Lab Notebook, Team Aalto-Helsinki 2014, September-October

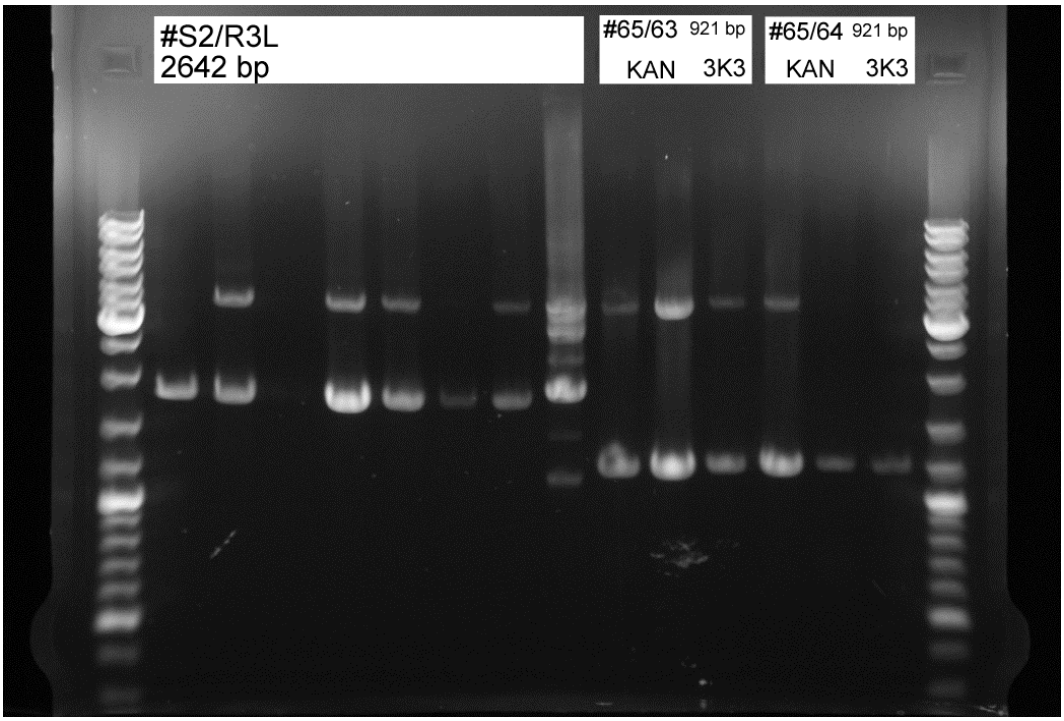
(NOTE: in the gel pictures we have used our own index numbers for the Biobricks)

1.9. p. 46-48

S2 and BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 (in pSB1T3) were religated (again) and transformed to Top10, 150 ul and the rest of the cells were plated. Based on the colony PCR (some of the sa mples had several band of which some might have been correct size) liquid cultures were made of four (7, 8, 11 and 17) colonies of S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 so they could be miniprepped, restricted with one enzyme and checked on a gel tomorrow.
Plates of BBa_K823004/BBa_E0430 and BBa_K823004/BBa_E0420 had many colonies (less on the ones with low copy pSB3K3 plasmid). A colony PCR of those would be made tomorrow.
Quikchange heat-shock transformation was done at VTT according to the Quikchange protocol (heat shock 40 sec) by using XL1-blue strain. Blue/white screening plate was prepared by pipetting 40 ul og 20 mg/ml X-gal and 40 ul of 0,1 mM IPTG and spreding them evenly on an ampicillin plate. The solutions precipitated when they were mixed but after a little while the white precipitation had cleared. There were three kinds of samples. P=PMK AP-HS transformation control, C=pWhitescript mutagenesis control and S=sample mutagenesis. 250 ul of C was plated on a blue/white screen AMP plate. 5 ul of P mixed with 200 ul of LB was plated on a KAN plate and 20, 100 and 200 ul of S was plated on AMP plates. The plates were put in the +37 °C incubator. /MS&OV

