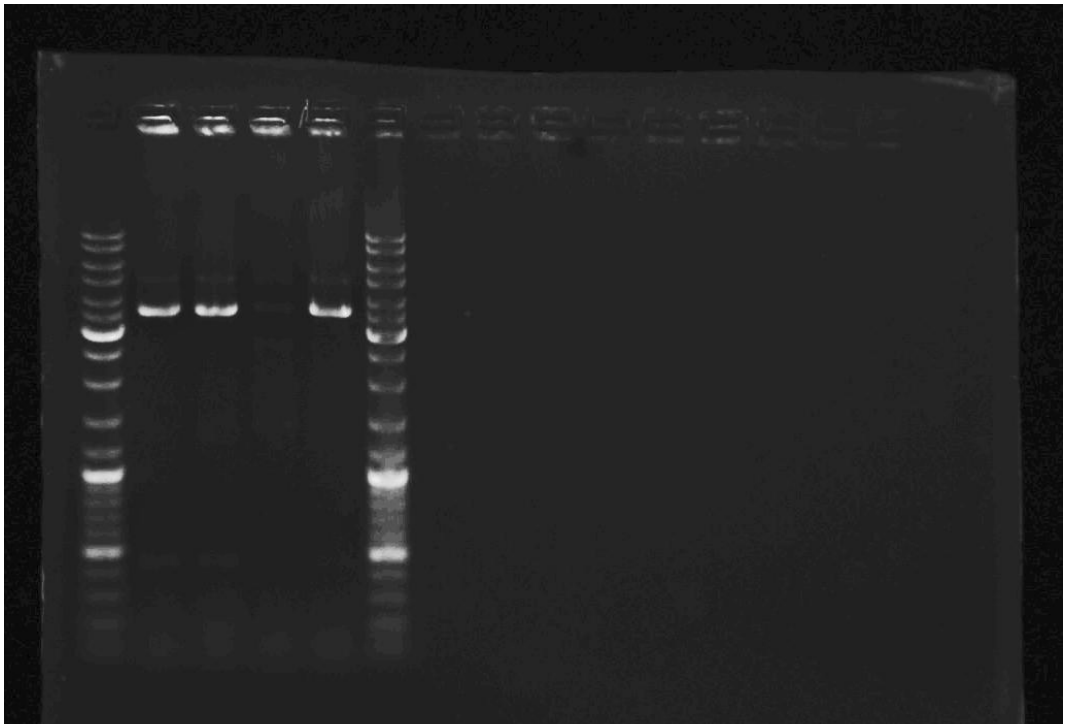
Lab Notebook, Team Aalto-Helsinki 2014, July

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

1.7. p.24

BBa_K592008+BBa_B0033 ligations had been successful [note later: hadn't been] and YYC912 plates had plenty of colonies. XL1-blue plates had very little colonies (slow growth rate in kanamycin?) The controls were empty, which is probably because a wrong tube of control ligation mix was used. Of colonies grown in YYC912 a colony PCR and gel run were conducted. /MI&MS



