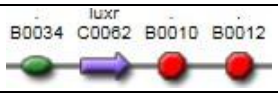
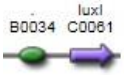
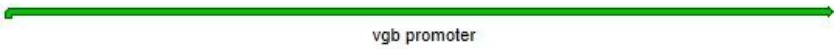
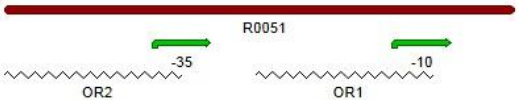
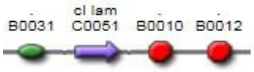
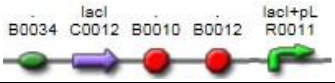
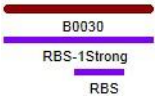
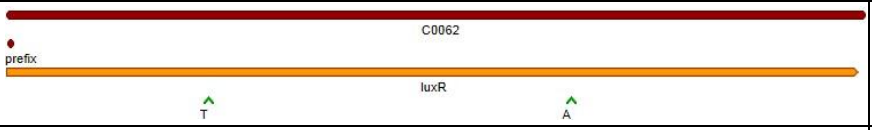
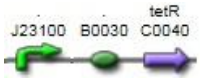
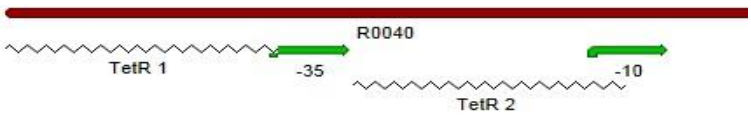
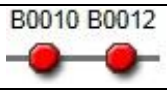
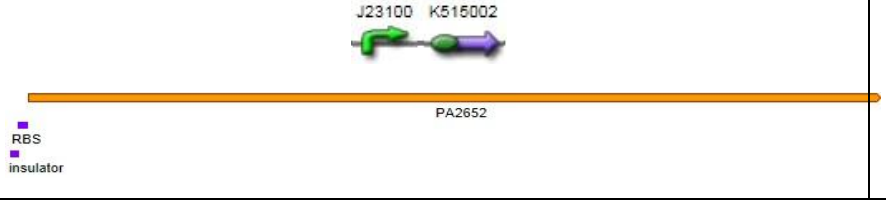
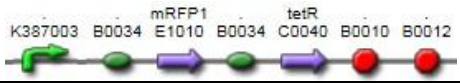
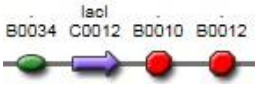
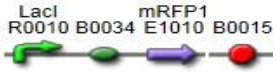
Notebook

Plasmid transformation:

The basic information and number of the biobricks we use from iGEM registry are showed in the below table：

Part	Structure	Length	Number
I0462		936bp	108OA
R0062		55bp	106OA
C0261		661bp	114CA
J23100		35bp	118CA
K561001		135bp	523CC
R0051	 promoter (lambda cI regulated)	49bp	106KA
P0151		932bp	110CA
Q04121		1370bp	120PK
B0030		15bp	101HA
C0062		756bp	104OA
K081010		749bp	210PA
R0040		54bp	106IA
B0034		12bp	102MA
B0015		129bp	123LAK
K515102		1759bp	501EC

K561002		1679bp	523AC
P0412		1308bp	306PA
J04500		220bp	412AAK
J04450		2070bp 2204bp	103AC（pSB1C3） 105AK(pSB1K3)

We transform these plasmids with standardized genes in batch, and change the schedule according to the following experiments.

3rd Jul: transform the first batch of plasmids, including 13 ones: 101HA, 102MA, 104OA, 106IA, 106KA, 106OA, 108OA, 110CA, 114CA, 118CA, 120PK, 123LAK, 210PA

4th Jul: select 5 monoclonal bacteria for each gene

13th Jul: transform plasmid 120PK

15th Jul: transform plasmid 501EC, 523AC, and 523CC

16th Jul: select 10 monoclonal bacteria for 501EC, 10 for 523AC, and 5 for 523CC

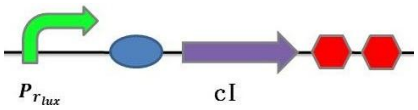
4th Aug: transform plasmid 103AC and 105AK, without coating plates

