NEU-CHINA-A iGEM2018

26/3/18-1/4/18

- Amplify pet28a plasmid

5/4/18

- Split primer into EP tube

9/4/18

- K12 resuscitation, cultivate at 37℃

10/4/18

- Transformation of pCDFDuet-1
- Cultivate K12 at 37℃

11/4/18

- Extraction of plasmid pCDFDuet-1

12/4/18

- pCDFDuet-1 plasmid digestion

15/4/18

- Reverse transcription PCR and PCR of IL-10

16/4/18

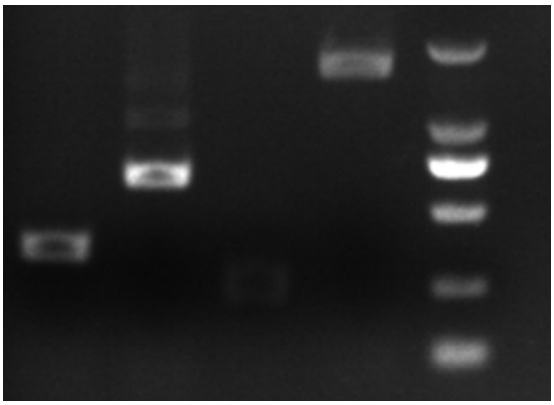
- Cultivate BL21

17/4/18

- Cultivate K12 and BL21

18/4/18

- Extraction of the genome of K12 and BL21
- PCR of Yebf, PnorV and NorR
- From left to right: yebf, yebf with wrong position, PnorV, NorR, DL2000 marker



20/4/18

- Purification of the PCR product of pNorV
- Digest it with pCDFDuet-1
- Purification of digestion product and transformation after connecting them

21/4/18

- Colony PCR of pNorV but the result is obscure
- Overlap PCR
- Purification of Yebf and IL-10 -failed

22/4/18

- PCR of Yebf and IL-10 and then purify them-succeed
- Overlap PCR of Yebf-IL-10 and them purify the product of Overlap PCR

23/4/18

- Colony PCR of PnorV

24/4/18

- Extraction of PnorV from pCDFDuet-1
- Purification of pCDFDuet-1 plasmid and NorV then digest, connect and transform them-failed

26/4/18

- Cultivate E.coli（pNorV - pCDFDuet-1）

27/4/18

- Extraction of PnorV from pCDFDuet-1
- Purification of pCDFDuet-1 plasmid and NorV then digest, connect and transform them

28/4/18

- Connecting the NorR and Pcdf(PnorV) and then transformation, the E.coli failed to grow

29/4/18

- Connecting of NorR and pCDF(PnorV) and then perform the electrophoresis
- Extracting NorRand pNorV from the genome of K12

30/4/18

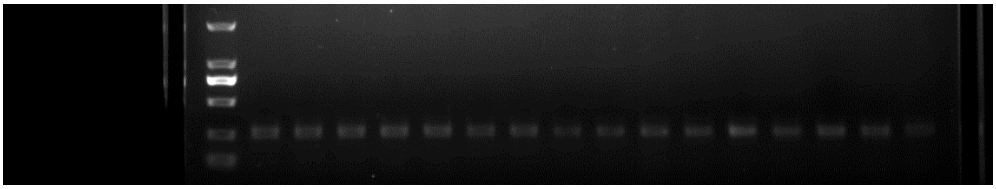
- Preparing experimental materials
- Alcohol precipitation and shaking preparation were carried out to prepare the plasmid the next day.

1/5/18

- Extraction of plasmid
- Digestion and then gel electrophoresis
- PCR purification of genome and use PS and MM for PCR
- PCR purification and digestion
- Digestion of plasmid and gel purification
- PCR purification of PCR product and then connecting and transformation

2/5/18

- Connecting the pNorV and plasmid and then conduct and colony PCR



- Cultivate the E.coli to prepare for plasmid extraction

4/5/18

- Digestion: the size of plasmid was right but the promotor was missing.
- Send three of plasmid to sequencing

6/5/18

- Result of sequencing :2 of them have a gap in 100%; the other one has a gap in99%

7/5/18

- Digestion of Plasmid No.3 and purified NorR
- Connection and transformation

8/6/18

- Colony PCR
- Cultivate the E.coli

9/5/18

- Extraction of NorR-pCDF.
- Digestion and sequencing

11/5/18

- PCR of Luciferase
- Purification of PCR product

12/5/18

- Digestion of pCDF and luciferase
- Connection and transformation
- Trasformation

