

Abbreviations and notes:

Col: Colony

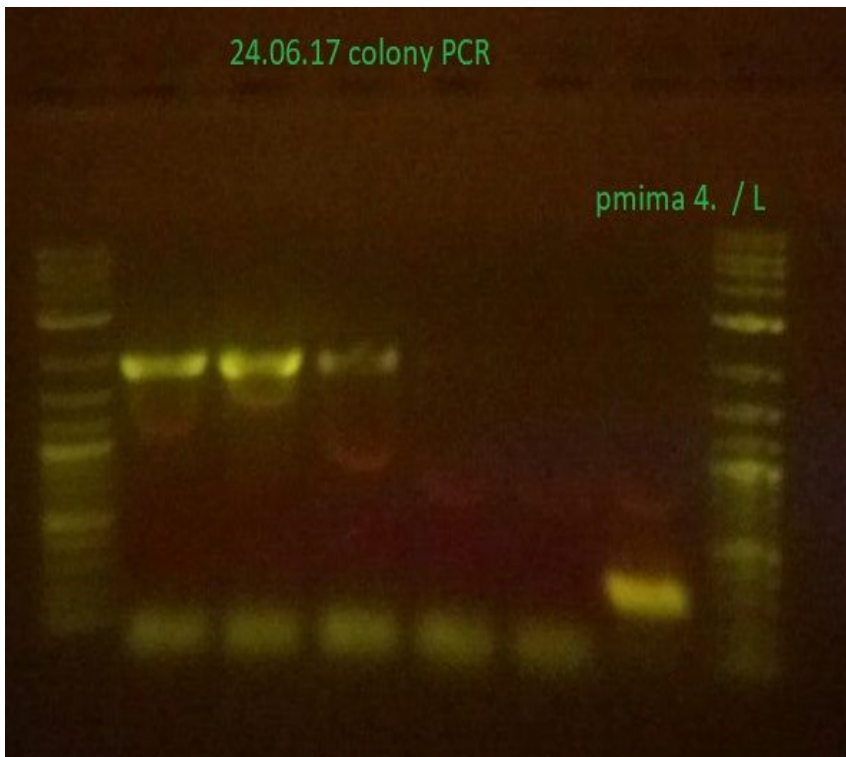
Ver: Verified

1:1000 atc corresponds to 250 ng/μl

2log is used as a ladder for all gel electrophoresis experiments.

21.6.17

Figure 1: Colony PCR was done for pmimA and 4th colony was seen to be verified from the gel photo.



29.6.17

Colony PCR was done for psB1C3 mimR. From the gel photo colonies 2,3 and 4 were seen very slightly but they were positioned on the right location. Therefore we thought that these colonies could go for the sequencing.

Figure 2: Colony PCR for mimR.

