Lab Diary

仇真 （ QIU Zhen ）

7.5-7.9

Try and make competence cells using $CaCl_2$ method. BL21(DE3) Δ sulA Δ lexA used as materials were streaked out from a former plate. The protocol used is from MOLECULAR COLONING: A LABORATORY MANUAL (the 3rd edition).

7.9-7.15

- Extra glycerol stocks of BL21(DE3) Δ sulA Δ lexA competence cells were made and stored at -80℃.

- New TASK received: transform yeasts as a luminophor.

  We Learned about YEAST genetics and selected a yeast strain (BY4741) and a vector (PYES2, promega) for transfromation. Protocols and formulations were offered by Nancy Liu. She is so nice and permitted us finishing transformation in her laboratory.

  Essential cell media for both E.coli and yeast were prepared, together with other solutions for yeast transformation.

7.15-7.26

- Renilla luciferase (Rluc for short) was got from J52008, an exsited part. The part was double digested with EcoRI and SpeI, meanwhile PYES2 was double digested using EcoRI and XbaI.
- Both products were taken to run gel electrophoresis. We chose 1KB ladder at first to get no distinctive marker stripes. It turned out the same when we took a second trial.In contrast, our sample's bands were clear.

  Then we took on gel purification. The products were used for overnight ligation, followed by transformation and mini prep. No positive sequencing results returned. We failed to cut off the Rluc sequence for using mismatched enzymes and buffers. We realized this after some other people had done.

- We started a new turn and sent our PYES2 with Rluc for sequencing. Three samples tested were all exactly the sequence in partregistry page.

7.27

Yeast transformation and TINA's birthday.

7.28-7.29

Targeted our substrates and decided practical conditions for expression and light emission.

7.30-7.31

We Visited OUC, discussed current project related puzzles and exchanged ideas on synthetic biology.

Great fun the outdoor baffet!

8.4-8.10

- In glass tube tests, a fittest condition for T7-Luxbrick expression in BL21 (DE3) was obtained. A suggested IPTG concerntration at last is 0.05 Mm at 30℃(the shaker open to let more fresh air in). Cell cultures became brighter in colder environment.
- Experiment carried out in larger scale failed twice the following days.
- New Task Received: Orthogonal Tests;

  Collecting plasmids;

  Making a list of following experiments;

8.11-8.19

- Transformation:

  In the beginning, BL21(DE3)ΔsulAΔlexA as hosts were transformed with wild type promoters, mutant 408 family promoters, or co-transformed with lexA408-VVD and 408 family promoters. The following day I streaked out a single colony onto new plates；

  Transforming these plasmids into trans5a successfully;

  Transforming these plasmids into BL21(de3) to get no positive results;

  In despair, I took another try and got it.

8.20-8.29

- streaking and cultivating cells on plates;
- streaking, or writing words recording cultivating conditions on plate;
- placing cells to grow 30℃,light exposure or dark state;
- due to phage infection, on most plates no colony could be seen.

9.1-9.5

I repeated former experiments and carried on. Meanwhile inoculate cells into 48 well plates, conditionally. The liquid sample was then transferred (centrifuged and resuspended in PBS) into microplate for fluorescent detection.