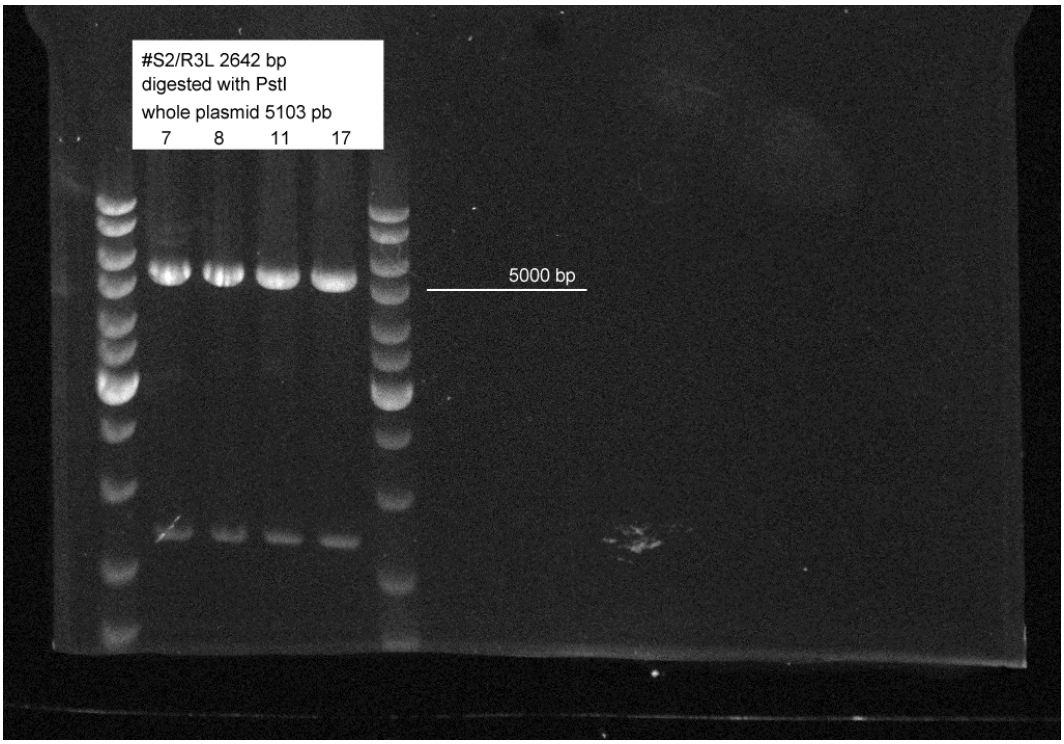
2.9. p 48

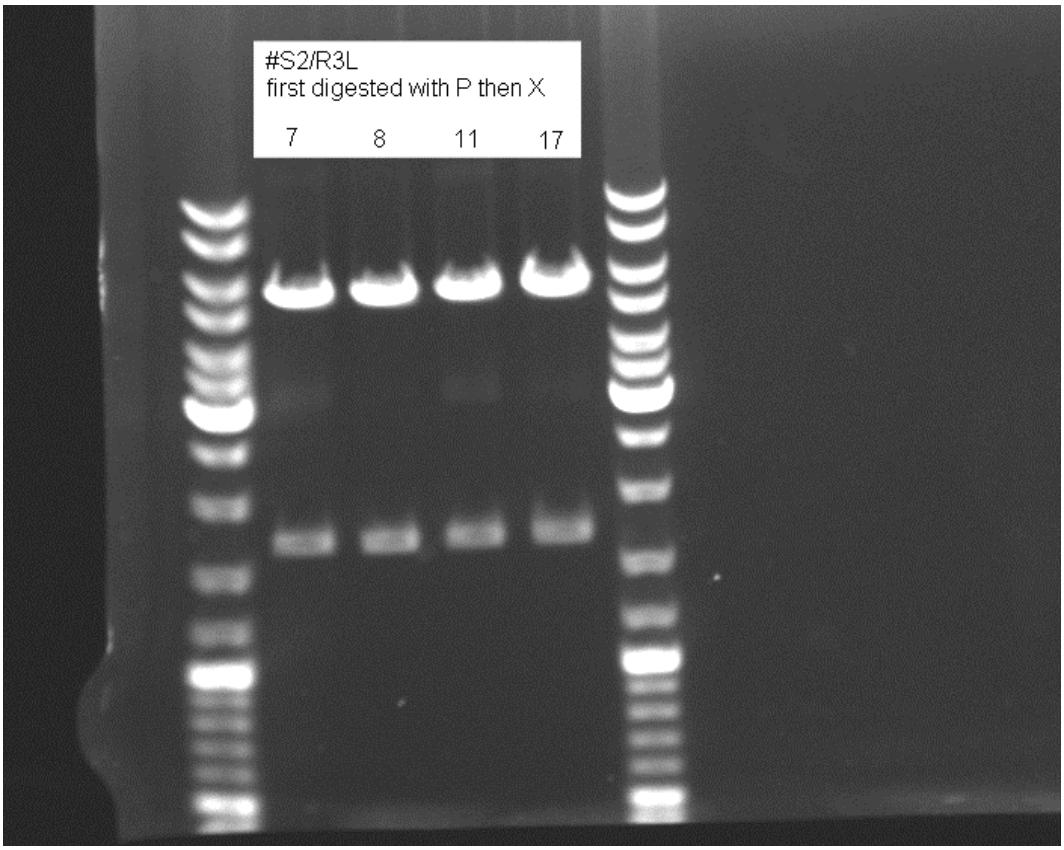
Colony PCR of S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 (done 1.9.), BBa_K823004/BBa_E0430 (in both backbones) and BBa_K823004/BBa_E0420 (in both backbones) was done. Liquid cultures made yesterday were miniprepped and the four samples of S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 plasmids were restricted with PstI according to the following protocol. 2 ug of DNA, 6 ul of 10x buffer, 0,6 ul of 100x BSA, 2 ul of enzyme and x ul of water so that the total volume is 60 ul. Tubes were incubated 3 h in +37 °C. A gel (0,7%, old ladder) of the colony PCR samples was run. /MS&OV



3.9. p. 48

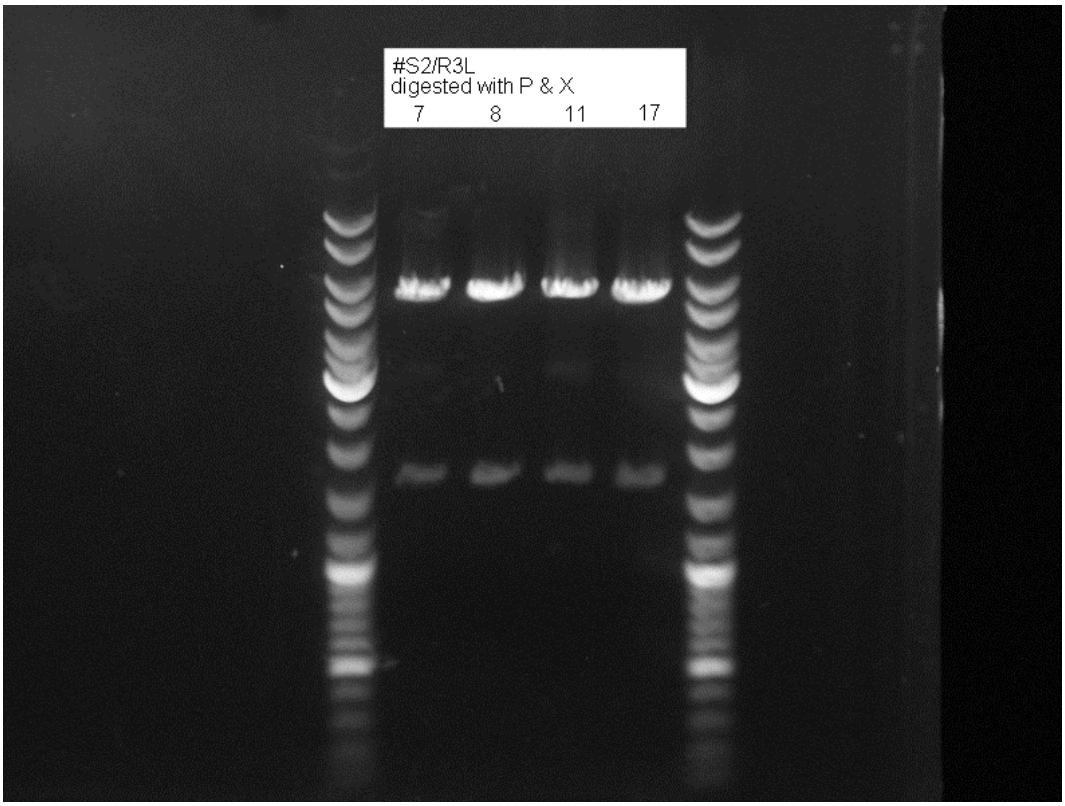
Plasmids digested yesterday (S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015, colonies 7, 8, 11 and 17 from 27./29.8.) were incubated in +80 °C to inactivate the enzyme and the samples were run on a gel. The plasmids were also restricted with XbaI (30 ul of the restriction mix, 3 ul of 10x buffer, 0,3 ul of BSA, 1 ul of XbaI and x ul of water so that total volume is 40 ul, incubation 3 h), just in case. A liquid culture of the sample 8 was made. A gel was run of the samples digested with two enzymes (separately) but as it looked the same as the one digested with only one enzyme, a new restriction with two enzymes (at the same time) was put to incubate overnight at +37 °C. The normal restriction protocol was used. /MS


#S2/R3L 2642 bp
digested with PstI
whole plasmid 5103 pb
7 8 11 17
5000 bp


#S2/R3L
first digested with P then X
7 8 11 17

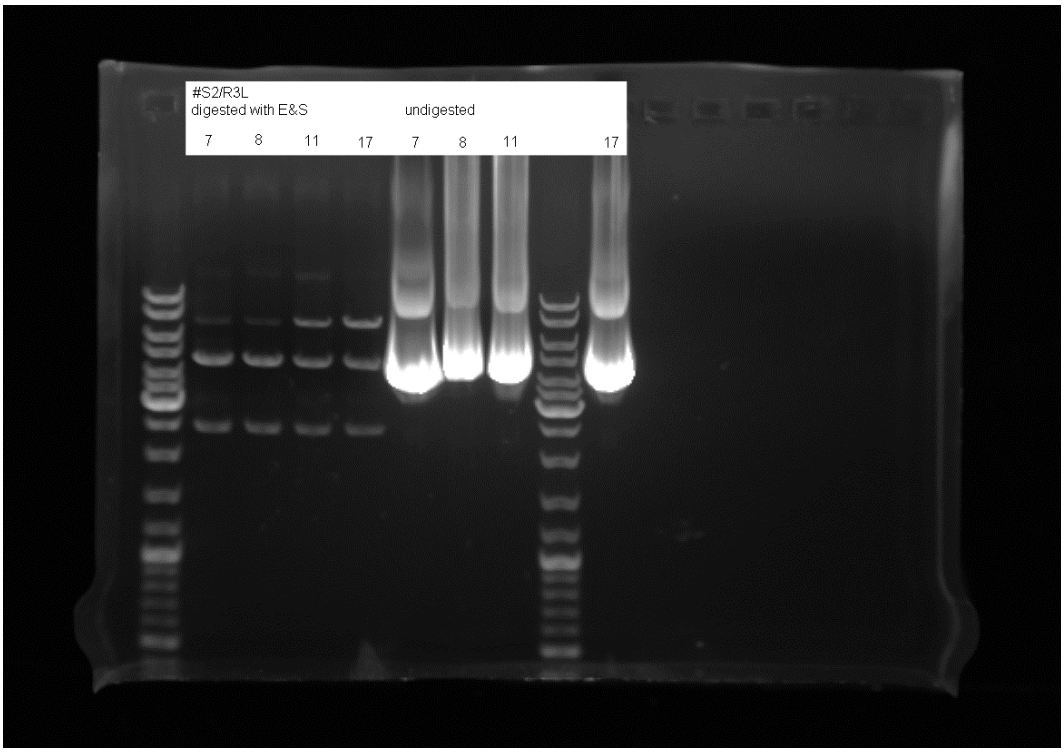
4.9. p. 48

Enzymes were inactivated by incubating the samples at +80 °C for 20 min and then they were run on a gel. The liquid culture made yesterday (sample 8) was miniprepped. The gel still looked the same as yesterday (bands at around 5000-5500 bp and ~ 1700 bp). WTF? /MS



5.9. p 48

All four samples (7, 8, 11 and 17) were restricted with EcoRI and SpeI so that it could be seen if the restriction sites can be found. The samples as well as an undigested sample (that was forgotten to dilute) were run on a gel. /MS

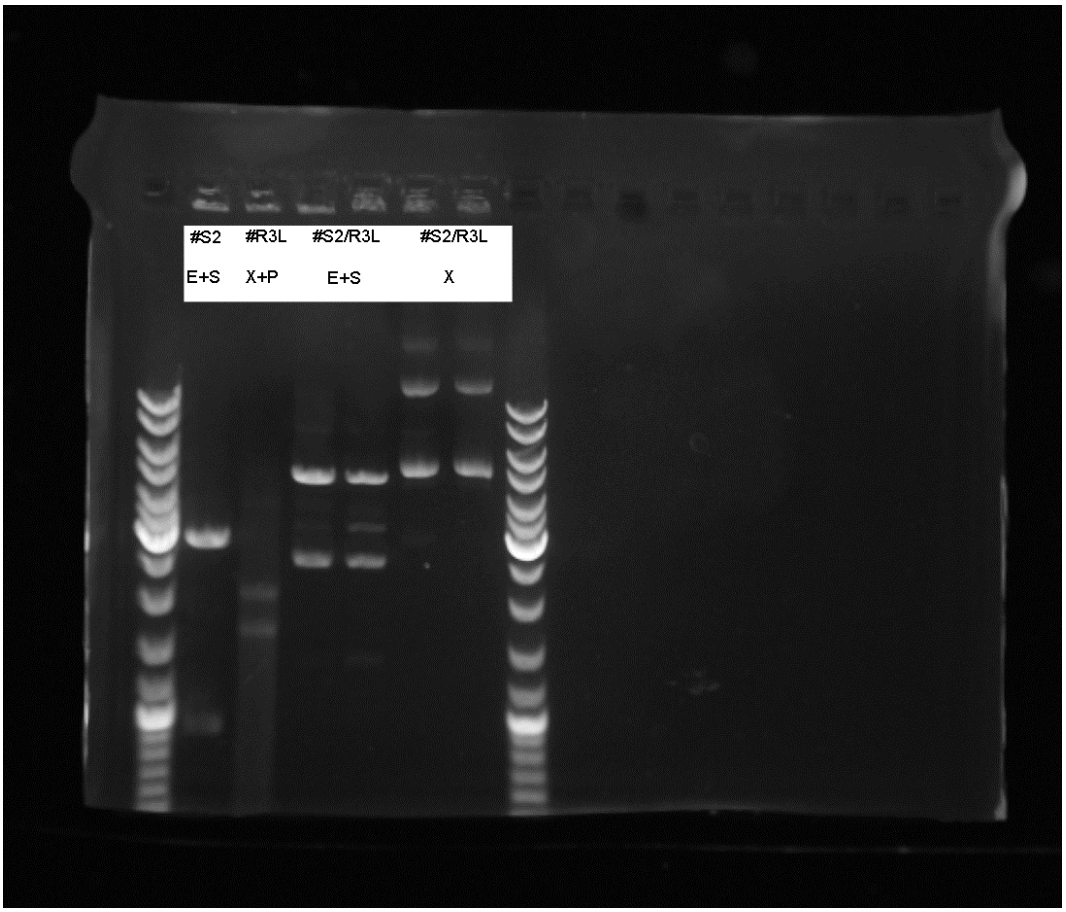


7.9. p 50

Liquid cultures of the test unit, BBa_K592009 and BBa_K823005/BBa_E0240 were made. S2, BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 and S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 (7&8) were restricted overnight at +37 °C so that S2 was restricted with EcoRI + SpeI, BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 with XbaI + PstI and S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 with EcoRI + SpeI and XbaI. /MS&LV

8.9. p. 50

The enzymes in the restriction mixes were inactivated and the samples were run on a gel. Previously restricted samples of S2 and BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 were taken to VTT for gel purification so that the ligation could be tried with purified inserts and backbone (remember alkaline phosphatase to prevent autoligation of backbone). /OV, MS&MI

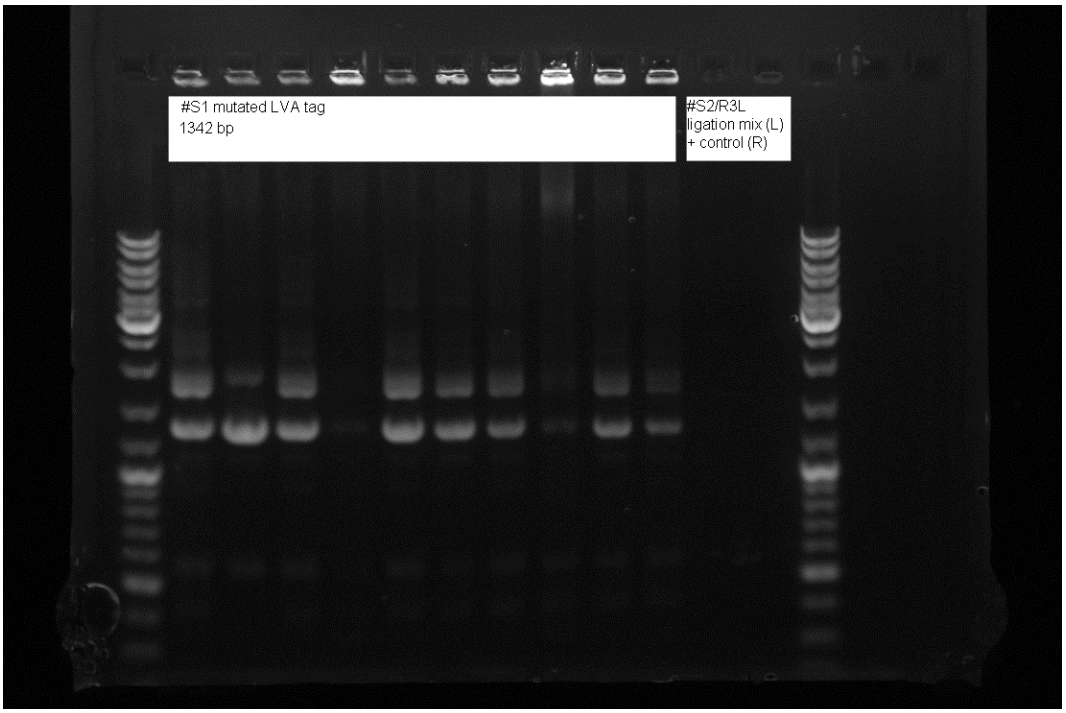


9.9. p. 50

The purified parts from VTT (in gel) S2 (650 mg) and BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 (300 mg) were extracted from the gel (1x washing, 5 min at +70 °C, elution to elution buffer 2 x 15 ul). The concentrations of the purified inserts were measured and they were quite low. The pSB1T3 backbone was treated with alkaline phosphatase (15 min +37 °C, 5 min +75 °C). Purified inserts were ligated to the backbone. Ligation was transformed to Top10. Liquid cultures were made of the mutation transformations and they were also inoculated onto a new plate. /MI, MS&OV

10.9. p 50

There were no colonies on the ligation plates so they were put back in the incubator. A colony PCR was done of the mutated transformants. A gel was run of the PCR samples, S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 ligation mix and the ligation control. The mutated samples seemed ok, ligation mix and control didn't show up on the gel at all. There still no colonies on the ligation plates in the afternoon so they were thrown away.
A liquid culture was made of BBa_K823008/BBa_K592016/BBa_B0015/BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 (colonies 1-4) for sequencing. /MI&MS



11.9. p. 50

Liquid cultures of BBa_K823008/BBa_K592016/BBa_B0015/BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 and mutated S1 were miniprepped. Sequencing samples were made of these two and S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015. New liquid cultures of the test unit and positive and negative controls were made by diluting the liquid cultures that were made yesterday. /MI (&OV)

15.9. p. 50

A new backbone pSB6A1 (from BBa_J04450) was transformed to Top10. 10 ul and 100 ul were plated. /MI&MS

16.9. p. 52

Liquid cultures of the test unit, negative control and all three Measurement Interlabs Study parts were made. /MI&MS

17.9. p. 52

Liquid cultures of the MI Study parts were discarded as useless since they were supposed to be measured in minimal media but the instructions specified them to be measured in LB (as we had done previously already). A miniprep of the pSB6A1 part was done (2 x 5 ml) after which the backbone was restricted for 3 h 40 min. The backbone was gel purified at VTT and measurements were conducted with the test unit (and controls). /MI&MS

18.9. p. 52

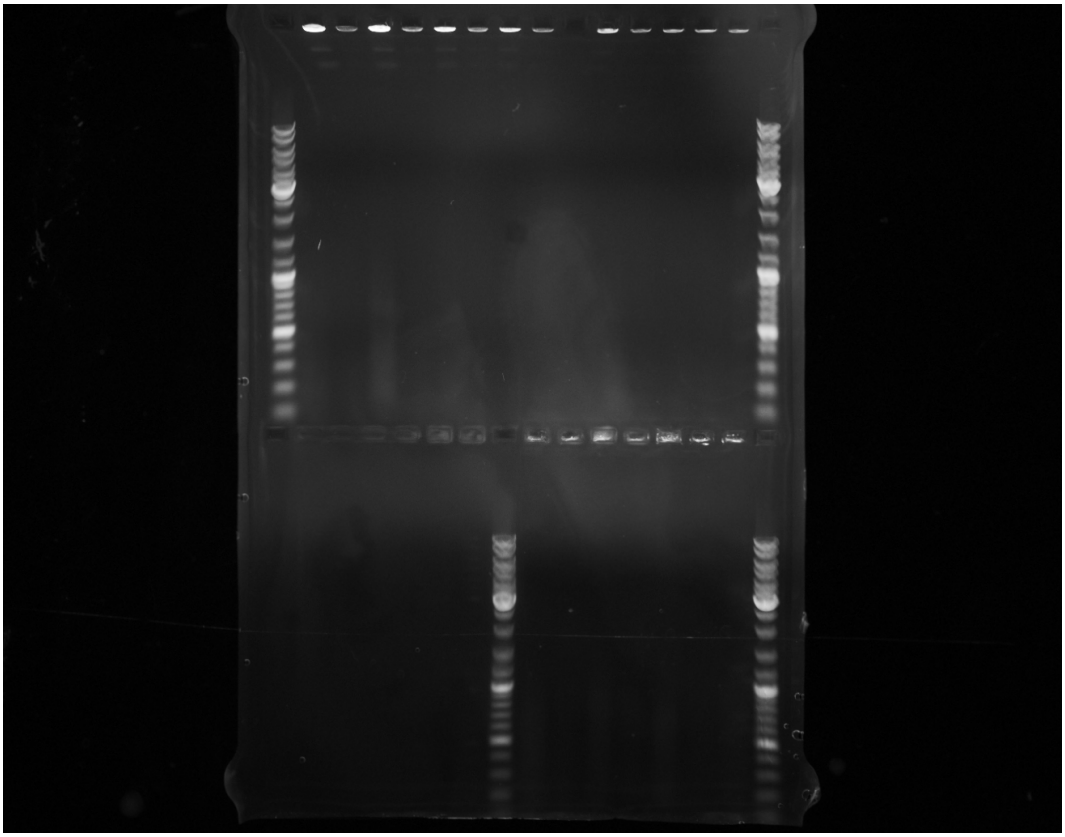
The gel purified backbone was extracted from the gel by using a gel purification kit. /MS

22.9. p. 52

BBa_R0040/BBa_B0034 + BBa_K082003/BBa_B0015 (in pSB1C3) and S2 + BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 (in pSB1T3) were ligated again and transformed to Top10. Sequencing samples (BBa_R0040/BBa_B0034, BBa_K082003/BBa_B0015, S2, and S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015) were prepared. /MI&MS

23.9. p. 52

The sequencing samples were taken to Meilahti. A colony PCR of yesterday's ligations was done. With S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015, two sets of primers (VF2/VR and 624F/M13R) so we could see if the pUC57 (from S2) plasmid had survived in the ligations and was the reason why couldn't get the ligation to work. CAM and TET plates were prepared. A gel was run of the PCR samples but for some reason there was nothing to be seen. The samples were saved for future use, just in case. /MI&MS

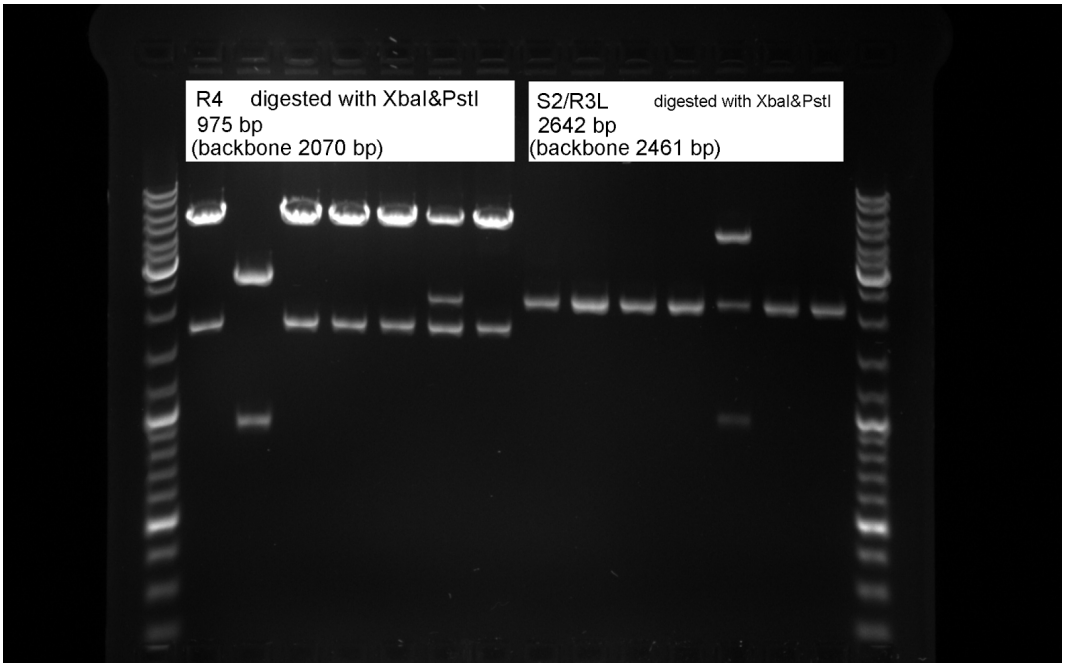


24.9. p. 52

Liquid cultures were made of the colony PCR samples. In addition liquid cultures were made of BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015, BBa_K082003/BBa_B0015 and BBa_R0040/BBa_B0034 for Sanni since she wanted to help us with the troublesome ligations. /MI&MS

25.9. p. 52

Liquid cultures were miniprepped. Colony PCR samples and BBa_K592006/BBa_B0034 (colony 4) were restricted with XbaI + PstI. The restricted samples were run on a gel. /MS