2.7. p. 26

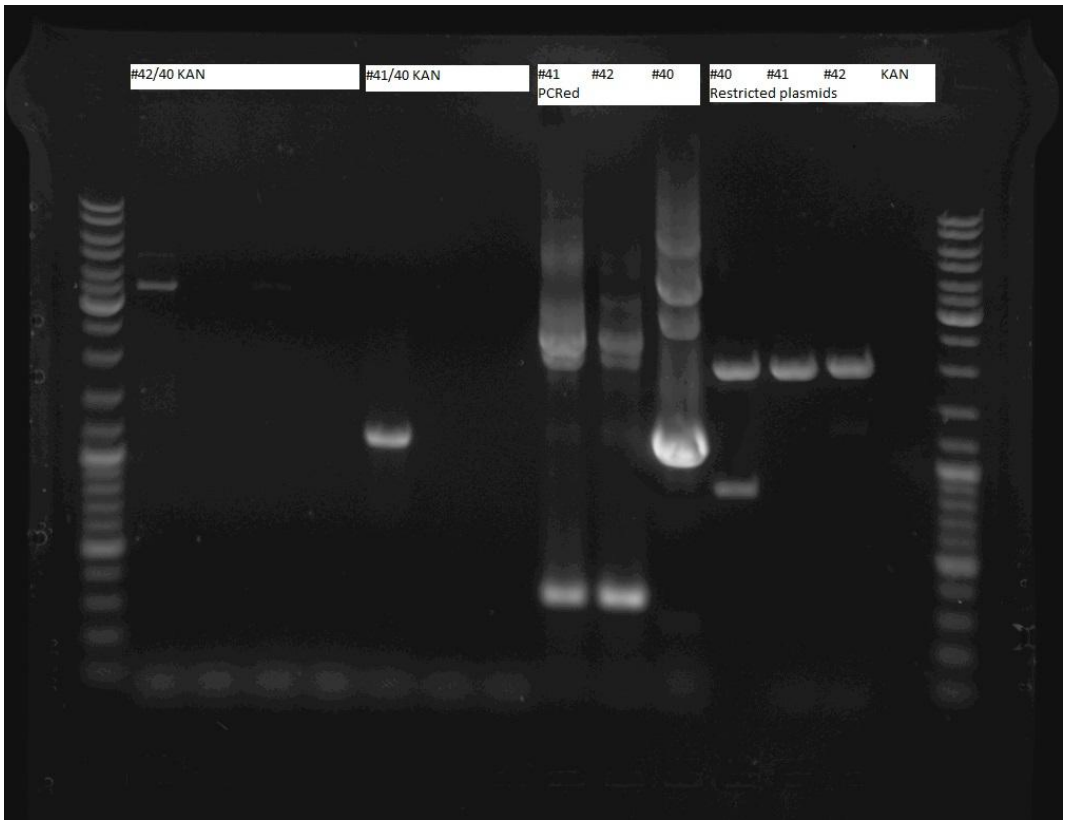
As the result of the gel made yesterday, the practice project we had been working on until now was decided to be ditched. Biobricks BBa_I20260, BBa_E0240, BBa_K823005, BBa_K823012, BBa_B0034, BBa_B0030, BBa_K819017 and BBa_K880005 were miniprepped. Measurement interlabs parts (BBa_K823005/BBa_E0240, BBa_K823012/BBa_E0240) were assembled in KAN backbone and also a control was made with KAN backbone only.

Biobricks BBa_R0051, BBa_I12007, BBa_R0065, BBa_C0040 and BBa_R0040 were transformed in XL1-blue. Ligated plasmids were transformed in YYC912. Appropriate ratio was noticed to be 0,7ul DNA and 45ul cells to make a successful electroporation. Transformed cells were spread on a plate and put in +37 °C to be incubated. /MI&MS

3.7. p.26

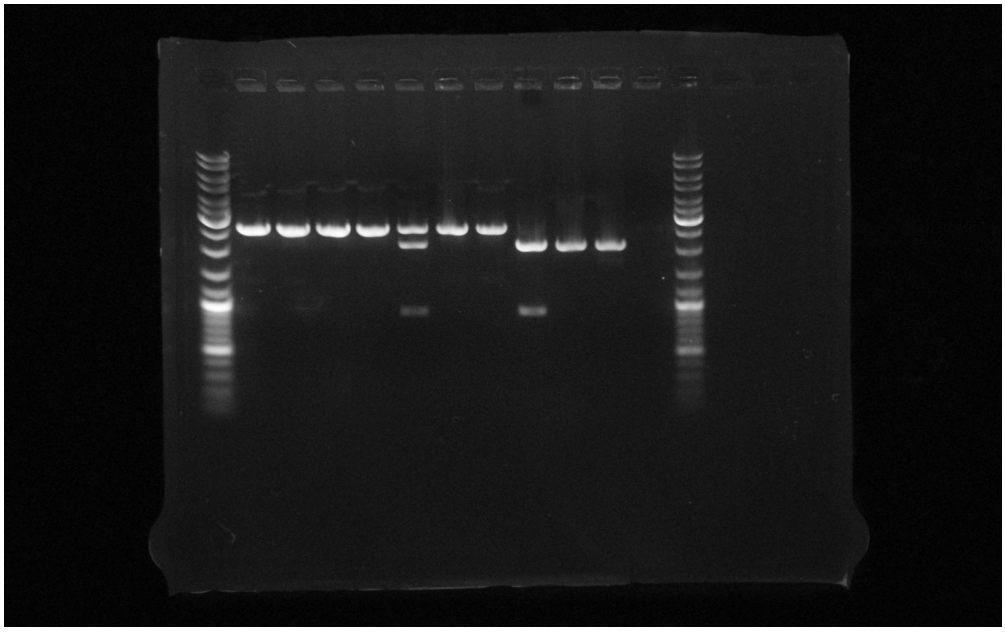
There were quite few Biobrick colonies, 150 ul could be better volume to be plated, or even 200 ul. Control plate had one white colony, ligation plates of 100 ul plating volume had some red colonies and only single white colonies. On the plates where the rest of the cell suspension was plated had quite good amount of colonies, and ¼ of them were white. From ligation plates 7 colonies were picked to colony PCR and gel run. In the gel there were also samples of PCR amplified plasmids