13/5/18

- Colony PCR of pCDF-luciferase
- Cultivate the E. coli and DH5α

- Extraction of plasmid
- Digestion –only three of the results shows bands at 1600bp

14/5/18
- Extraction of plasmid and digestion

16/5/18
- Amplification of myrosinase from the plasmid synthesized from the company

27/5/18
- The gel purification, digestion of myrosinase
- Digestion, connection and transformation of pet-28a

28/5/18
- Colony PCR of myrosinase

29/5/18
- Extraction of plasmid containing myrosinase and digestion to verify

4/6/18
- Extraction of plasmid pCDF-PnorV-NorR

6/6/18
- PCR of YebF-mIL10-Flag
- Purification of PCR product
- Digestion and connection then transformation

7/6/18
- Transformation of YebF-mIL10-Flag

8/6/18
- Colony PCR of YebF-mIL10-Flag
- Cultivate the E.coli

9/6/18
- Extraction of plasmid
- Digestion-failed
- Colony PCR-failed

11/6/18
- Overlap-PCR of purified yebf and IL-10

12/6/18
- Digestion
- Purification
- Connection and transformation

14/6/18
- Colony PCR

18/7/18-10/8/18：

- Construction of plasmid：
  YebF+mIL10（PCDF-Duet1）
  mIL10 （PCDF-Duet1）
  YebF+hIL10（PCDF-Duet1）
  hIL10（PCDF-Duet1）
  Luciferase（PCDF-Duet1）
- Expression of myrosinase

20/8/18

- preparing for the experimental materials ,transformation of BL21

21/8/18

- Colony PCR

22/8/18

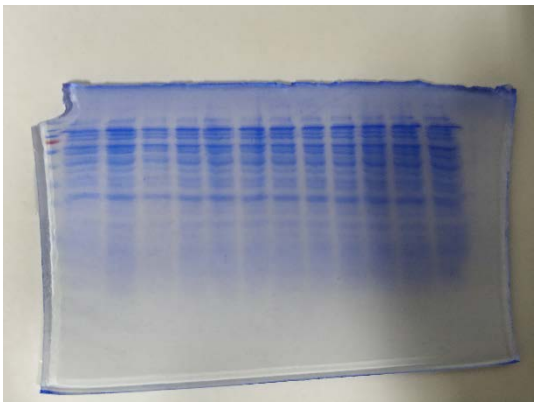
- Cultivate the E.coli

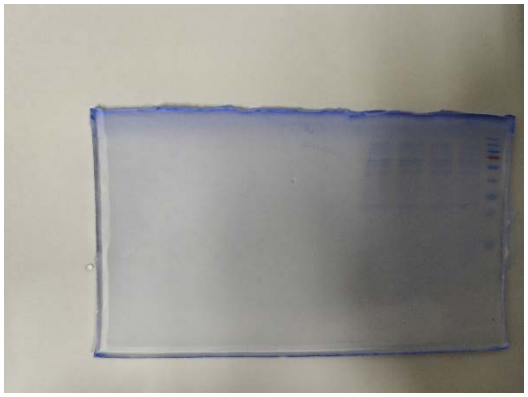
23/8/12

- Dilute the bacterial solution to an OD of 0.2 and incubate at 37 ° C to an OD of 0.5.
- IPTG gradient induction, 0 0.25 0.5 1 mg/ml, 30 ° C (with ice pack) for 4 hours and 16 hours.
- For the four-hour batch, 1 ml of the culture supernatant was collected, and the supernatant was collected by ultrasonication, and the SDS-PAGE was run, and then stained with Coomassie brilliant blue.

24/8/18

- After 16 hours of bacteria collection, 1 ml of the culture supernatant was collected, and the supernatant was collected by ultrasonication, and the sds-page was run, and then stained with Coomassie brilliant blue.





25/8/18-30/8/18

- Repeat the expression expriment

8/9/18

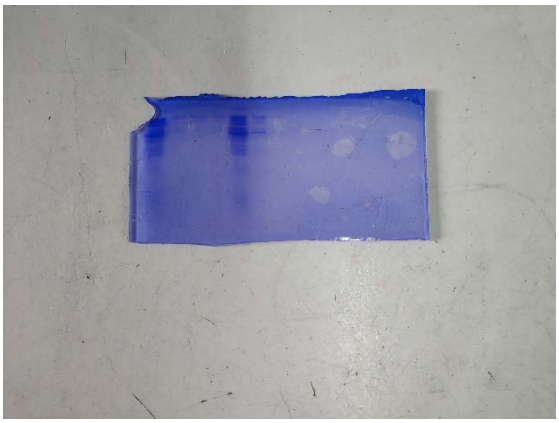
- Transformation of E.coli by Blue/RFP/GFP/PyeaR from the kit

