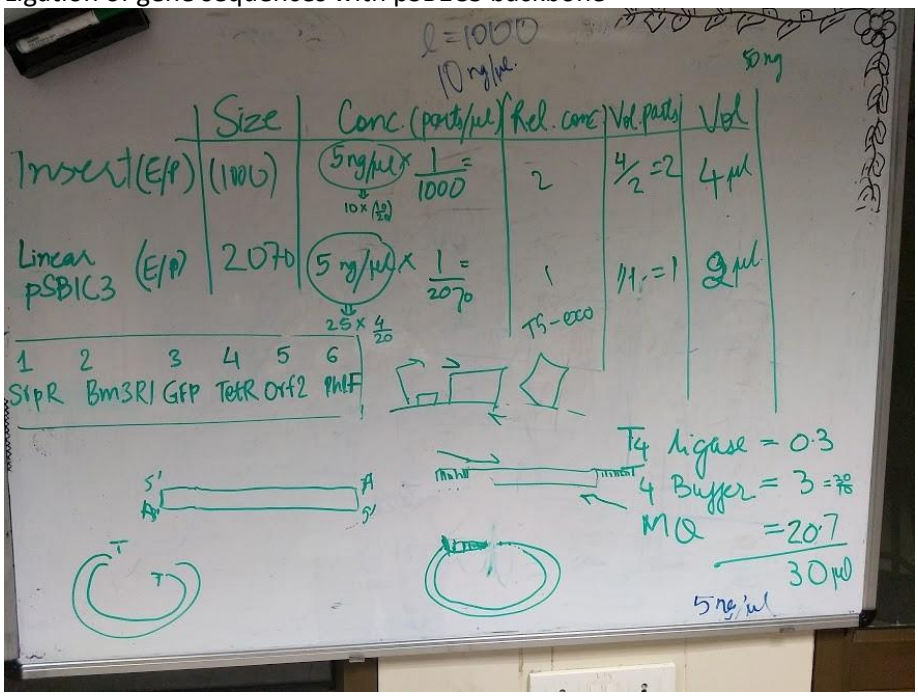
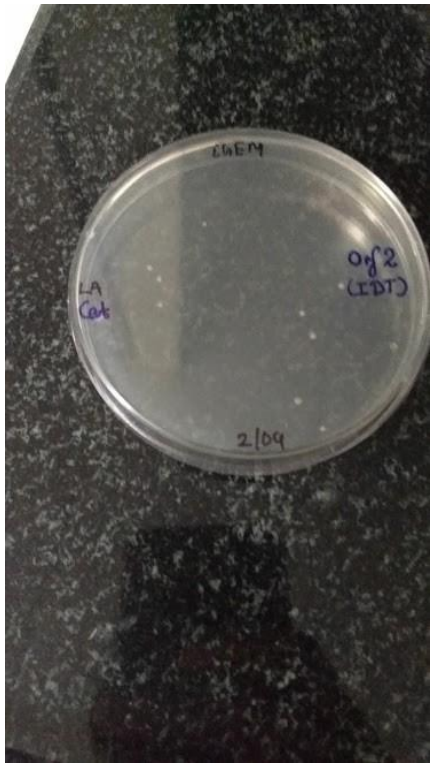
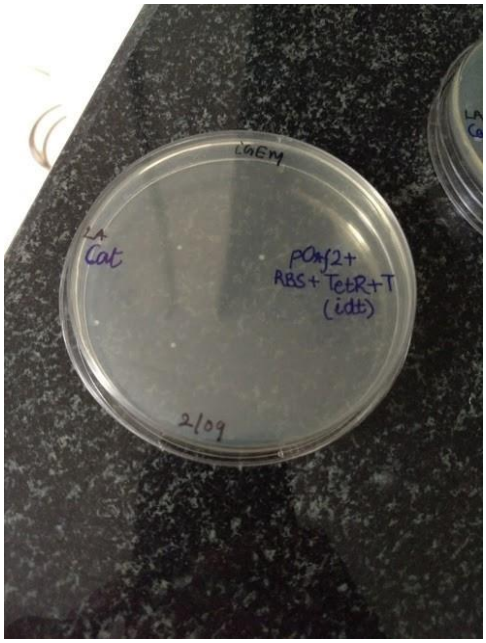
September--
02/09

- Double digestion of linear gene sequences received from IDT(10ul of 10ng/ul) and linearized pSB1C3 backbone (4ul of 25ng/ul)
- Gel electrophoresis of the above mentioned sequences to verify their presence
- Ligation of gene sequences with pSB1C3 backbone
- 

- Transformation of ligated inserts after 3hrs of ligation at 37°C and further overnight ligation

03/09

- Only 2 of the transformed plasmids displayed colonies: Orf2 and TetR
- 

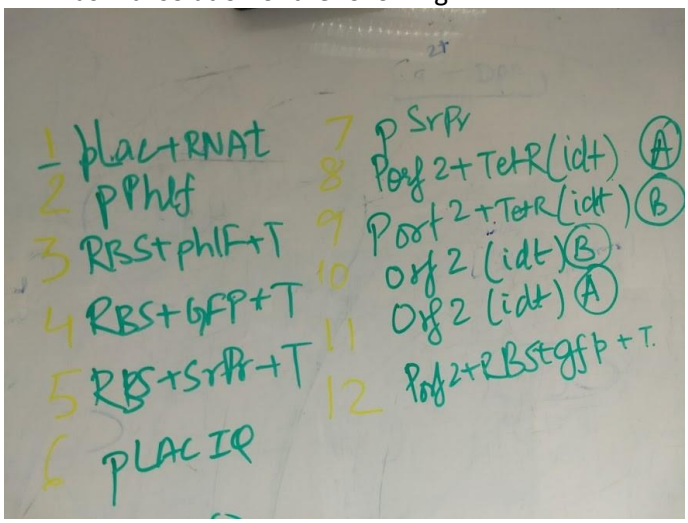


- Autoclave of tips, MCTs and 25ml flasks
- Transformation of parts ligated overnight, i.e. Orf2, GFP, PhlF, SrpR, TetR and BM3R1
- New digestion of all the sequences received from IDT; similar to yesterday
- Ligation of the digested parts with pSB1C3 backbone