8th Aug: transform plasmid 306PA and 412AAK, without coating plates

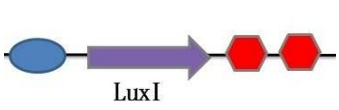
10th Aug: transform plasmid 314EAK, without coating plates

Constructions of standardized plasmids

pR-cI:



LuxI-Ter：



5th Jul: cultivate 106OA, 110CA, 114CA and 123AK in LB medium

6th Jul: extract the plasmids; use enzymes to digest 106OA(S & P), 110CA(X & P), 114CA (E & S), 123LAK (E & X) in 37℃ for 2 hours; extract the fragments;

ligation the purified fragments with T4 DNA lingase over night

7th Jul: transform pR-cI and LuxI-Ter

8th Jul: there is only one bacterium on the plate of LB with Amp; we find lawn on the plate of LuxI-Ter; then pick up the lawn, cultivate pR-cI in LB medium

9th Jul: extract plasmids of pR-cI; use X&P enzyme digestion to detect and the result is positive; transform LuxI-Ter again

10th Jul: there is still lawn on the plate of LuxI-Ter, no monoclonal bacteria; cultivate the transformed products of LuxI-Ter in LB medium

11th Jul: cultivate LuxI-Ter in LB medium; detect plasmids by electrophoresis and it shows single and bright stripe; transform plasmids

12th Jul: lawn on the plate of LuxI-Ter again

13th Jul: relink LuxI-Ter at 16℃ for 2h; transform it and cultivate it in LB medium

14th Jul: extract plasmids and detect it. It shows a single stripe

5th Aug: cultivate 114CA and 123LAK in LB medium

6th Aug: extract the plasmids; use enzymes to digest 114CA (E & S) and 123LAK (E & X) at 37℃for 2h; extract 114CA; dispose 123LAK at 80℃ for 15min

7th Aug: transform LuxI-Ter and cultivate it in LB medium

9th Aug: extract the plasmids and detect them by electrophoresis

13th Aug: transform plasmids of LuxI-Ter

14th Aug: select 9 bacteria of LuxI-cI and detect the bacteria solution by PCR; cultivate LUxI-Ter1, 2, 3 in LB medium

15th Aug: extract plasmids and detect it by PCR; transform them again

16th Aug: select 6 bacteria of LuxI-Ter and cultivate them in LB medium

17th Aug: extract the plasmids and detect them by electrophoresis; use enzymes to digest LuxI-Ter(E&P) at 37℃ overnight

18th Aug: extract the gel; link LuxI-Ter with pSB1C3 and transform this at 16℃ for 2h

19th Aug: no bacteria

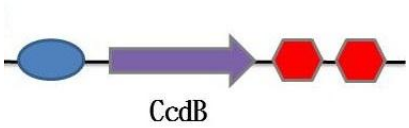
1st Sep: use enzymes to digest LuxI-Ter

2nd Sep: gel-extract LuxI-Ter and link it to pSB1C3; transform it

3rd Sep: only one bacterium; select it

17th Sep: deliver it to company to sequence it

R-CcdB-T



5th Jul: steak on the plate of pEntr-3C

6th Jul: select monoclonal bacterium to cultivate in LB medium

7th Jul: extract plasmids and amplify gene CcdB

Program

94℃	5min	
94℃	30s	32 cycles
54℃	30s	
72℃	25s	
72℃	5min	
4℃	∞	

Extract gel and standardize gene CcdB by standard primer EX-Standard-F/SP-Standard-R

Extract gel and link it to carrier pMD18-T

Links' system

pMD18-T	0.5ul
CcdB fragment	8ul
Solution I	1.5ul

8th Jul: transform

9th Jul: select monoclonal bacteria and detect its liquid by PCR. One of the results is positive

10th Jul: deliver T-CcdB2 to sequence it

12th Jul: cultivate it in LB medium

13th Jul: extract plasmids; use enzymes to digest it and extract gel; link CcdB and 123LAK; transform

14th Jul: after it grows into lawn, cultivate the transformed solution in LB medium

15th Jul: detect the solution by PCR but it shows no specific stripe; transform again

16th Jul: it still grows into lawn

5th Aug: cultivate T-CcdB2 and 123LAK in LB medium

6th Aug: extract plasmids and digest them by enzymes; extract CcdB gel; dispose 123LAK in 80℃ for 15min

7th Aug: transform CcdB-Ter and cultivate the solution in LB medium

9th Aug: extract plasmids and detect them with electrophoresis; cultivate 102MA in LB medium