BBa_E0240, BBa_K823005 and BBa_K823012, restricted plasmids BBa_E0240, BBa_K823005 and BBa_K823012 and KAN backbone.
Electrocompetent cells were made of Top10 strain. /MI&MS



4.7. p. 26

Despite the fact that according to the gel made yesterday only one ligation was successful, all 7 ligation samples were miniprepped and restricted. A gel was run with the samples and also restricted separate pieces of BBa_E0240, BBa_K823005 and BBa_K823012 were added as a reference. The gel gave the same (bad) result as the colony PCR. /MI&MS



7.7. p. 26

The new Ykä strain (=YYC912) was decided to be blamed for strange results and the same ligations were decided to be tested in Top10 strain. Ligations BBa_K823005/BBa_E0240 and BBa_K823012/BBa_E0240 (2.7.14) were transformed in Topi (=Top10). Also from already restricted pieces the following constructs were assembled: BBa_K823005/BBa_E0240 in pSB1A3, BBa_K823012/BBa_E0240 in pSB1A3, BBa_K823005/BBa_E0240 in pSB1T3, BBa_K823012/BBa_E0240 in pSB1T3 and controls for these. AMP control gave sparks in electroporation twice so it was given up from transforming. To test the transformation efficiency, three most dilute samples of Sanni's plasmid serial dilution were used. Two plates were made from each sample, plating volumes were 150 ul and 10 ul. The plates were put in the +37 °C incubator. /MI, MS, OV, LV