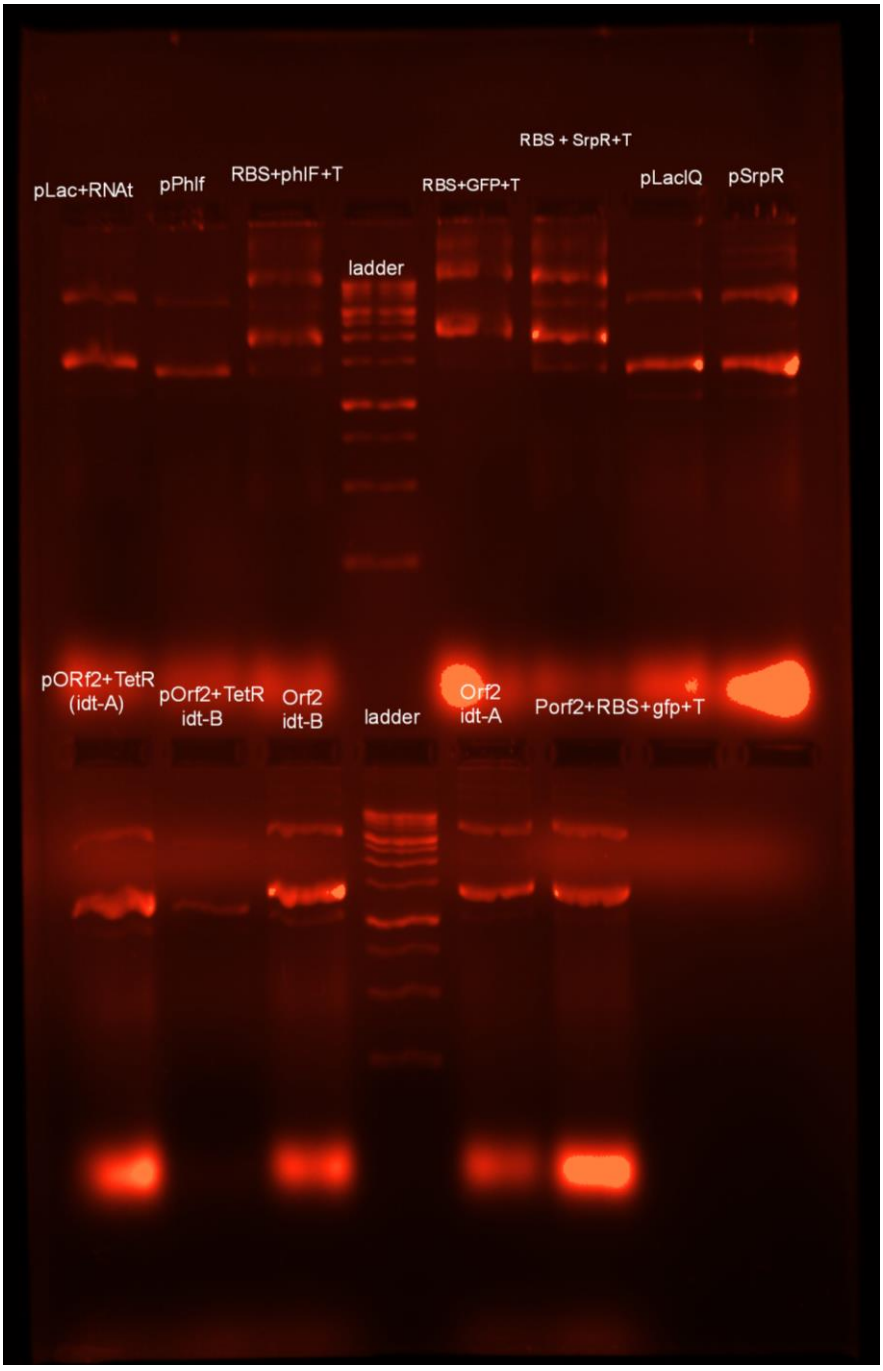
04/09

- 5 of the transformed plasmids displayed growth on plates

- Plasmid isolation of the following

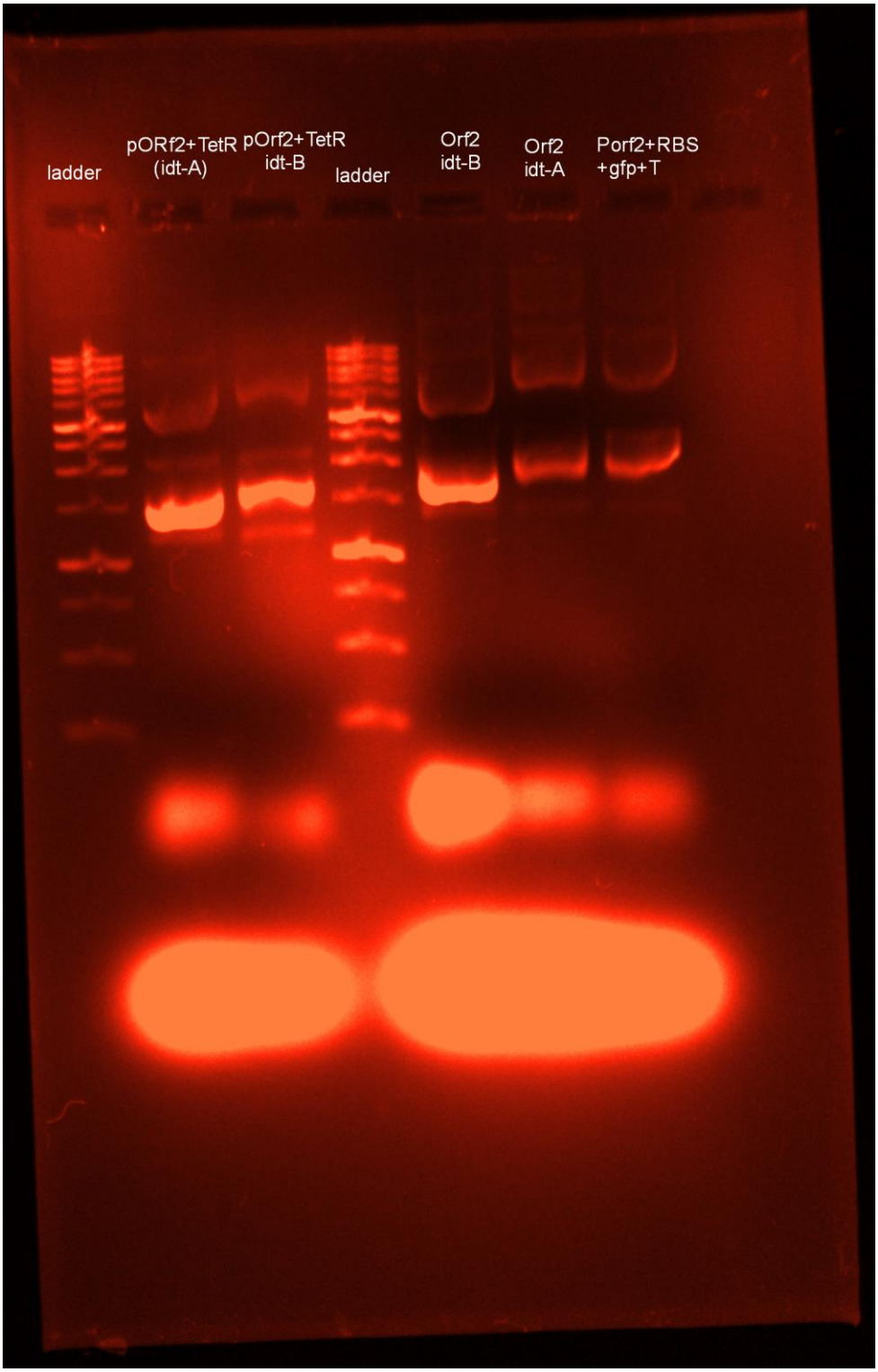


- Gel run of the following:-

- 

05/09

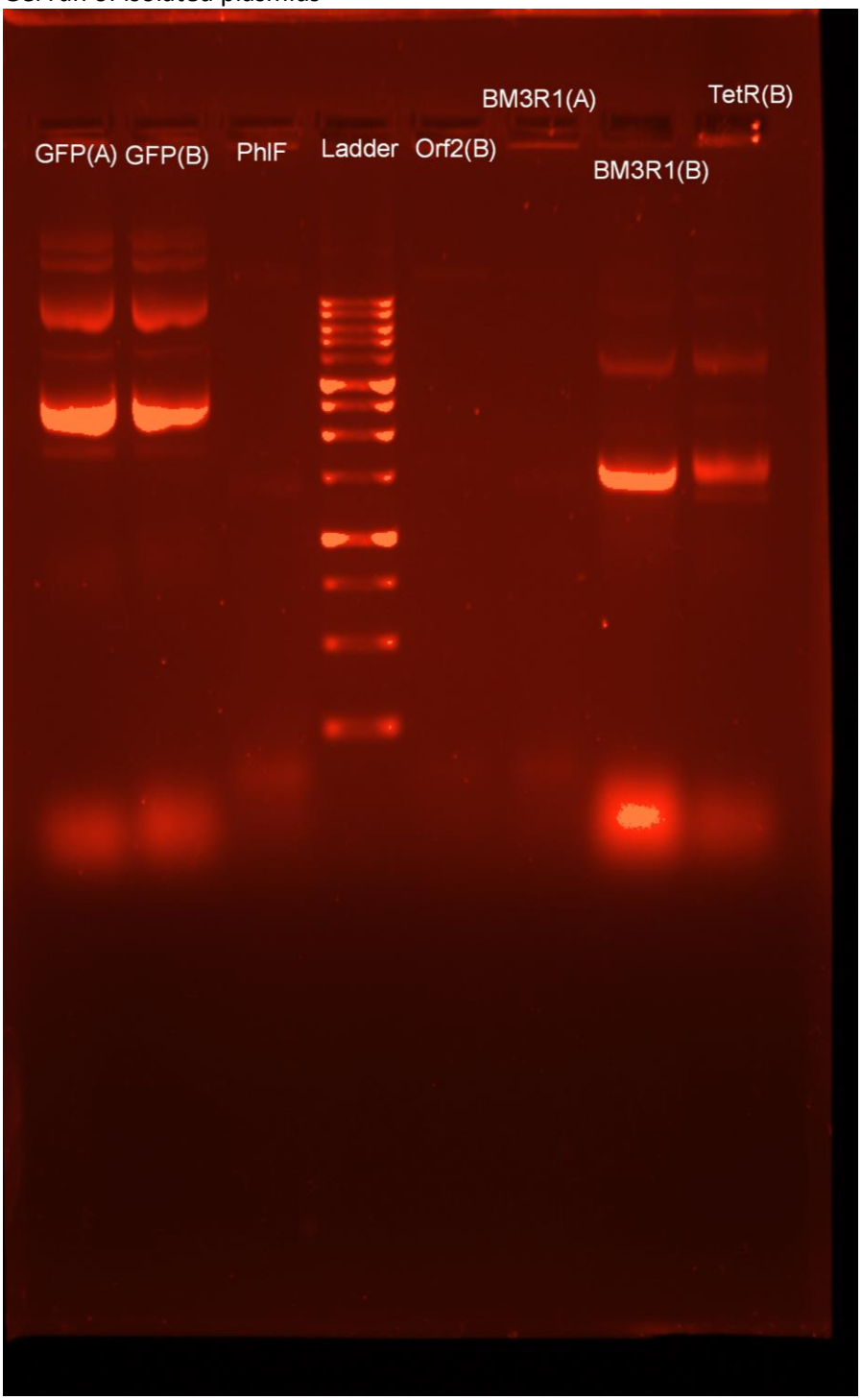
- Single digest of the following :-

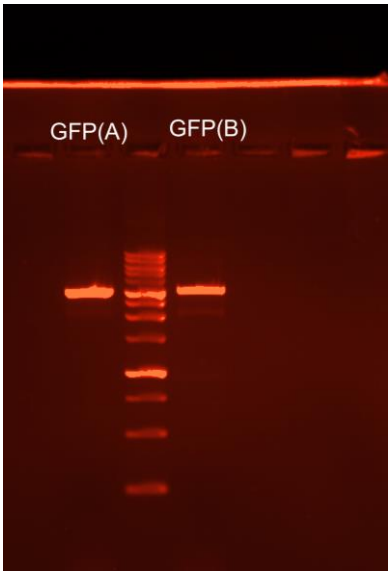


- The result shows that the digestion was not proper as we still observe supercoiled and nicked only
- Observation made that supercoils do not always run to the same length
- Autoclave of LB, LA, MCTs and tips