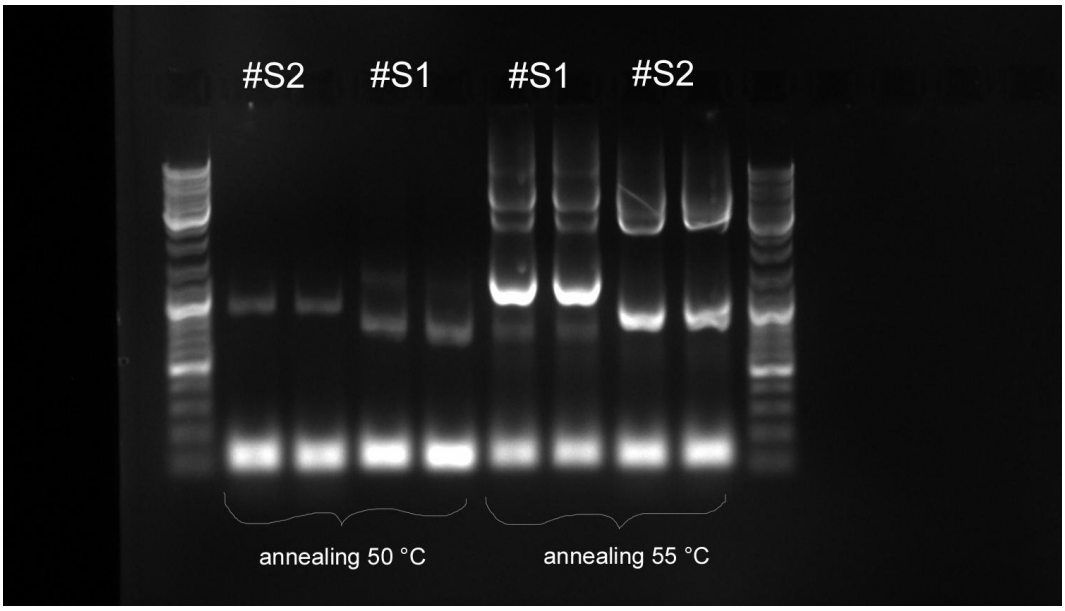


26.9. p. 52

BBa_K823008/BBa_K592016/BBa_B0015 + BBa_K592006/BBa_B0034 (in pSB1C3) were ligated together. The OR site primers were prepared. The ligation was transformed in Top10 and the plates were put in the +30 °C incubator. /MS

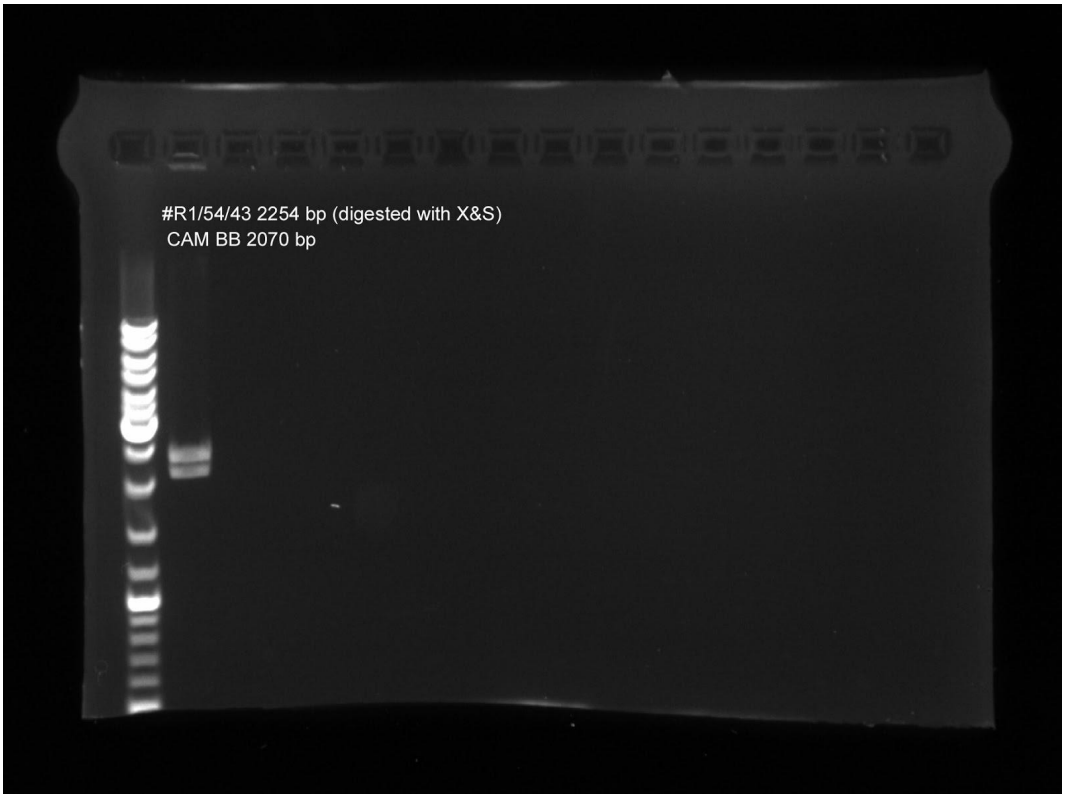
28.9. p 52-54

A liquid culture of the ligation BBa_K823008/BBa_K592016/BBa_B0015/BBa_K592006/BBa_B0034 was made. The OR sites were PCRed with the new primers. The protocol was same as colony PCR except the denaturation temperature was reduced to 95 °C and two annealing temperatures (50 °C and 55 °C) were used (two programs). On the gel, the product looked about the right size so the samples were restricted with EcoRI + PstI and the restricted product was gel purified and cut out of the gel. /MS&LV



29.9. p. 54

The liquid culture of BBa_K823008/BBa_K592016/BBa_B0015/BBa_K592006/BBa_B0034 was miniprepped and it was restricted with XbaI + SpeI. The restricted sample was then run on a gel and it looked good -> successful ligation, yay! :) The OR sites that were cut out of the gel were extracted and ligated to pSB1C3 backbones. The ligations were transformed to Top10 and 150 ul and the rest were plated. /MS

