iGEM IIT DELHI 2014 NOTEBOOK

Week 1:

Day 1: SAFETY AND BASIC LABORATORY TRAINING

- Learned how to manage glass wares before starting the experiment.
- Operation of weighing balance machine properly.
- Preparation of Luria broth and Luria agar.
- Learned the measures to keep in mind while we are doing the autoclave.
- How to operate autoclave.
- Plating of Luria agar.
- Fixing by paraffin tape to avoid contamination.
- E.coli K12 itself contains nrfA. So in absence of biobrick (as we didn't receive the biobrick yet) of nrfA, we planned of doing PCR of nrfA gene. For that we Transferred E.coli strain K12 in 100ml Luria broth via loop or pipette tip and left it in the shaker/incubator for 6-7 hours.
- Streaked the above culture on a new agar plate and left overnight to grow.(master plate prepared)

Day 2: PELLETING

- Strain taken and transferred to a test tube (5ml.) and incubated at 100 rpm for 4 hours.
- Centrifuged to get pellets
- GENOMIC DNA EXTRACTION

Day 3: GENOMIC DNA EXTRACTION

- ELECTROPHORESIS

Day 4: AGAROSE GEL ELECTROPHORESIS

- COMP CELL PREPARATION

Week 2:

Day 5: COMPETENT CELL PREPARATION

- 3A ASSEMBLY
- COMPONENTS OF KIT
- BIOBRICK IMPLIMENTATION
- HELP (iGEM) CHECK FOR METHODS AND PROTOCOLS

Day 6: Efficiency of comp cell

Day 7, 8, 9: BUFFER PREPARATIONS

- 13 buffers of various types prepared

Week 3

Day 10, 11, 12: MEDIA PREPARATION

- 8*200ml LB

- 4*250ml LA 4*10ml PLATING(Antibiotics: Amp, Kana , Cat , Tet)
- 30*5ml LB Test Tubes

Day 13, 14: PROMOTER and RBS searching

- Searched best Constitutive Promoter and RBS
- Their plasmid backbone studied

Week 4

Day 15: PLANNED on 3A assembly.

- Different sub steps to be taken in 3A assembly.
- Small flow chart made.

Day 16: TRANSFORMATION

- Transformed RBS and Promoter in the comp. cells
- Spreading on Amp. Plates
- we tried to grow Nrf A to grow in LB test tube
- Cys I has restriction sites HOW TO SOLVE?

Day 17: CHECKING

- **********FAILURE**********
- No growth on the Amp plates {where it was supposed to be}
- Growth in the LB media{only for check that transformation took place}

Day 18: STREAKING on Amp PLATES.

- Growth is observed so Transformation was efficient enough.
- CONCLUSION: Either naming of Antibiotic Plates was wrong or some Plating problem.