07/09

- Preparation of plasmid Soln. 1
- Isolation of following plasmids obtained on 04-09
- TetR – A,B; GFP – A,B; PhlF; BM3R1 – A,B; Orf2 – A,B

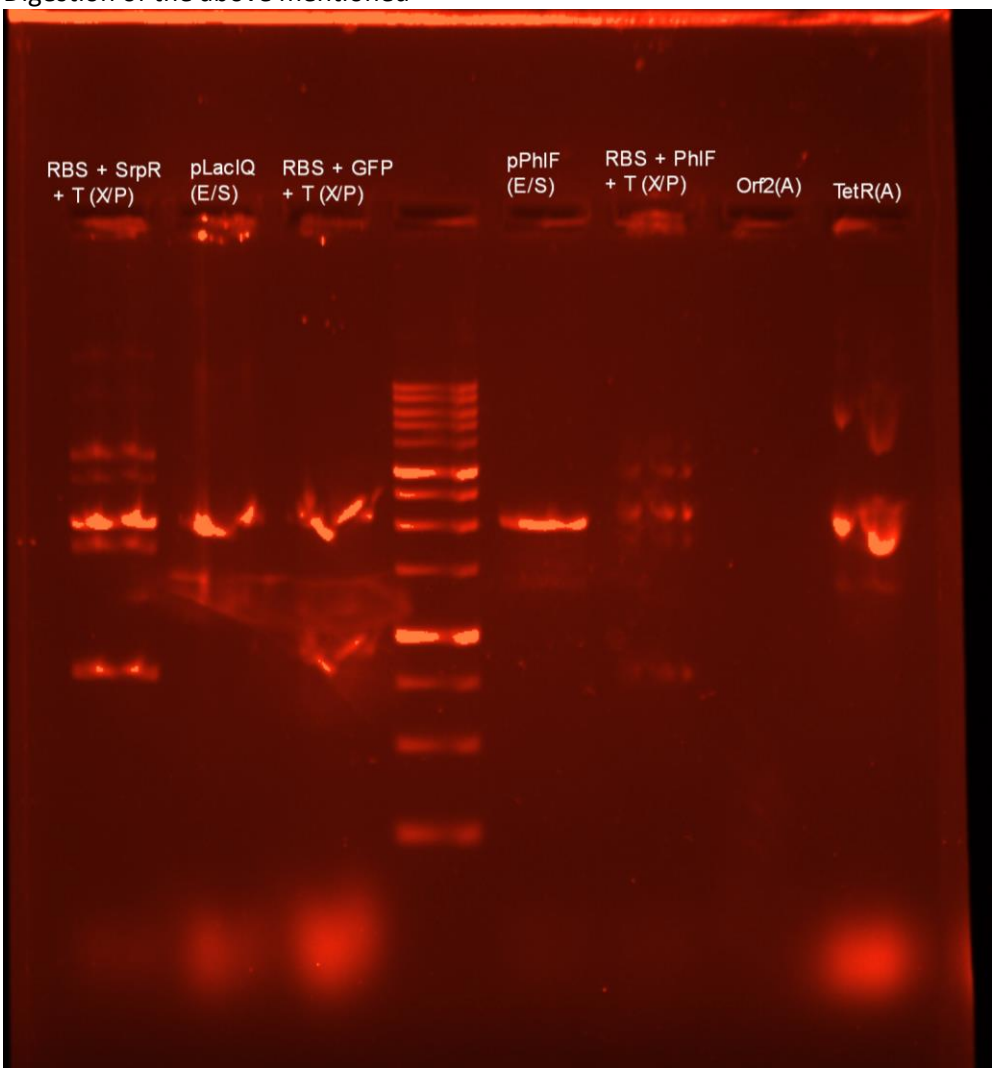
- Inoculation for Interlab
- Double digest of fresh IDT parts with E/P - Orf2,TetR,SrpR,PhlF, BM3R1 and GFP and pSB1C3
- Gel run of isolated plasmids-
- 



- confirms GFP biobrick
- Ligation of IDT parts with backbone by Kshitij

08/09

- Plasmid isolation of pLac, RBS + GFP + T, pPhlF, RBS + SrpR + T (parts received from Glasgow)
- Digestion of the above mentioned



- Ligation of the following with 1:4:4 ratio :- pPhlF + RBS + GFP + T, pLac + RBS + PhlF + T, pLac + RBS + SrpR + T
- Transformation of biobrick parts ligated by KSTR with ratio 2:1 and parts isolated from kits.
- INTERLAB completed successfully by straining our eyes and soul till 2PM on next day.

09/09

- Successful colonies obtained in plates transformed yesterday
- Inoculation of all the parts required for Interlab