11th Aug: extract plasmids and cut them with enzymes; extract gel; link CcdB and 102MA to become R-CcdB

12th Aug: transform R-CcdB and cultivate it in LB medium

13th Aug: extract R-CcdB and detect it by electrophoresis; transform plasmid and cultivate it in LB medium

14th Aug: coat plate

15th Aug: select 8 bacteria

16th Aug: detect solution by PCR

17th Aug: detect solution by PCR and No.3 and No.8 are positive

19th Aug: R-CcdB8 is not well prepared; extract plasmids and digest it with enzymes; extract gel and link R-CcdB3+123LAK overnight

20th Aug: transform and coat plate

21st Aug: select 2 bacteria

22nd Aug: cultivate R-CcdB-T1 and R-CcdB-T2 in LB medium

23rd Aug: R-CcdB-T2 is not well prepared; extract plasmids of R-CcdB-T1 and detect it by enzyme digestion

3rd Sep: extract plasmid, digest (E&P)，purify except CcdB-Ter, link to pSB1C3, pSB1K3, CcdBC(T-CcdB2+ pSB1C3), CcdBK(T-CcdB2+ pSB1K3), R-CcdBC(R-CcdB3+ pSB1C3), R-CcdBK (R-CcdB3+ pSB1K3), R-CcdB-TC(R-CcdB-T1+ pSB1C3), R-CcdB-TK(R-CcdB-T1+ pSB1K3)

4th Sep: Transformation, and culture it in medium

4th Sep: transform and shake bacteria

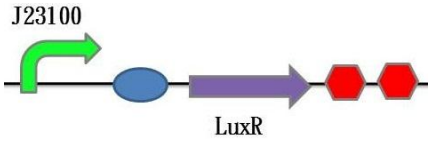
5th Sep: Extract the plasmid, and detect by electrophoresis

6th Sep: transform ligation results again, coat plate

7th Sep: picking the bacteria

12th Sep: deliver CcdBK1、CcdBC1、R-CcdBC1、R-CcdBK1、R-CcdB-TK、R-CcdB-TC1、CcdB-Ter to company to sequence

LuxR



5th Jul: cultivate 108OA in LB medium

6th Jul: extract plasmids and digest108OA(X&P) and 114CA(S&P); link 108OA+114CA to be LuxR-I overnight

7th Jul: transform LuxR-I

8th Jul: select 6 monoclonal bacteria

9th Jul: detect the solution by PCR

10th Jul: retransform and cultivate in LB medium

11th Jul: extract plasmids of LuxR-I and detect it by enzyme digestion

12th Jul: retransform LuxR-I and coat plate

13th Jul: LuxxR-I grows in lawn; cultivate transformed solution in LB medium

14th Jul: extract plasmids of LuxR-I

5th Aug: cultivate 108OA in LB medium

6th Aug: extract plasmid of 108OA; digest 114CA (E&S) and 108OA (E&X) with enzymes

7th Aug: transform LuxR-I and cultivate the solution in LB medium

9th Aug: extract plasmid of LuxR-I and detect it with electropherosis

10th Aug: cultivate 118CA in LB medium

11th Aug: extract plasmid and digest 118CA(S&P) and 108OA(X&P); link 118CA+108OA to become LuxR

12th Aug: transform LuxR and cultivate the solution in LB medium

13th Aug: extract plasmid and detect it with electropherosis

15th Aug: transform plasimd LuxR and cultivate the solution in LB medium

16th Aug: coat plate of LuxR

17th Aug: select 8 bacteria of LuxR

18th Aug: detect the solution by PCR

19th Aug: cultivate transformed solution of LuxR in LB medium

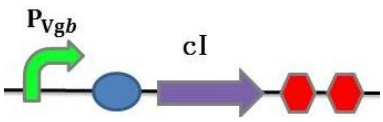
20th Aug: extract plasmids and detect them by PCR

21st Aug: transform plasmid LuxR

22nd Aug: select 3 bacteria and cultivate LuxR2 in LB medium

23rd Aug: extract plasmids and detect them with enzyme digestion

pV-cI



17th July: shake bacteria 523CC

18th Jul： extract plasmid of 523CC and detect it with electropherosis, digest 523CC（S&P） with enzymes overnight