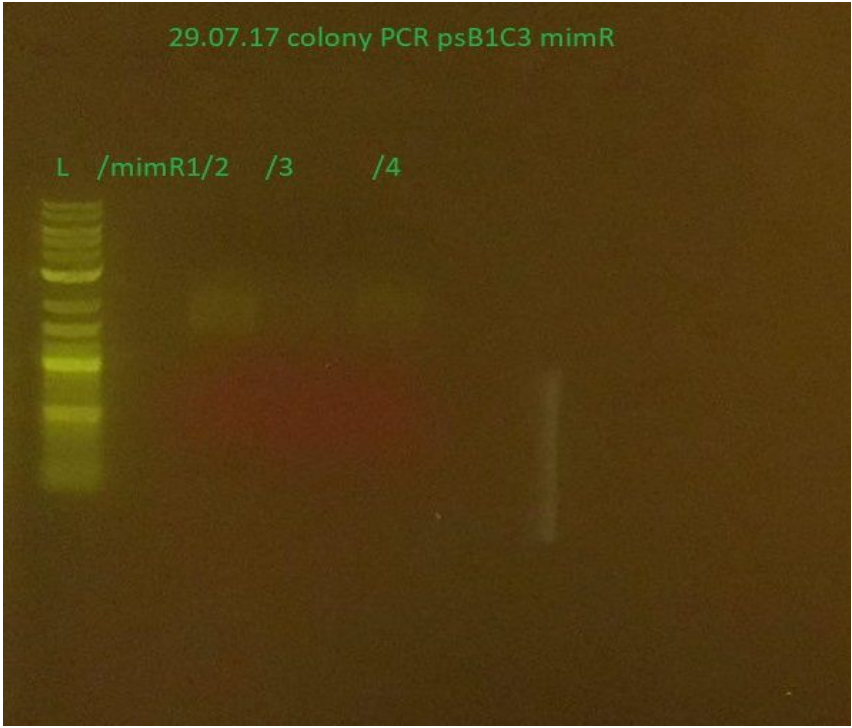


Figure 3: Colony PCR for pmimA



30.7.17

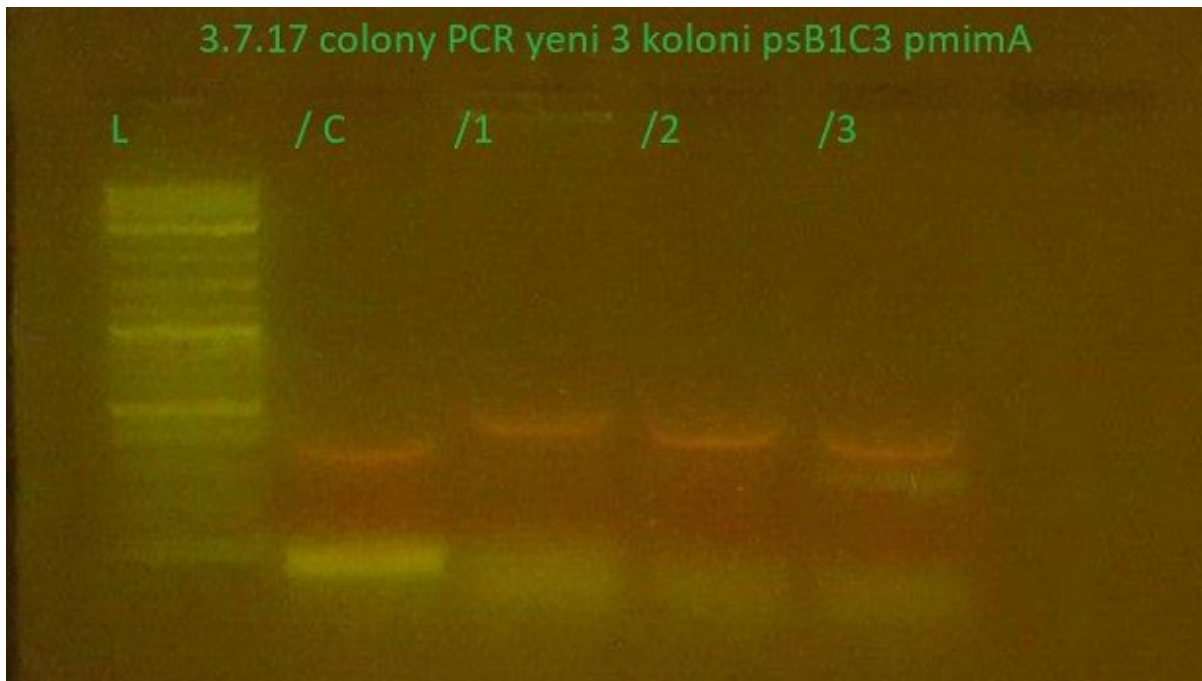
psB1C3 mimR 2-3-4 colonies are sent to sequencing.
Sequencing result was negative.

7.7.17

psB1C3 pmimA 4. colony was sent to be verified by sequencing.

For psB1C3 pmimA new 3 colonies were chosen again and 3rd colony was seem to be verified from the gel photo. Therefore 3rd colony was sent to sequencing on 7.7.17.

Figure 4: Colony PCR for pmimA

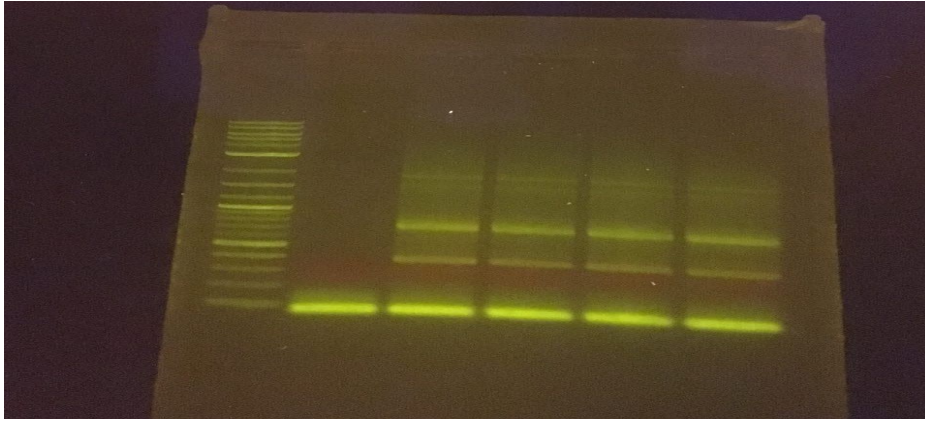


11.7.17

mimR coming from IDT was runned on the gel to be controlled.

In order to clone mimR into the pSB1C3. mimR PCR was conducted. The concentration was 8 ng/ul.

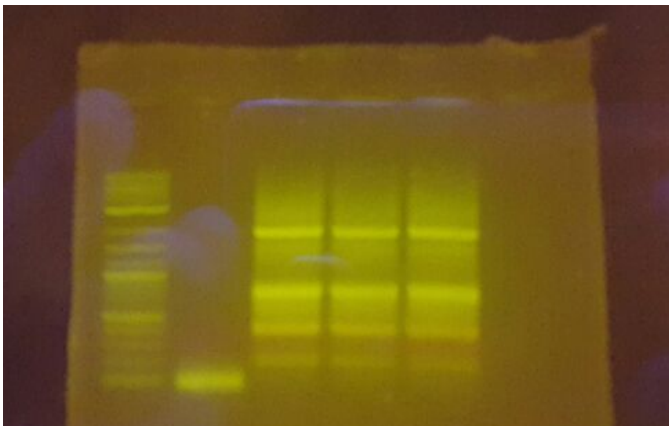
Figure 5: PCR results for mimR



12.7.17

In order to raise 8 ng/ul concentration, PCR experiment was conducted again. Concentration was raised to 36 ng/ul.

Figure 6 : PCR results for mimR

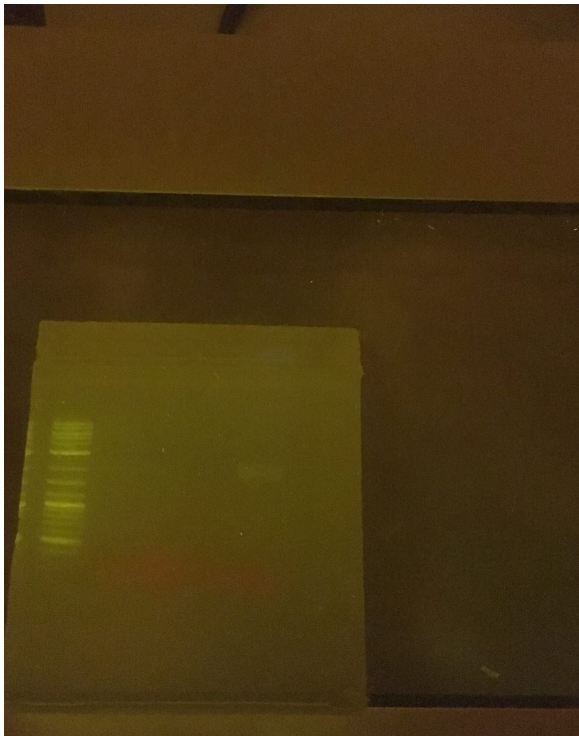


13.7.17

Transformation was conducted.

4.7.17

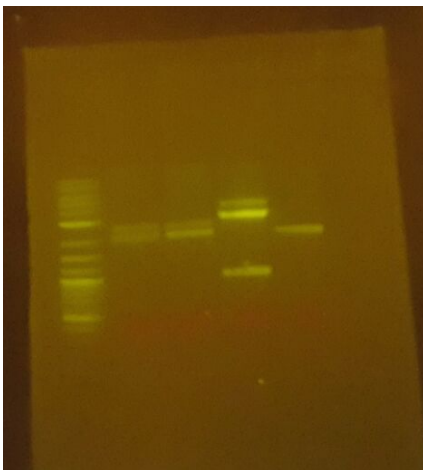
Figure 7: Colony 1-2-3-4 digestion



Colony 5 and 6 culture were prepared.

15.7.17

Figure 8: Colony 2-3-5-6 digestion



Colony 5 was verified from the digest and it will went for sequencing.

17.7.17

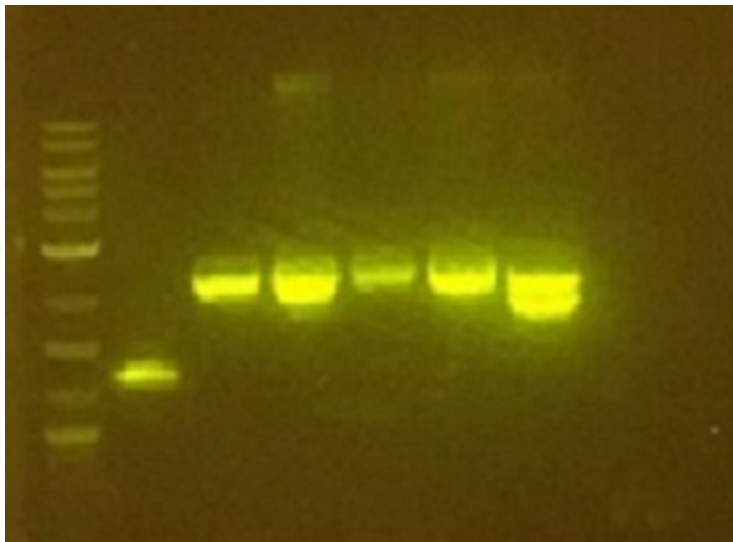
psb1c3 mimr 5th colony was sent to sequencing.

20.7.17

MimR pSB1C3's miniprep was conducted. (It was eluted with 30ul)

Figure 9: 4 HxIR pSB1C3 and mimR pSB1C3 and AlkR's PCR product was cut with XbaI and SpeI enzymes. Gel: AlkR PCR, HxIR pSB1C3 1-2-3-4, mimR pSB1C3 was all cut by XbaI and SpeI.

1282 bp/2062-496-144 bp/2062 bp-1790 bp

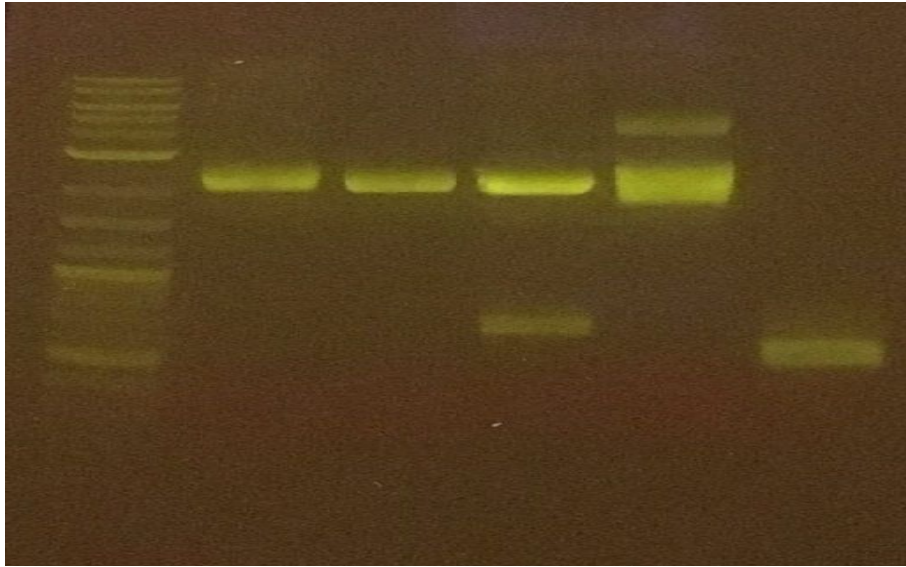


Cell culture was prepared with newly selected 3 colonies from MimR pSB1C3.

21.07.17

MimR pSB1C3's miniprep was conducted.

Figure 10: The HxIR's PCR and mimR pSB1C3 and Hxlr pSB1C3 5-6-7 which we already have was digested with XbaI and PstI enzymes. Gel: HxIR psB1C3 5-6-7 , MimR pSB1C3 and HxIR PCR was all digested with XbaI and PstI enzymes.



pSB1C3 mimr 5th colony was verified with the sequencing.

24.7.2017

Figure 11: For pmimA PCR for pza.

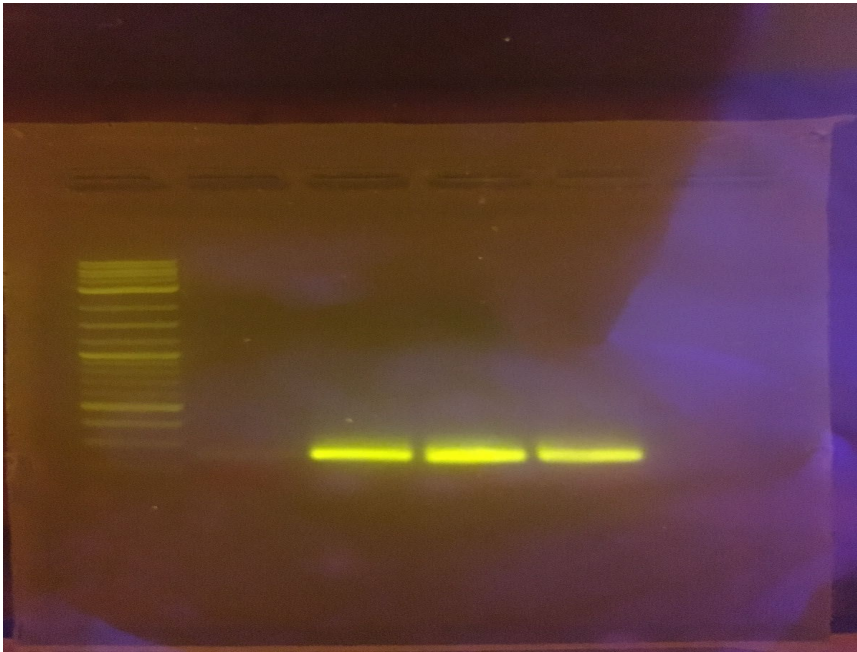
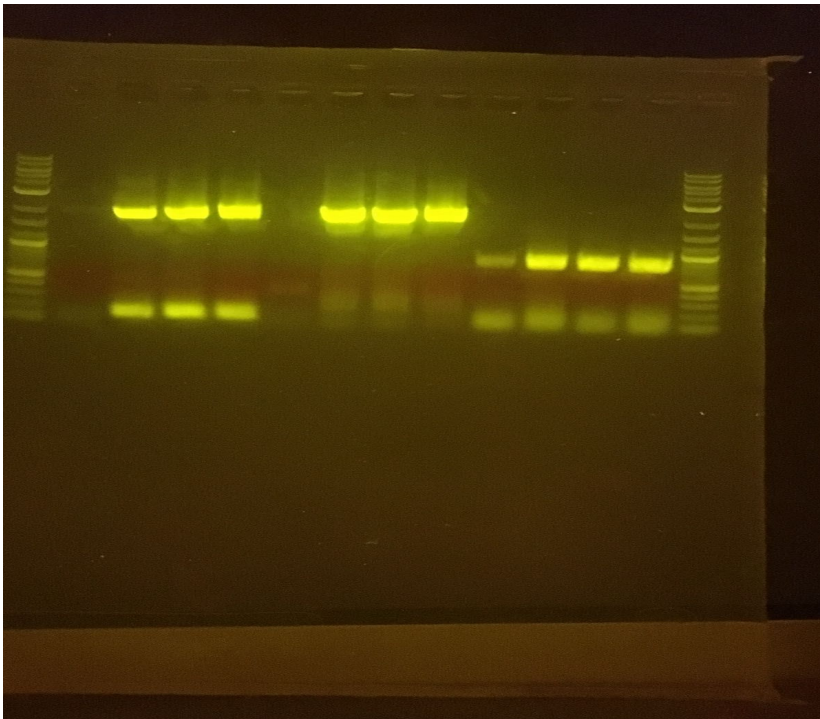


Figure 12: For pza; Pza PCR, mimR PCR, sfGFP PCR C 1 2 3,



Pza-mimr-pmima-sfgfp gibson assembly was performed.

25.7.17

Miniprep conducted for pSB1C3 pmimA 4th colony.

In order to verify pSB1C3 pmimA 4th colony we performed restriction digestion. pmimA was digested with XbaI and pstI. Also we used an iGEM part to obtain pSB1C3 backbone. Then we digested that part with XbaI-pstI and EcoRI-PstI.

28.7.17

pza pmimA mimR induction started.

31.7.17

pmimA mimR induction was tried again with 0.4 ul isopropanol and 0.4 acetone.

1.8.17

Induction was tried again with the addition of atc when dilution was performed and 10 ul acetone and 10 ul isopropanol was added.