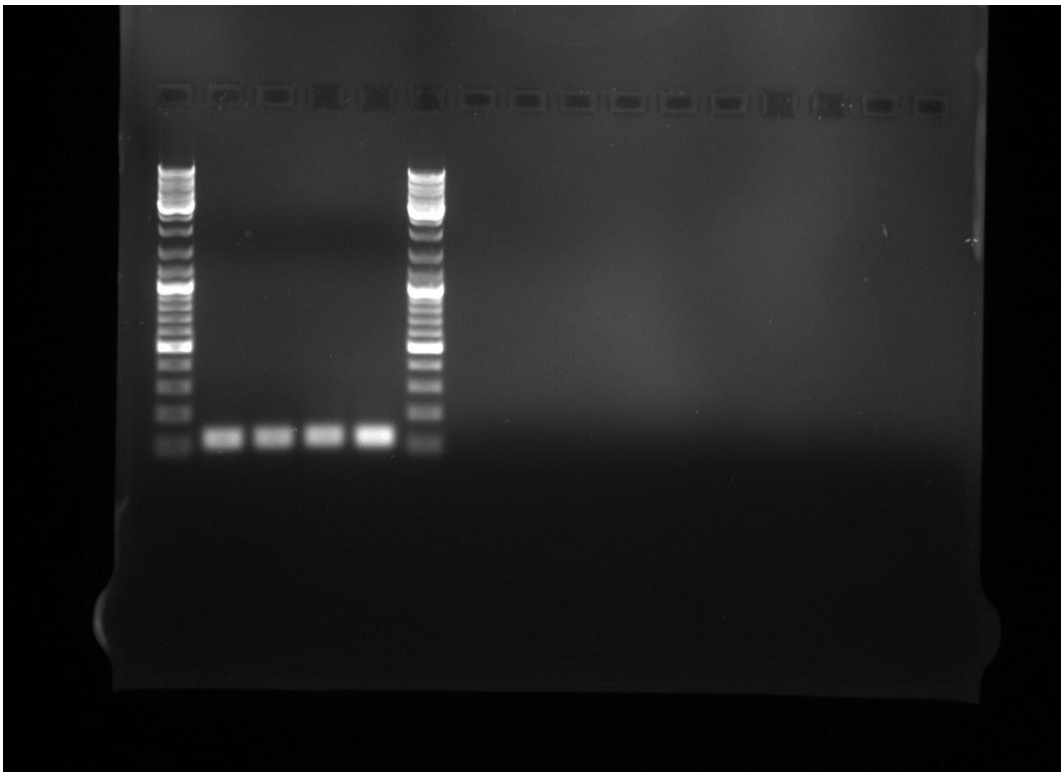


30.9. p. 54

There was only one colony in the plates so the ligase might have been too diluted (1 unit/ul). New ligations were made using a stronger ligase (200 units/ul) and they were transformed to XL1-blue. 150 ul and the rest were plated. /MS

1.10. p. 54

There were still no colonies on the plates so a new ligation was made with a total volume of 5 ul (1 ul backbone; 1,4/2,0 ul of insert; 0,5 ul of buffer and 0,5 ul of undiluted ligase). The ligation was put in +16 °C overnight. A gel was run of the OR site samples, just in case, but it looked ok! BBa_K823008/BBa_K592016/BBa_B0015/BBa_K592006/BBa_B0034 was prepared to be sent to iGEM HQ. /MS



2.10. p. 54

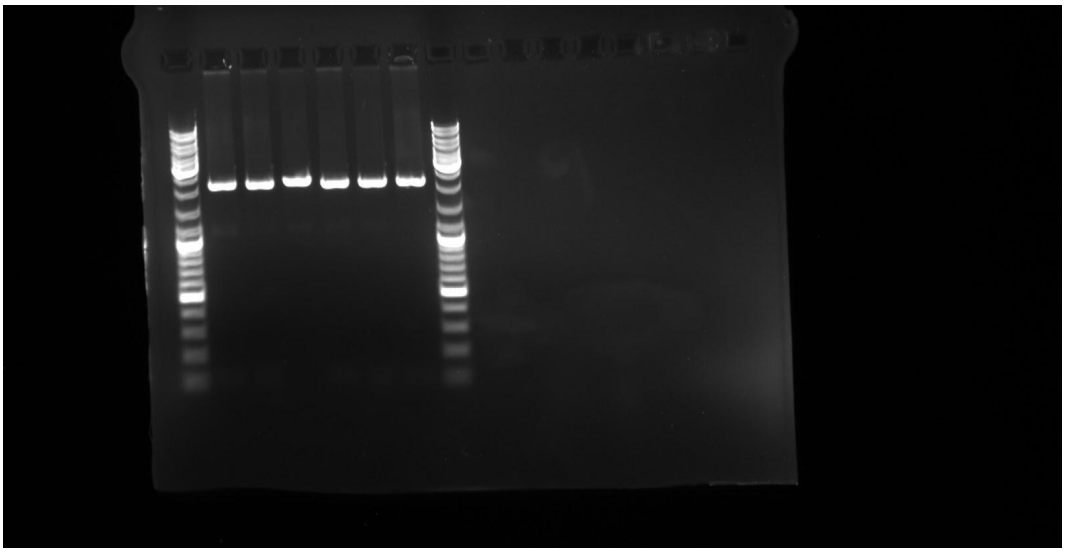
The overnight ligated parts were transformed to XL1-blue. 150 ul and the rest were plated. /MS