19th Jul: extract plasmid, link 523CC+110CA to become pV-cl

20th Jul: transform pV-cl and cultivate the solution in LB medium

21th Jul: there is no bacterium on the plate of LB, transform again, and cultivate the solution in LB medium

22th Jul: no bacterium

23th Jul: redo our work from the beginning; cultivate 523CC and 110CA in LB medium

24th Jul: extract plasmid of 523CC and 110CA; digest 523CC（S&P）and 10CA（X&P）with enzymes

25th Jul: after detecting them with electrophoresis, extract the gel, link 523CC+110CA to become pV-cl, transform and cultivate the solution in LB medium

26th Jul: there is still no bacterium on the plate of LB

27th Jul: bacterium appears, select 10 monoclonal bacteria

28th Jul: detect the solution by PCR, no result

5th Aug: cultivate 523CC and 110CA in LB medium

6th Aug: extract plasmid of 523CC and 110CA, digest 523CC（S&P）and 10CA（X&P）with enzymes for 2h, link 523CC+110CA to become pV-cl,

7th Aug: transform and cultivate the solution in LB medium

9th Aug: extract plasmid of pV-cl, and detect it with electrophoresis

29th Aug: detect plasmid by PCR

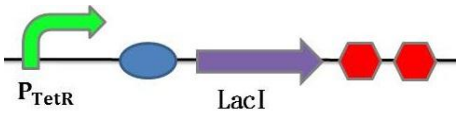
1st Sep: digest 523CC（S&P）and 10CA（X&P）with enzymes,

2nd Sep: after detecting them with electrophoresis, extract the gel, link 523CC+110CA to become pV-cl

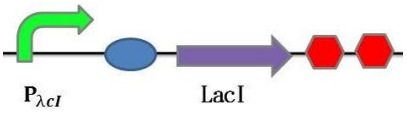
3rd Sep: transform and cultivate the solution in LB medium

4th Sep: extract plasmid of pV-cl and detect it with electrophoresis, detect it by digestion.

pT-LacI



pC-LacI



10th Aug: cultivate 106IA、106KA and 306PA in LB medium

11th Aug: extract plasmid of 106IA、106KA and 306PA, digest 306PA（X&P）、106KA（S&P）、106IA（S&P）with enzymes for 2h, after detecting them with electrophoresis, extract the gel, link 106IA+306PA to become pT-LacI and 106KA+306PA to become pC-LacI.

12th Aug: transform and cultivate the solution in LB medium

13th Aug: extract plasmid of pC-LacI and pT-LacI, detect them with electrophoresis

14th Aug: redo the work, transform and cultivate the solution in LB medium

15th Aug: extract plasmid of pC-LacI and pT-LacI, detect them with electrophoresis

16th Aug: digest 306PA（X&P）、106KA（S&P）、106IA（S&P）with enzymes for 2h, after detecting them with electrophoresis, extract the gel, link 106IA+306PA to become pT-LacI and 106KA+306PA to become pC-LacI.

17th Aug: transform and cultivate the solution in LB medium

18th Aug: extract plasmid of pC-LacI and pT-LacI, detect them with electrophoresis

29th Aug: detect plasmid by PCR, pT-LacI positive, pC-LacI negative

30th Aug: transform pT-LacI and pC-LacI, coat plate

31th Aug: select monoclonal bacteria of pT-LacI, lawn on the plate of pC-LacI, do colony PCR

1st Sep: cultivate pT-LacI1 in LB medium

2nd Sep: extract plasmid of pT-LacI, digest and then do gel extraction, link pT-LacI1+pSB1C3 , pT-LacI1+pSB1K3

3rd Sep: transform pT-LacIC、pT-LacIK，coat plate,

4th Sep: select 5 monoclonal bacteria of pT-LacIK, cultivate in LB medium

5th Sep: extract plasmid of pT-LacIK, digest and then do gel extraction, link pSB1C3+pT-LacIC

6th Sep: select 5 monoclonal bacteria

8th Sep: deliver pT-LacIC1 to company to sequence it

Promoter PureI

8th Jul: amplify promoter PureI from template DNA

Program

94℃	5min	
94℃	30s	┐
54℃	30s	├ 32 cycles
72℃	38s	┘
72℃	5min	
4℃	∞	

Link it to T-Vector after purified

9th Jul: transform T-PureI

10th Jul: select 5 monoclonal of T-PureI from plate and detect the solution by PCR