10/09

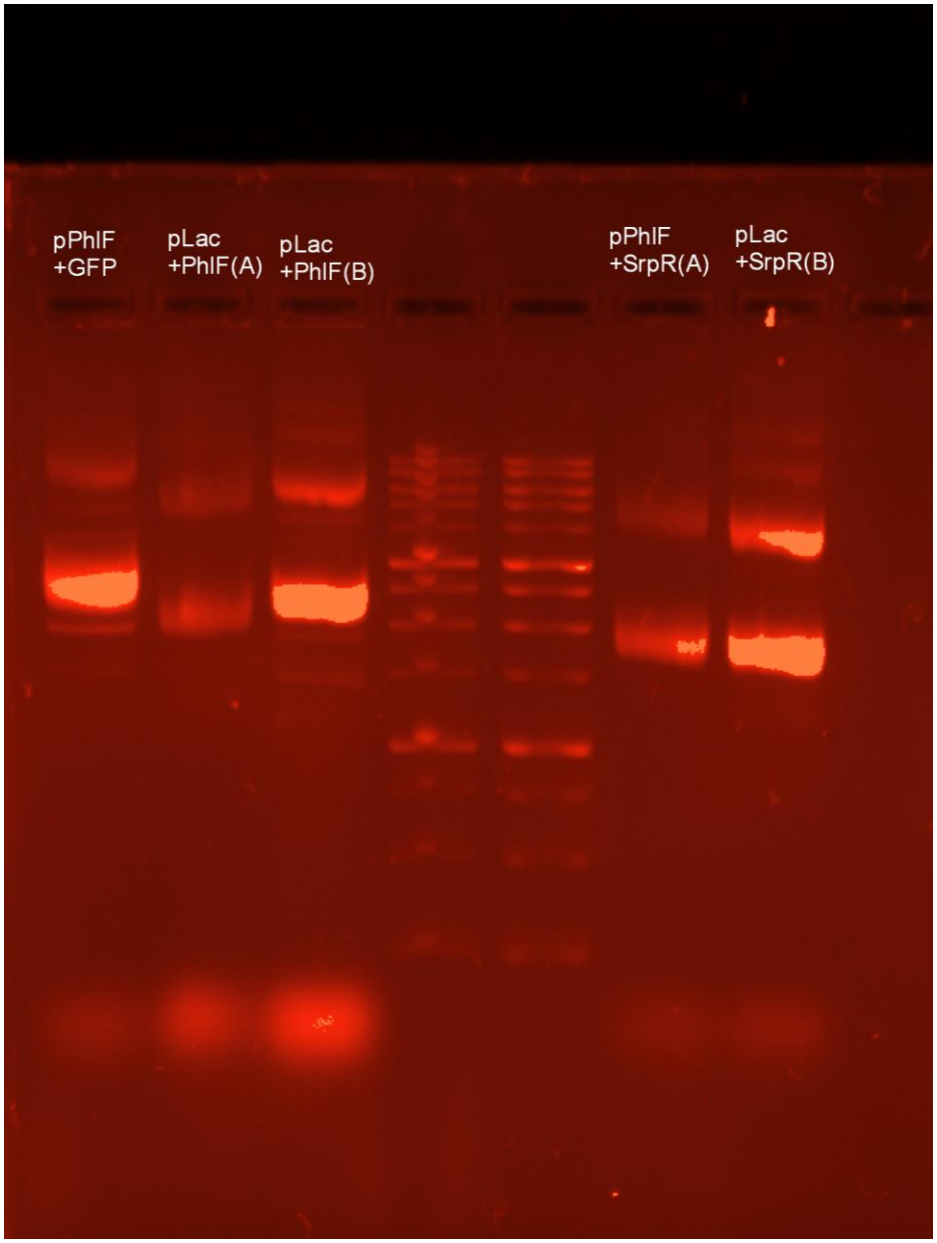
- Transformation of pPhlf + RBS + GFP + T, pLac + RBS + PhlF + T and pLac + RBS + SrpR + T
- Preparation of more than 72 LB tubes and MCTs
- OD measurements of Interlab parts under spectrophotometer
- OD measurement of Interlab parts under plate reader and comparison with spectrophotometer results

11/09

- Colonies obtained successfully in parts transformed yesterday
- Inoculation of ligated parts
- Fresh inoculation of Interlab parts

12/09

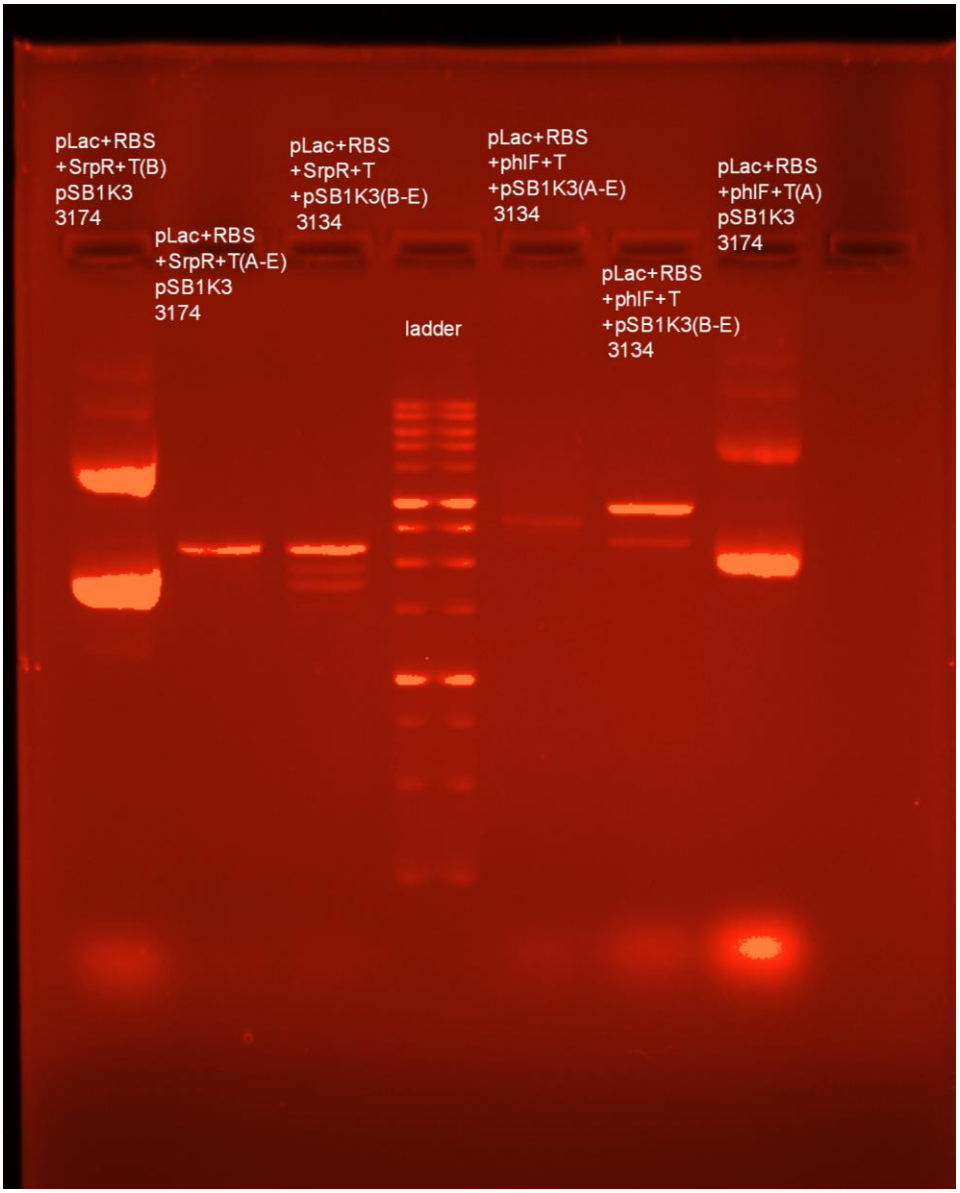
- Gel run of isolated plasmds



- Interlab data obtained for final day and data compiled for all the processes done for interlab

