# Week 5

Day 19, 20, 21: PROBLEM SOLVING

- 4*250ml LA
- 4*10ml Antibiotic Plates PLATED

Day 22: CULTURING

- Promoter and RBS grown in Amp media test tube.
- 5 Biobricks grown in Chloramphenicol media test tube
- Promoter in clump give pink colony……..

Day 23, 24: SEARCHING

- NOx and SOx preparation methods
- Detection methods of above.
- How NYMU solved cysI problem
- Pink color in promoters is due to RFP downstream to promoter sequence.

# Week 6

Day 25: STREAKING

- Streaked 7 plates and Incubated for 16 hours at 37 degree C
- Taped by Paraffin next day and store at 4 degree C

Day 26: BLUNDER and CULTURING

- Added wrong buffer so all culture wasted.
- One more thing we came to know our promoter itself contain RBS.
- Again cultured all the 5 biobricks and promoter.

DAY 27: PLASMID ISOLATION by QIAGEN KIT

DAY 28:

- we were ready to do Digestion and Ligation but the linearized plasmid backbone was not in sufficient quantity
- So TRANSFORMED all the four plasmids to multiply their no.

# Week 7

DAY 29: DIGESTION + LIGATION

- No growth observed on any plate because “Linearized Plasmids do not undergo transformation.
- So finally decided to do 3A Assembly {digestion +ligation} from whatever available and then transform them to multiply. Once we got cells then their Plasmid Isolation to get linearized plasmid back.
- Less promoter was added to NOx digestion process.

DAY 30: TRANSFORMATION

- Transformation of NOx and Sox cells to get plasmids growth.

DAY 31:

- Found no growth on NOx plate and 2 small colonies on SOx plate
- We ruined our plasmid backbones.

DAY 32:

- Decided to SEARCH bio bricks having plasmids resistance of Kanamycin and Tetracycline and to transform them to get Required Backbones.
- Transformation of 2 bio bricks.

# Week 8

DAY 33:

- Growth in K tube and growth in K PLATES.
- So decided to run Transformation for T again.

DAY 34:

- We came to one conclusion that might be the plate is wrong.
- So decided to check by streaking 4 different antibiotic resistant strands.

DAY 35:

- CAT and TET growth was observed and no growth for Amp and Ken.
- So decided to streak the colonies on CAT and TET plates.
- 4 Test tubes and 1 plate with Quadra sections of 4 different colonies {1 was red suspected to be the Clone!}