11th Jul: deliver T-PureI, T-PureI1 and T-PureI5 to be sequenced

14th Jul: get the results of sequencing and redesign primer to synthetize

3rd Aug: amplify promoter PureI on template DNA and extract gel; standardize promoter by standard primer

6th Aug: amplify promoter PureI on template DNA and extract gel; digest it by enzymes

7th Aug: extract plasmid of 103AC、105AK，PCR standardize promoter by standard primer ,than extract gel; digest 103AC（E&P），105AK（E&P），PureI（E&P）and then do gel extraction, Transform PureI and cultivate the solution in LB medium

8th Aug: extract gel of pSB1C3、pSB1K3，link PureI+pSB1K3 to become PureIK, link PureI+pSB1C3 to become PureIC, transform and cultivate the solution in LB medium

9th Aug: extract plasmid of PureI, detect it with electrophoresis

10th Aug: relink PureIC、PureIK, transform and cultivate the solution in LB medium

12th Aug: transform PureIC，cultivate the solution in LB medium

13th Aug: extract plasmid of PureIK

14th Aug: redo the standardization work of PureI, after extraction, use PCR to plus A, link to T-Vector to form st-PureI，

15th Aug: transform st-PureI，cultivate the solution in LB medium

16th Aug: coat plate,

17th Aug: select10 monoclonal bacteria of st-PureI，

18th Aug: do PCR to detect the bacteria solution

27th Aug: cultivate PureI、PureIK and st-PureI in LB medium

28th Aug: extract plasmid

29th Aug: detect plasmid of PureI、PureIK、st-PureI by PCR

2nd Sep: transform st-PureI，cultivate the solution in LB medium

3rd Sep: select 5 monoclonal bacteria of st-PureI

4th Sep: do PCR to detect the bacteria solution

Gene NOX

15th Jul: do PCR to get gene NOX from template

Program

95℃	5min	
95℃	30s	┐
54℃	30s	├ 32 cycles
72℃	90s	┘
72℃	10min	
4℃	∞	

Link it to T-Vector after purified

16th Jul: transform T-NOX，cultivate the solution in LB medium

17th Jul: select8 monoclonal bacteria of T-NOX

20th Jul: do PCR to detect the bacteria solution, number 1, 3 positive

21th Jul: deliver T-NOX1、T-NOX3 to company to sequence it

3rd Aug: redo do PCR to get gene NOX from template, after purified, use PCR to standard gene NOX

6th Aug: PCR gene NOX, after extraction, digest with enzymes NotI & SpeI, cultivate at 80℃ for 15min, link NOX+pSB1AK3

7th Aug: standard gene NOX, gel extraction, digest, transform NOX，cultivate the solution in LB medium

8th Aug: link NOX+pSB1C3 to become NOXC, NOX+pSB1K3 to become NOXK, transform NOXC、NOXK

9th Aug: extract the plasmids and detect them by electrophoresis

10th Aug: relink NOXC、NOXK, transform and cultivate the solution in LB medium

12th Aug: extract the plasmids and detect them by electrophoresis, transform NOXC，cultivate the solution in LB medium

13th Aug: extract the plasmids of NOXK, detect them by electrophoresis

14th Aug: standard gene NOX, gel extraction, use PCR to plus A, link to T-Vector

15th Aug: transform st-NOX, cultivate the solution in LB medium

16th Aug: coat plate

17th Aug: select10monoclonal bacteria of st-NOX

18th Aug: do PCR to detect the bacteria solution

27th Aug: cultivate NOX、NOXK、st-NOX

28 th Aug ; extract plasmids

29th Aug: detect genes by conducting PCR on plasmids

31th Aug: cultivate pET-28a

1st Sep: extract plasmids from pET-28a, and have them digested by EcoRI&Xhol

2th Sep: extract from gel, amplify NOX,T-NOX3 by PCR, change the site for enzyme digestion, extract from gel, digestion by enzymes, link NOX and pET-28a, link NOX3 and pET

4TH Sep ： transform NOX-pET NOX3-Pet, cultivate them using transforming solution

5th Sep: extract plasmids from NOX-pET and NOX3-pET, transform DH5α、BL21 seperately

Gene CsgD

18TH Jul: extract gDNA from λ K12 roughly, and amplify gene CsgD based on the extracted gDNA, extract from gel