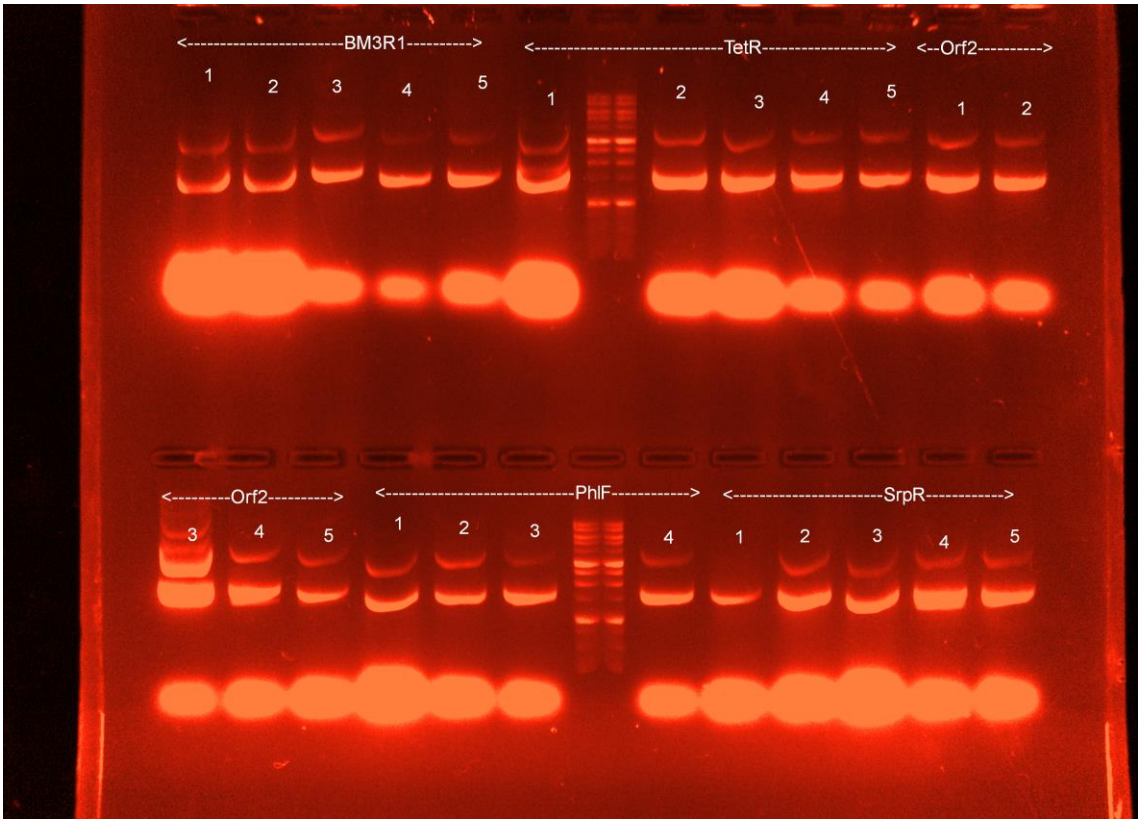
13/09

- Single digest of pLAc + Srpr and pLAc + phlf --- found out to be wrong in gel run