3/9/18-7/9/18

- Repeat the expression experiment but with some changes during the inducement
- 50 ml of bacterial culture, after 16 hours of induction, collect all supernatants, centrifuge at 3000 rpm for 5 min, freeze-dry

9/9/18

- Result of Sds-page



- Take the freeze-dried product and dissolve it with 2 ml of water to conduct Western Blot
- Colony PCR; cultivate E.coli for a night

10/9/18

- Result
- Plasmid extraction

11/9/18

- Transformation of E.coli by Blue/RFP/GFP/PyeaR

12/9/18

- Plasmid construction of the original NO line, connect the promoter and RFP/blue pigment protein together by overlapping;
- Take 1ul of RFP and blue plasmid from kit for PCR and take NO plasmid for PCR.

14/9/18

- PCR of NO，RFP and Blue

15/9/18

- Re-do the connection transformation

16/9/18

- Colony PCR of RFP

17/9/18

- Amplify the plasmid by cultivate E.coli

18/9/18

- Succeed in the expression of Blue

23/8/18-18/9/18:

- construction of plasmid：
  maz-F (pCold I)
  Lysis (pCold I)
  maz-E (pet28a)
  GFP (pet28a)

- Expression of Myrosinase:
- Kill switch inducement in the plate (Characterization):
- Expression of maz-F、Lysis:

22/9/18

- Transformation of plasmid come form ShangHai Jiao Tong university
- PCR of in-fusioned PyeaR plasmid

23/9/18

- In-fusion of PyeaR-Blue
- Cultivate the E.coli containing PyeaR

24/9/18

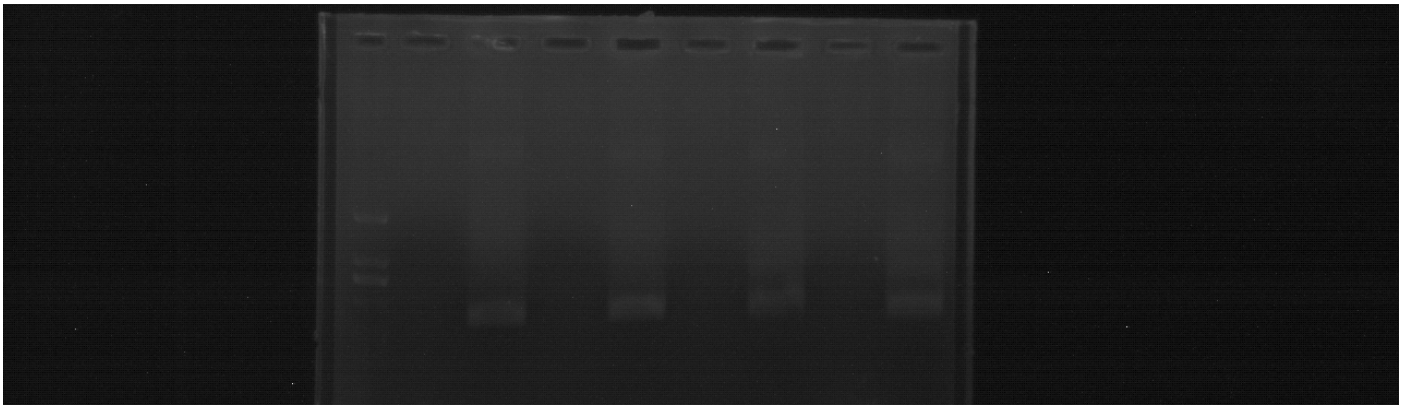
- succeed in expression of PyeaR-GFP

25/9/18

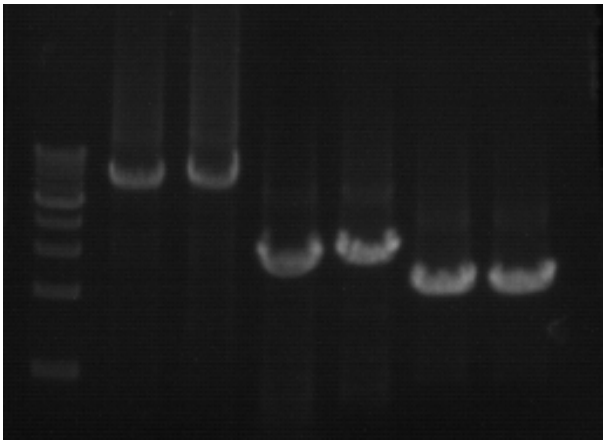
- Insert pyear-blue and compare that with GFP

