Week 11

4 July:

- Prepared Plasmid Miniprep for NOx and SOx.
- GEL RUN for plasmids of NOx and SOx was prepared in noon.

- **Sequence:**

S0x	N0x

In gel electrophoresis, the band of Sox was detected but band of N0x was not detected.
This may be due to error during plasmid prep. Therefore re-inoculated again.

5 July:

- Growth was observed in tube of NOx of previous day.
- Plasmid isolation of NOx was done
- Gel run for NOx done

GEL RUN RESULT:
No band for NOx. ☹
Digestion of SOx was done to check correctness of 3A assembly.
Overnight digestion was carried out at 37^0C

6 July:

GEL run electrophoresis was done for checking digestion of S0x.
RESULT: Only one band was obtained. Moreover the band was of plasmid.

- 10 plates of Kana were prepared.
- Promoter, NrfA and SQR were inoculated
- Incubated for overnight growth at 37^0C in shaker.

7 July:

Plasmid isolation was done and elution was carried out in 1.5 MCD
Result:

- Elution was very less for NOx & SOx (less than 20 ul)
- Promoter was only 45.

We observed that eluted product was always less than elution buffer, mainly because of absorption taking place.
Gel run was done again (Order: Promoter, NrfA and SQR)
A significant concentration was observed.

3A assembly method was performed, however it did not yield results again.
4 tubes were inoculated, 2 for promoter, 1 for SQR and 1 for NrfA

8 July:

Plasmid isolation was done again. Elution in 1.5 MCD. Eluted volume for promoter was about 70 ul while for nrfA and Sox was 40 ul.

To check proper functioning of enzymes EcoR1 {E}, Pst1 (P), Xba1(X) and Spe1(S), digestion was carried out using them.

9 July:

- The gel run showed that except Spe1 remaining 3 enzymes were working well
- Used new working sample of Spe1

10 July:

Digestion carried out

Ingredient	Promoter(Pn)	NrfA(N)	Promoter(sP)	SQR(S)
Water	23	23	23	23
NEB Buffer 2.1	5	5	5	5
DNA	20	20	20	20
Ecor1	1	0	1	0
Pst1	0	1	0	1
Spe1	1	0	1	0
Xba1	0	1	0	1

- Digestion left for 90^0C min at 37 followed by 20 min at 80^0C in PCR program.