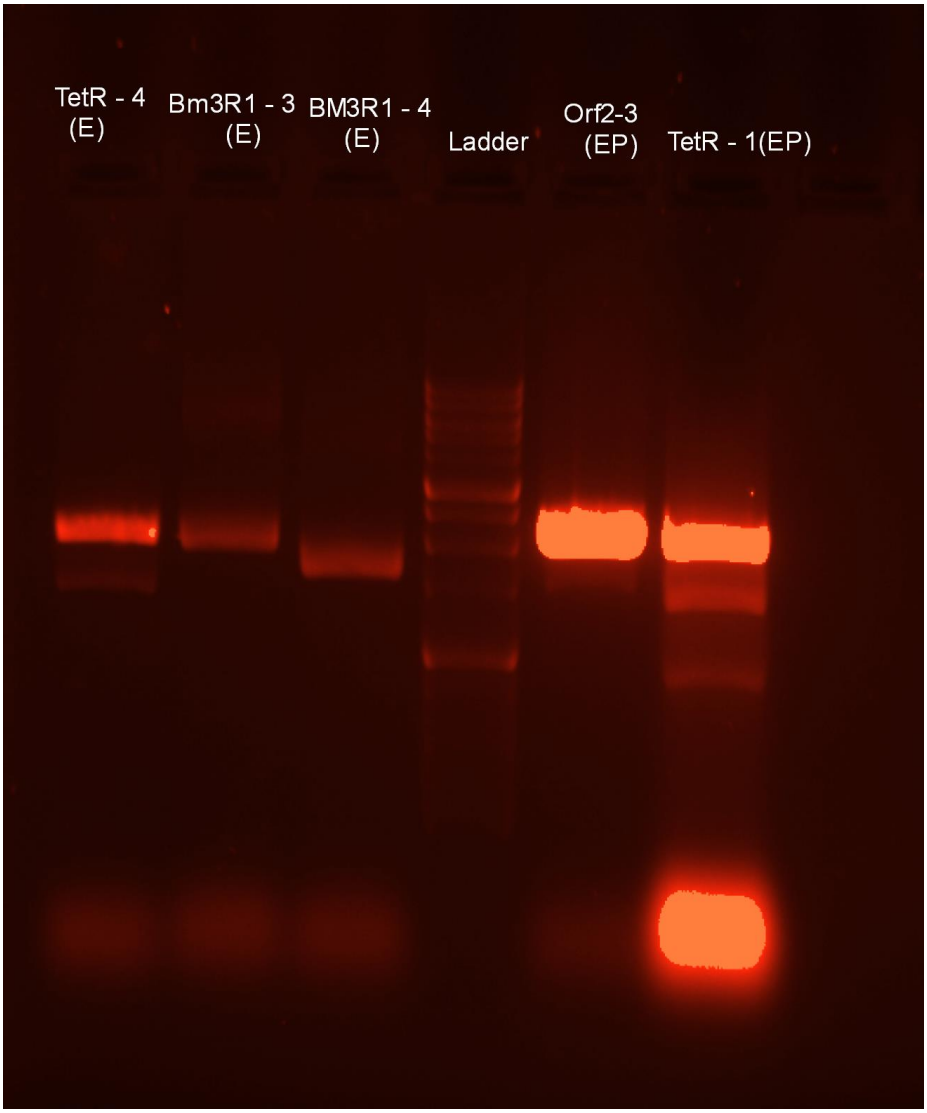
- 

- Pphlf + GFP speculated to be confirmed
- 24 plasmid isolations from idt parts ligated by KSTR

14/09

- 

- Digestion of TetR-4(E); BM3R1-3,4(E); Orf2-3(E/P) and TetR-1(E/P)isolated yesterday



-
- TetR1 found to contain 2 plasmids, rest all were incorrect
- Single digest of pPhlF + GFP to get it confirmed
- Gel Electrophoresis of :-
- 1 – pPhl + RGT (E)
- 2 – RBS + Phlf + T (X/P)
- 4 – pLac (E/S)
- 5 – RBS + SrpR + T (X/P)

15/09

- Inoculation of pOrf2 + GFP
- Double digest of following:-
- PLac, RBS+PhlF+T, RBS+SrpR+T, pSrpR
- Ligation of pLac + SrpR, pLac + PhlF, pSrpR + GFP
- Decision to transform nicked TetR (IDT) to obtain its colonies

16/09

- Colonies received for pOrf2 + GFP and pPhlf + GFP - confirmed biobricks
- Lack of glow in Orf2 + GFP suggests faults in the development of DNA by IDT

17/09

- Inoculations of IDT parts to try and ligate them

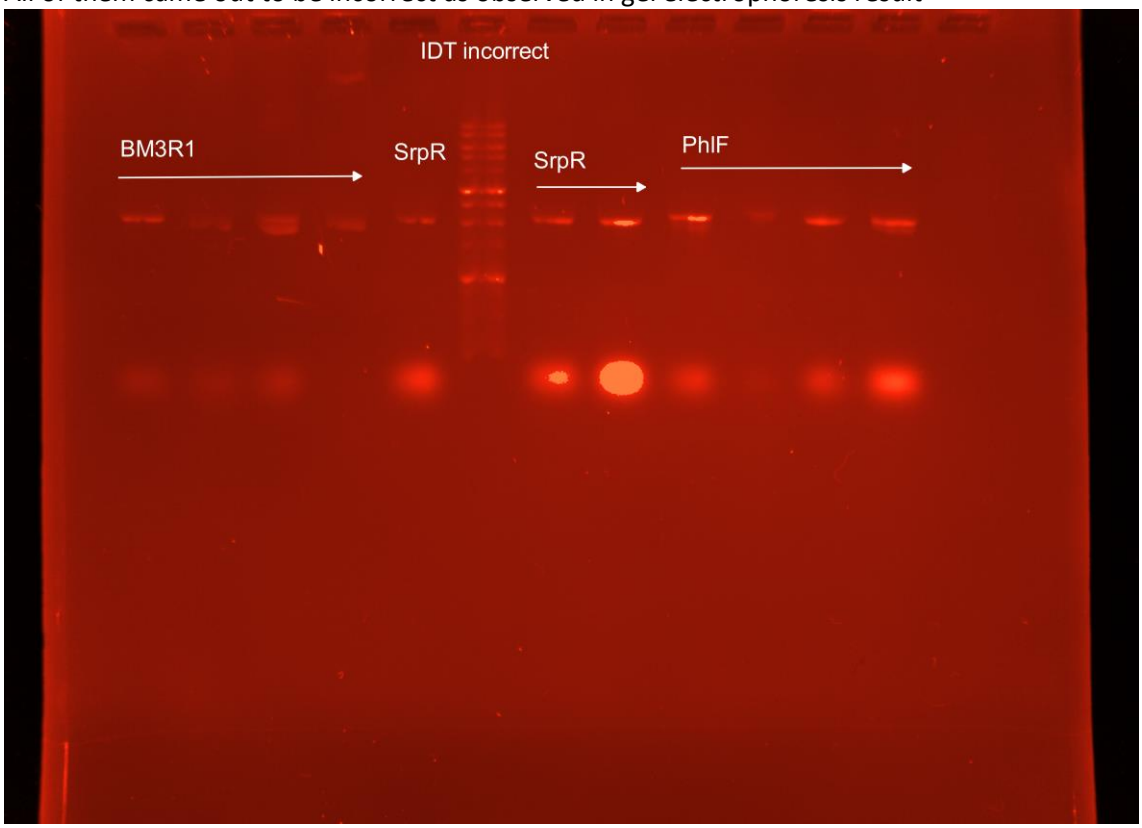
18/09

- Discussion over track selection and further steps and measures to take to overcome the repetitive failures faced in ligating IDT parts

19/09

- 36 Plasmid isolations and their gel run
- 12 selected for digestion after gel electrophoresis

20/09

- All of them came out to be incorrect as observed in gel electrophoresis result
- 

21/09

- Ligation of BM3R1(IDT),SrpR(IDT),PhlF(IDT), and plac+RBS+PhlF+T, pSrPr+RBS+GFP+T, pLac+RBS+PhlF+T