1/10/18

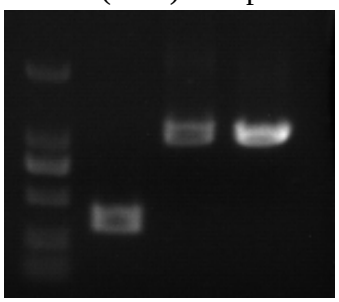
- Two types of yebf plasmid backbones were used for PCR, and two types of yebf were each purified



The figure above shows the plasmid skeleton of two types of yebf, yebfO-syebfO



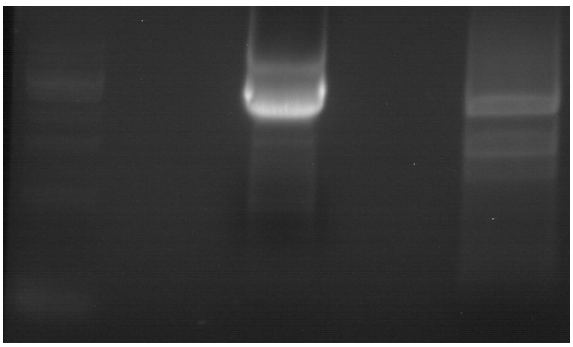
The figure above is TF (GFP) & mp-YG & mp-YB & mp



The figure above is shows the success of Overlap-PCR
Restriction enzyme digestion and ligation of the yebf series, pyear-GFP mutation

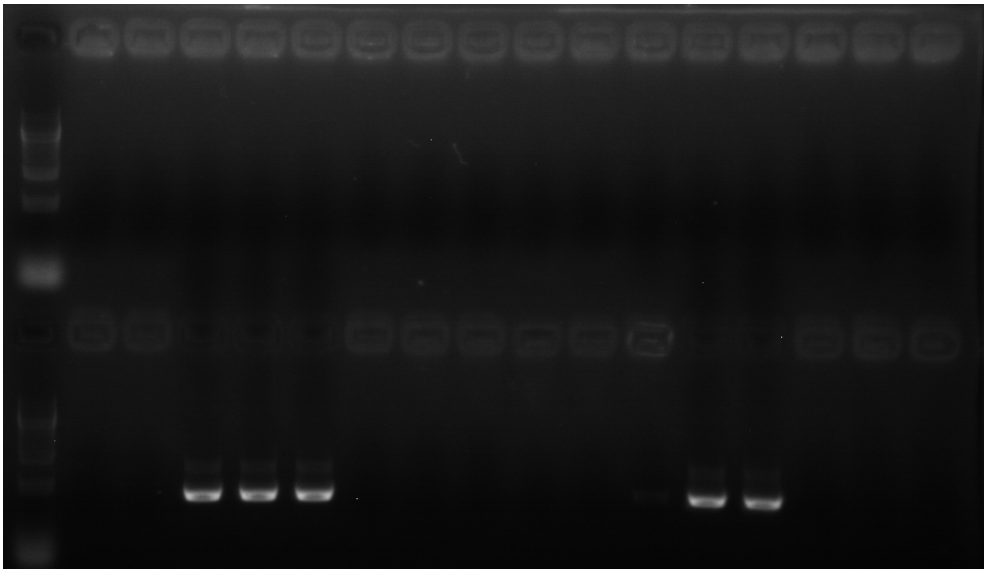
3/10/18

- DH5α failed to grow，so we decided to use BL21 for transformation
- PCR by MM and PS



4/10/18

- Succeed in growing



Transformation of Yebf（with RBS mutation and TF）

5/10/18

- The first row is yebf-GFP
- The second row is Syebf-GFP, the last 8 is pyear-blue
- The third row is pyear-blue
- Yebf-GFP: take No.11/12/13 to cultivate for 20h at 30°
- Syebf-GFP: take No.4/5/6 to cultivate for 20h at 30°
- Pyear-blue: take No.17 to cultivate for 20h at 30°

20/9/18-6/10/18:

