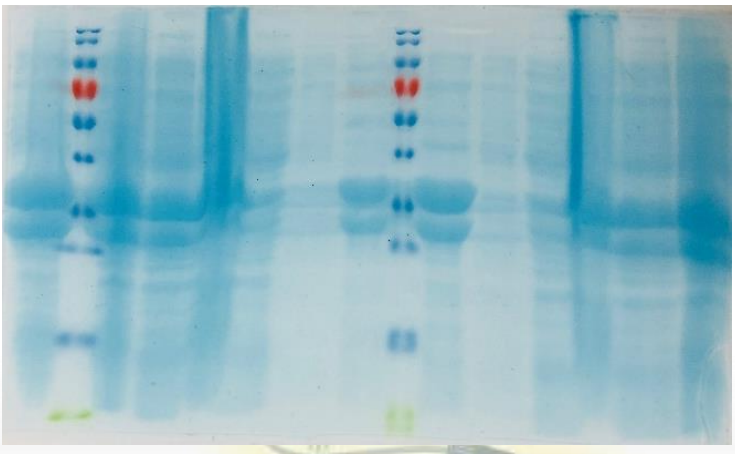
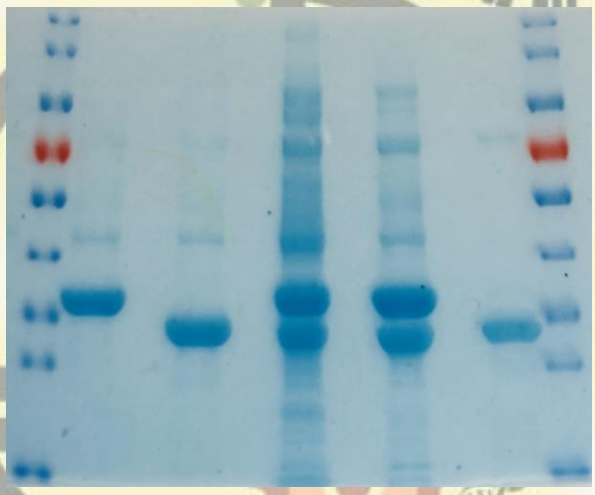


Figure 2 pet28a-BirA-sfGFP-avitag

10/8/2019

Purified furtherly to acquire sfGFP-Avitag and biotinylated sfGFP.

SDS-PAGE analysis:



(Form left to right: Marker, BirA enzyme, sfGFP-avitag, supernatant of pBGa lysate, pBGa lysate purified by Nickel column, pBGa lysate purified furtherly, Marker)

10/9/2019

Quantified the concentration of target protein by Bradford method.

HABA test diagrammatic sketch as follows: