Week23

Plasmid isolation for Cys1 and SQR clone was done.
Our PCR for Cys1 was successful.

PCR for Cys1 was successful
Digestion of SQR Clone and Cys1 by Pst1 was done.
However this PCR was also unsuccessful ☹

Plasmid Prep

- NrfA clone
- NrfA biobrick
- Promoter Biobrick
- SQR Biobrick

PCR was done for Cys1
We observed a Faint band at 1700 bp.

Now we performed a different kind of PCR of Cys1 (50 ul)

We put actual biobrick as well as the previous PCR product as templates.

Standardization of NOX and SQR clone:
SQR and N0x clones were initially prepared in pSb1k3 so we had done
Inoculation and overnight incubation
Plasmid prep.
Overnight Digestion of these clones with Ecor1 and Pst1 was done
Digested part is loaded on 1% agarose and Gel extracted for ensuring correct part
Ligation of these bio-bricks with pSB1C3 (which was prepared earlier for this purpose)
Gel run to confirm the clone.

Week 24

For SOx clone final
SQR clone was inoculated and left for overnight incubation.
Plasmid preparation of this clone is done and overnight digestion with Ecor1 and Pst1.
Digested part is loaded on 1% agarose and Gel extracted for ensuring correct part
Now we got our Part A which is a ligated product of Promoter and SQR gene.

For part B
PCR were run with variations in Buffer
We modified our buffers for proper PCR yield after a series of failures
This time we added DMSO along with buffer and succeeded in PCR.
This followed a series PCR for amplification of Part B and sufficient quantity of Cys1 gene is obtained.
This amplified gene is digested overnight with Xba1 and Nse1 (this time we were right in enzyme selection fortunately).

Digested part is then loaded on 1% agarose and Gel extracted for ensuring correct part

For Plasmid backbone
Inoculation of biobrick having pSB1C3 is done
Plasmid Preparation and Digestion with Ecor1 and Pst1 enzymes. Digested part is loaded on 1% agarose and Gel extracted for ensuring correct part
Now we get final linearized backbone in sufficient amount.

Week 25

For Final S0x clone:
As we had prepared all 3 parts in sufficient quantity as gel extracted product
We performed agarose gel run to cross check our parts.

Ligation for finalized NOx, SQR and S0x clone:
Ligation reaction for attaching part A and part B in the linearized plasmid was made and left overnight.
We performed agarose gel run to cross check our parts.
Transformation of ligated clones were performed and plates were left to be incubated overnight.
Nice growth was found on transformation plates and we took 2 clones of each product (to save time if a false clone occur) and inoculated and left for overnight incubation.
All the 6 clones were also plated while taking them for inoculation for further use.
Plasmid preparation for all the 6 clones was done
We performed Digestion reactions as follows:
N0x and SQR clones were digested by EcoR1 and Pst1.
Whereas 2 different digestion reactions were performed for final S0x clone
Reaction one was with Ecor1 single digestion.
Second Reaction was double digestion with Ecor1 and Pst1.