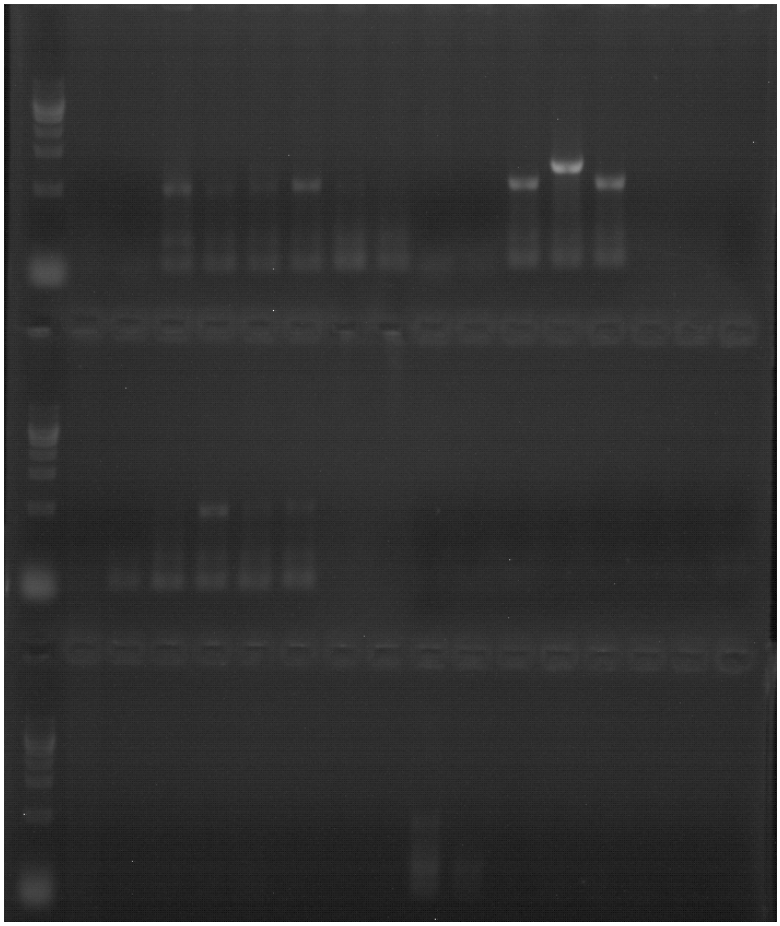
- Construction of plasmid：

  maz-F (pet28a)
  Lysis (pet28a)

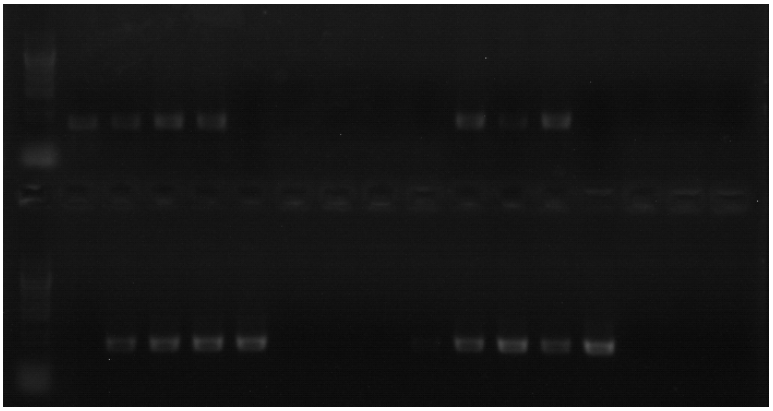
- Expression of Myrosinase：

- Kill switch inducement in the plate：

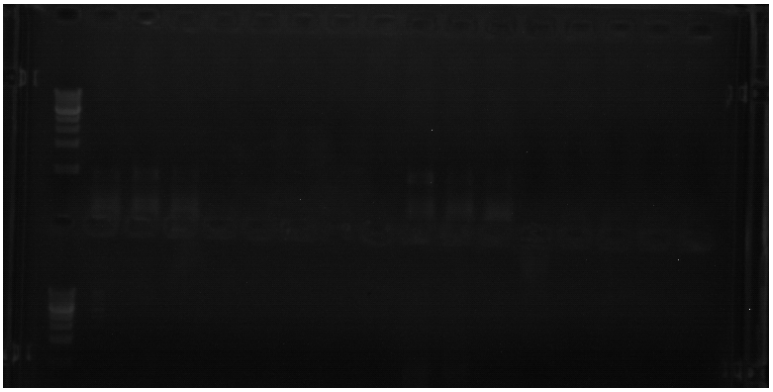
  kill switch :Growth curve



The figure below is TF-GFP



The figure below is PyeaR-Blue



6/10/18

- YG11 has fluorescence

8/10/18

- Colony PCR of YG11

9/10/18-11/10/18

- Yebf series and pyear-blue:PCR-PCR Purification-digestion-PCR Purification-connection-transformation-colony PCR-cultivate at 37℃ for 12h

12/10/18

- pyear-blue:succeed
- yebf:failed

12/10/18-14/10/18

- Repeat the experiments of Yebf: failed