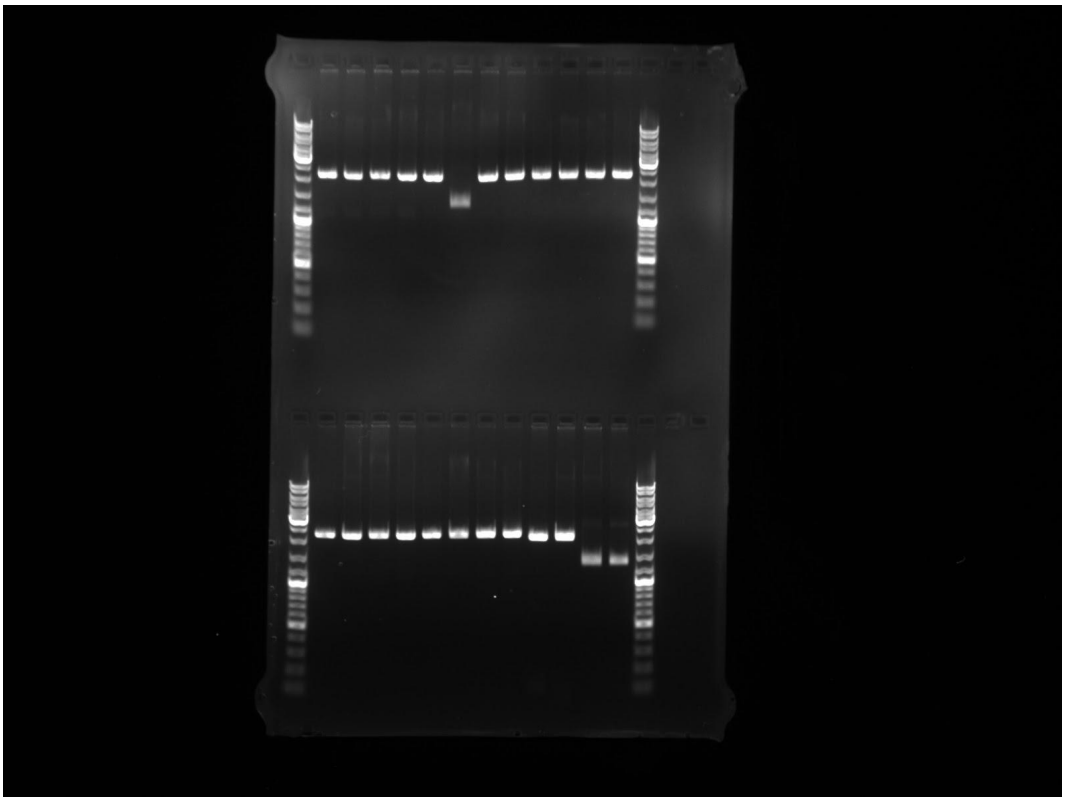
3.10. p. 54

Liquid cultures were made of 8 colonies (3 x OR(S1), 5 x OR(S2). /MI&MS

4.10. p. 54

Two of the 8 colonies picked turned out to be red but miniprep of the rest were made. The plasmids were restricted with XbaI, EcoRI, PstI and SpeI separately and with EcoRI + PstI. The samples were run on (2 different) gels. A liquid culture of Top10 was made so that competent cells could be made. Colony 6 of OR(S1) and colony 1 of OR(S2) looked good and new liquid cultures were made of them. /MI&MS





5.10. p. 54

Electrocompetent cells were made of Top10. The OR site liquid cultures were miniprepped and prepared to be sent to iGEM HQ. /MI&MS

6.10. p. 54

Samples were prepared for sequencing and the new bricks were sent to iGEM HQ. /MI&MS

8.10. p. 56

The plasmid BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015, constructed by Sanni, looked good on a gel so it was restricted with XbaI and PstI. BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 was reinoculated onto a clean plate and new liquid culture was made. BBa_K823008/BBa_K592016/BBa_B0015 + BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 were ligated together in pSB6A1 backbone overnight. /MI&MS

9.10. p. 56

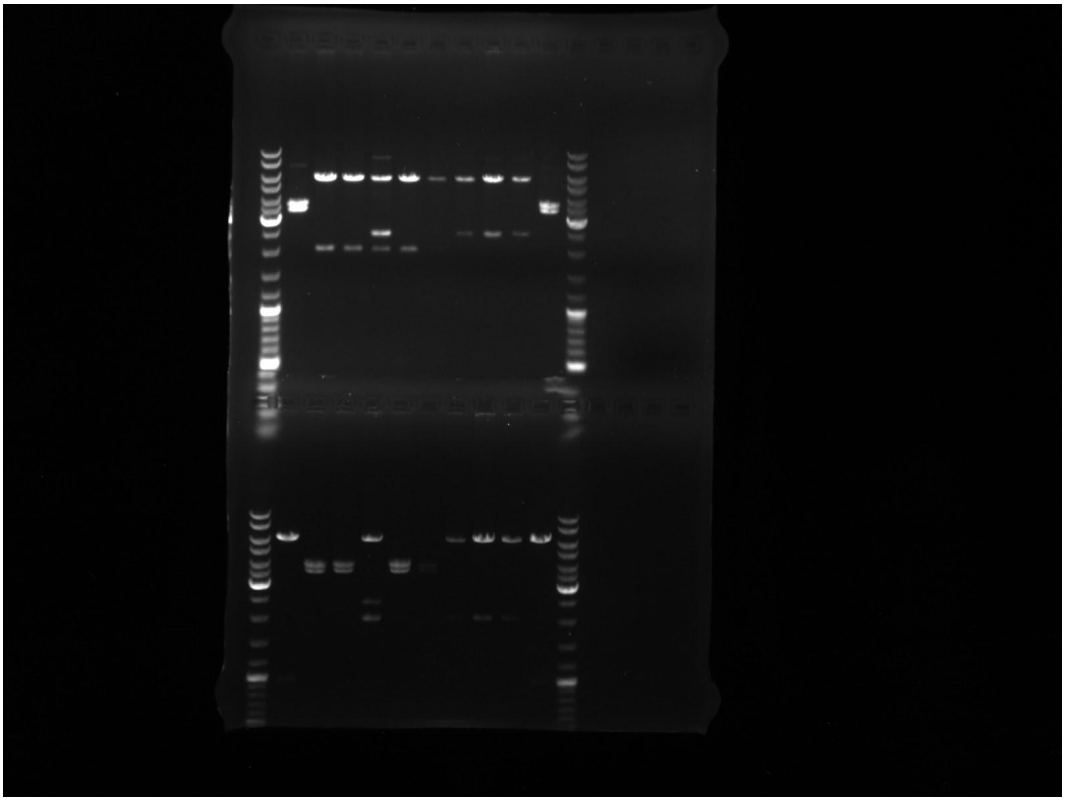
The ligated BBa_K823008/BBa_K592016/BBa_B0015/BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 and the control were transformed to Top10. 150 ul and the rest were plated. The liquid culture of BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 was miniprepped. /MS

10.10. p. 56

The plate where 150 ul of BBa_K823008/BBa_K592016/BBa_B0015/BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 was plated had plenty of colonies (also satellites and some red ones), of which 10 were picked for liquid cultures. The control plate also had a few colonies but a lot less than the actual ligation plate. /MS

11.10. p. 56

The Liquid cultures were miniprepped and the plasmids were restricted with EcoRI + SpeI and XbaI + PstI. The samples were run on a gel but it looked bad. Reinoculations from the liquid cultures were however made, just in case. /MI&MS



13.10. p. 56

Religation of BBa_K823008/BBa_K592016/BBa_B0015 + BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 in pSB6A1 was done by using 4 ul of BBa_K823008/BBa_K592016/BBa_B0015, 4 ul BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015, 0,8 ul of ligase, 1 ul of ligation buffer and 0,2 ul of backbone. Ligations were transformed to Top10. /MI

14.10. p. 56

The lab work was decided to be freezed due to time running out. /MI&MS