22/09

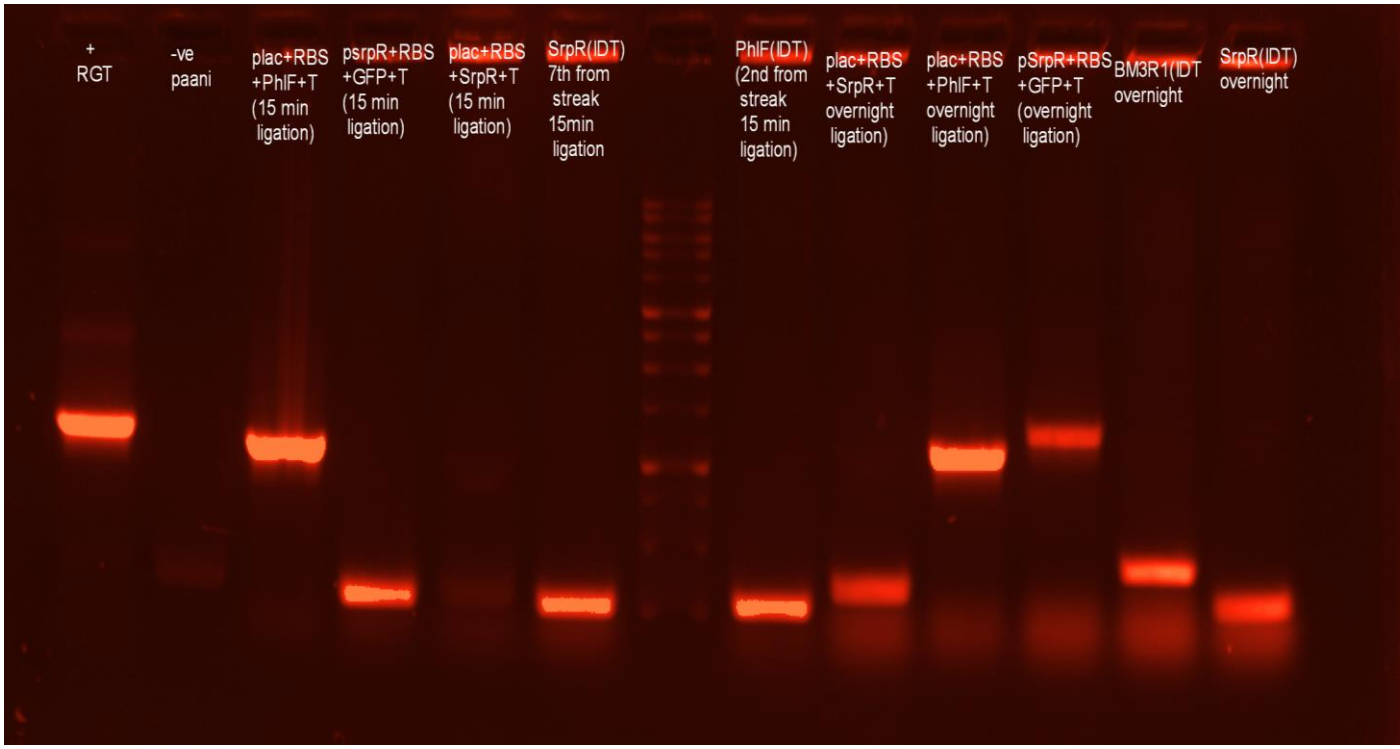
- Transformation of the parts ligated yesterday

23/09

- Not much colonies obtained in the transformations
- 15 min ligation of pLac+RBS+SrpR+T, pSrpR+RBS+GFP+T, pLac+RBS+PhlF+T
- Transformation of the 15 ligated parts

24/09

- Again a few colonies obtained in 15 min ligated parts' transformation
- cPCR of the few colonies obtained in overnight ligated transformations and 15 min ligated transformations



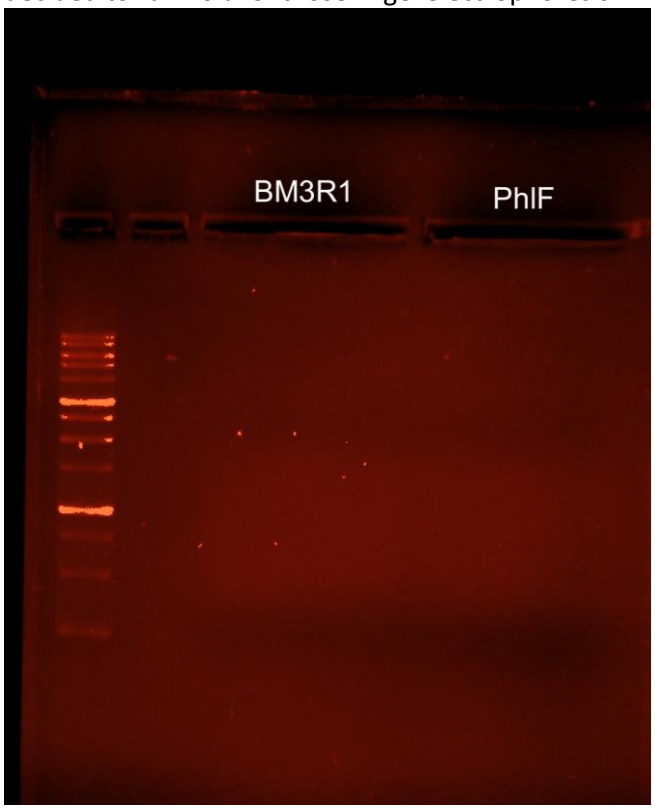
- Confirmation of colonies obtained for pLac+RBS+PhlF+T, pLac+RBS+PhlF+T, pSrpR+RBS+GFP+T (in KANAMYCIN) ; biobricks confirmed
- Streaking of confirmed colonies to revive them

25/09

- Plasmid isolation of pPhlF+RBS+GFP+T

28/09

- Due to repeated failures in attempt to obtain BM3R1, SrpR and Phlf biobricks from IDT we decided to run 40 ul of those in gel electrophoresis



- IDT vials did not contain any DNA even remotely similar to BM3R1 or PhlF; a rare probability that SrpR might be correct
- Gel extraction of SrpR
- Digestion of pLac, pSrpR, RBS+SrpR+T, RBS+PhlF+T

29/09

- Ligation of pLac+RBS+SrpR+T and pSrpR+RBS+PhlF+T from parts digested yesterday
- Transformation of the ligated parts

30/09

- Colony PCR of the few colonies obtained in the transformations; only to obtain result of a failed ligation