19TH Jul: standardize CsgD2 based on CsgD , extract from gel

20th Jul: link CsgD,CsgD2 to T-Vector

21TH Jul: transform T-CsgD,T-CsgD2, coat plates

22TH Jul: bacterial plaques are formed with both T-CsgD and T-CsgD2, retransform T-CsgD,T-CsgD2, coat plates

23th Jul: both can still develop into plaques

11th Oct: re-extract gDNA from λ K12 using new compound solution

12th Oct: re-amplify gene CsgD from gDNA

13TH Oct: extract CsgD from gel, standardize gene st-CsgD, extract from gel, and conduct enzyme digestion on st-CsgD

14th Oct: conduct PCR plus A reaction on CsgD,st-CsgDC at 72℃ for 90mins, link to T-Vector,link enzyme-digested st-CsgD to Psb1c3

15TH Oct: transform CsgD, st-CsgD, CsgDC, cultivate them in transforming solution

16th Oct: CsgDC is not equally distributed; coat CsgD,st-CsgD on plates

17th Oct: pick 10 monoclones each for CsgD,st-CsgD

18th Oct: detect CsgD,st-CsgD using PCR , st-CsgD3 shows positive

31th Oct: cultivate st-CsgD3

1st Sep: extract plasmids from st-CsgD3 ,detect using enzyme digestion

3nd Sep: re-collect fragmentated CsgD by gel to have it amplified using PCT, gel re-collection

4th Sep: standardize gene CsgD, gel re-collection, enzyme digested at 37℃ for 2h and at 80℃ for 15min, linked to pSB.

5th Sep: transform CsgDK, CsgDC , coat plates

6th Sep: no plaque of CsgDC, pick the white one from 2 plaques of CsgDK

7th Sep: extract plasmids from CsgDK1, detect by enzyme digestion

8th Sep; have CsgDK1 sequenced by a sequencing company

14th Sep: cultivate CsgDK1

15TH Sep: extract plasmids from CsgDK1, enzyme digestion

16th Sep: gel re-collection for CsgDK1, link to pSB1C3

20th Sep: transform CsgDC, coat plates

21th Sep: pick up 5 plaques

22th Sep: detect using PCT with all showing positive

23th Sep: cultivate CsgDC1,CsgDC2

24TH Sep: extract plasmids from CsgDC1, CsgDC2, detect by electrophoresis

25th Sep: add CsgDC to the 96-holes plate, have it dry in cold vacuum. Submit

Gene pflB、fdhF

19th Jul: amplify fdhF、pflB from Gdna of λ K12, gel re-collection, link to T-Vector after plus a reaction

21th Jul: transform T-fdhF、T-pflB, cultivate them in transforming solution

22th Jul: coat plates

23th Jul: pick 10 plaques each

25th Jul: detect using PCR

13th Oct: re-amplify pflB、fdhF from gDNA

14TH Oct: gel re-collection for pflB、fdhF

21th Oct: amplify pflB, gel re-collection

22th Oct: pre-mutation, fragmentate pflB into 4 pieces using mutation primer, electrophoresis

23th Oct: induce site-specific mutation on pflB, digest pflB on each site using mutation primers, gel re-collection

24th Oct: link fragmented pieces using overlapped elongation PCR, conduct electrophoresis on each step and re-collect

25th Oct: link pflB

26th Oct: amplify mutated pflB using end primers, electrophoresis, failure

27th Oct: re-link pflb fragments

28th Oct: detect mutated spflB

29th Oct: standardize fdhF、spflB based on standard primers, re-collect fdhF、spflB followed by enzyme digestion, link to pSB1K3、pSB1C3

1st Sep: transform, cultivate in transforming solution

2nd Sep: re-transform, coat plates

3rd Sep: no plaques

4th Sep: re-amplify pflB、spflB、fdhF, change enzyme digestion sites

5th Sep: gel re-collection for pflB、spflB、fdhF, enzyme digestion, link to pET-28a vector.

6th Sep: transform pflB-pET、spflB-pET、fdhF-pET, coat plates

7th Sep: no plaques on plates, re-transform

8th Sep: no plaques again

9th Sep: re-amplify pflB、fdhF using PCR, gel re-collection, enzyme digestion overnight

10th Sep: link to Pet-28a vector

11th Sep: transform, cultivate in transforming solution

12th Sep: extract plasmids followed by electrophoresis, transform to DH5α and BL21 separately.