Week12

11 July:

Gel run was done to check digestion

Morning:

Sequence: Pn, sP, N and S

Result:

- Single Bands of promoters around 3kb.
- 4 bands in case of N and S.

Conclusion:
Maybe due to incomplete digestion, therefore left it to digest till night.

Night:
Sequence: same
Result same
Conclusions:

- Some problem in Mini-prep
- Might be the columns for N and S were interchanged leading to 4 bands of similar lengths.
- Main problem is a 2.0 kb band.
- Might be some concatamers.

The promoter we were using till now **BBa_J23100** which is cloned or supplied in plasmid **BBa_J61002** which is Incompatible with RFC 10

- A new promoter had to be found so research time ON. It's incompatible because it has sites far away from each other.

12 July:

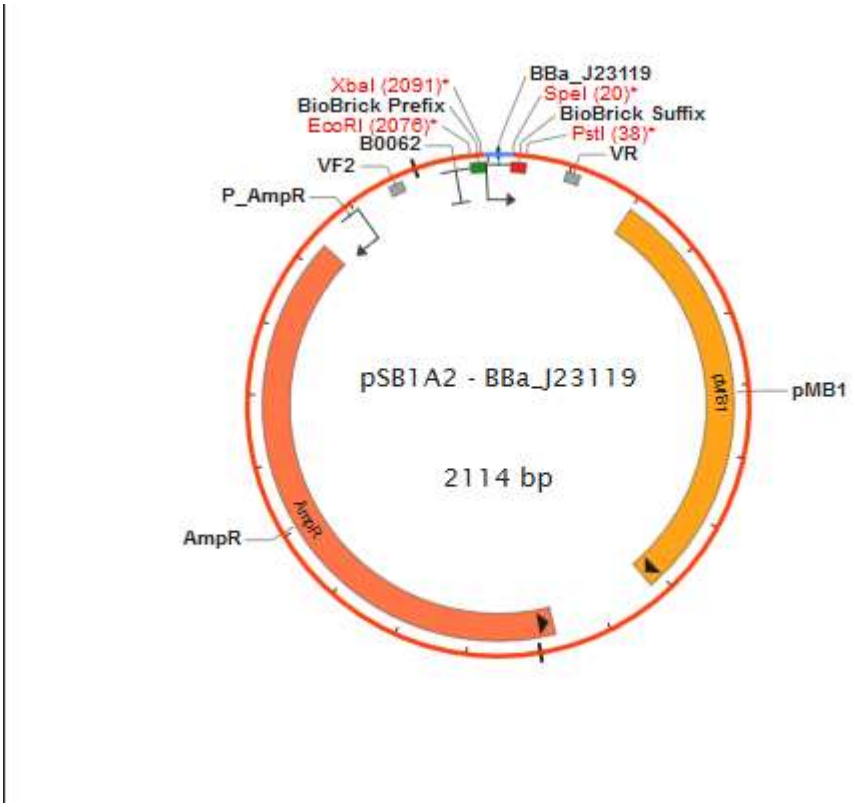
- Transformation of promoter

  BBa_J23119

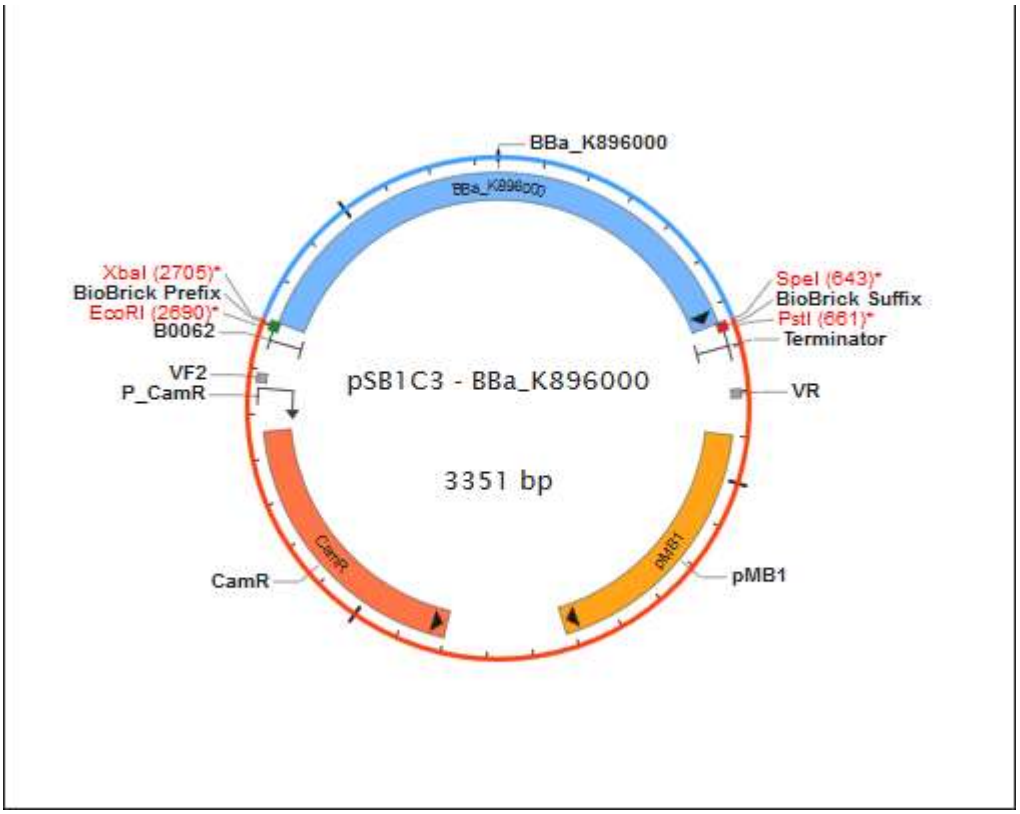
  Present in 2 plasmid backbones

Location	Plasmid backbone	Tube name	Plate name	Antibiotic
Plate4-17B	pSB1A2	1	J23119 Amp 12/7/14	amp
Plate3-17O	pSB1C3	2	J23119 Cat 12/7/14	cat

- Transformation of NrfA and SQR.



Plasmid Structure



SQR Biobrick+plasmid_backbone

14 July:

Digested parts were run on gel run electrophoresis.

- Digestion was left for 90 min at 37 followed by 20 min at 80 degrees in PCR program

July 15

After digestion of part a part b and linearized plasmid.
Ligation of these parts was done following the protocol of 3A assembly

Order:

Name	N0x	S0x
Part A(Promoter)	9.9	9.9
Part B(gene)	NrfA 11.7	SQR 11.7
Plasmid Backbone(Kana)	6	6
T4 DNA Ligase Buffer	3	3
T4 DNA Ligase	1	1

Problem

- Ligase enzyme was found frozen.
- Used another ligase enzyme.

July 17

- Gel run for ligated product.
- In order

Ladder	Ladder	N	S	S

Ladder 4ul
N, S 1ul
Dye 1ul

Expected gel run result

LIGATION band Lengths::
N0x::
Promoter 35+60
NrfA 1437
pSB1K3 2204
total 3.7kb

LIGATION band Lengths::
S0x::
Promoter 35+60
SQR 1281
pSB1K3 2204
Total 3.58kb

Result of gel run:
Very light bands on top