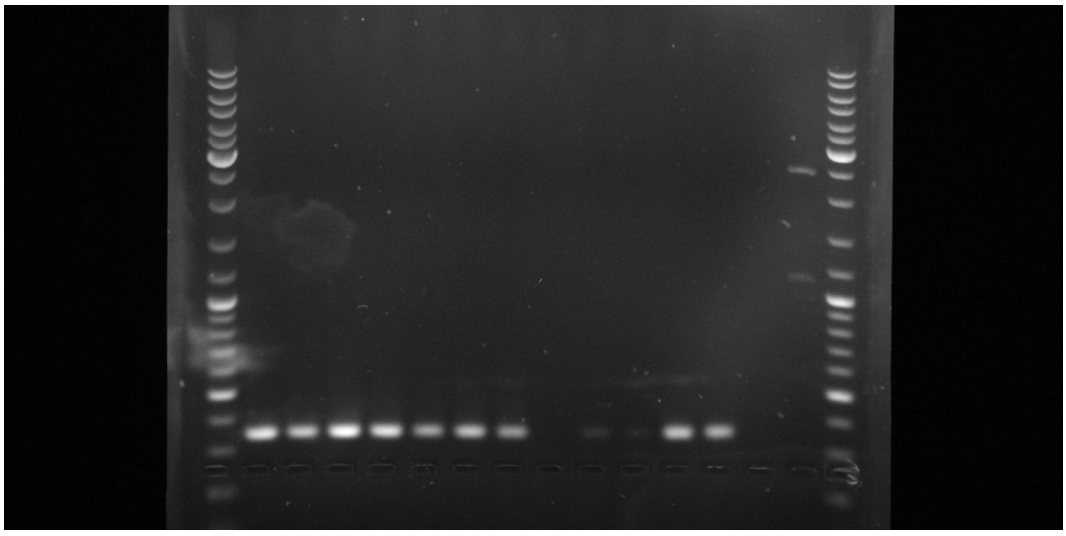
8.7. p. 28

On the plates grown overnight the colours of the colonies weren't clear enough, so the plates were put back in the incubator. On AMP plates the difference in colour was visible after about 20 hours of incubation. On TET plates the difference could not properly be seen and the colonies were very small, so the plates were put in the +30 °C incubator for another night. On KAN plates the growth was so weak that they were thrown away. The colony PCR was postponed to the next day.
A liquid culture of Top10 was made to make competent cells.
Biobricks BBa_K105004, BBa_K592016, BBa_K592006, BBa_K082003, BBa_K823008, BBa_K098991 and BBa_C0051 were transformed.
AMP, TET and CAM plates were prepared. /MI&MS

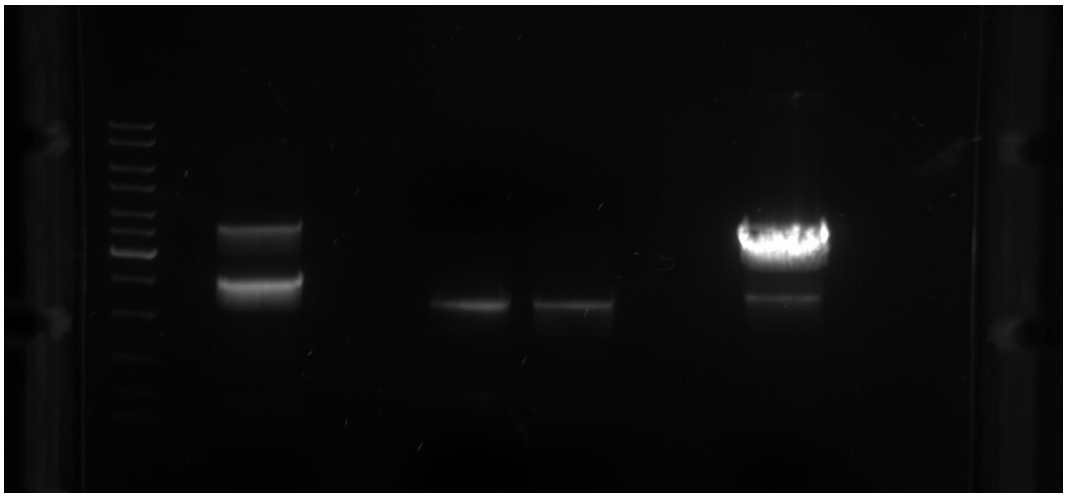
9.7. p.28

Dilutions were made of the Top10 liquid culture (1:100, 6x100ml). About 30 tubes á 100 ul electrocompetent cells were prepared.

A colony PCR was made of 12 colonies (8 AMP, 4 TET). Also a sample of miniprepped YYC912 was run to see if it had a plasmid of its own and a sample of the TET backbone to see if the restriction was complete. The gel showed that the ligations had not been successful (insert length appeared to be 350bp), YYC912 didn't have a plasmid and that TET backbone was OK.



TET, CAM ja AMP plasmid backbones were digested in big volume and run on a gel to purify at VTT. /MI, MS, LV & OL



14.7. p. 28

Biobricks BBa_R0051, BBa_K105004, BBa_I12007, BBa_R0065, BBa_C0040, BBa_R0040, BBa_K592016, BBa_K592006, BBa_K082003, BBa_K823008, BBa_K098991 and BBa_C0051 were inoculated from a plate to 3 ml of LB medium for miniprepping. Pieces cut from the gel purification gel (CAM, TET and AMP -backbones) were dissolved and the DNA was extracted using the DNA Gel Clean-up kit. /OV&MI

15.7. p. 30

Biobricks BBa_R0051, BBa_K105004, BBa_I12007, BBa_R0065, BBa_C0040, BBa_R0040, BBa_K592016, BBa_K592006, BBa_K082003, BBa_K823008, BBa_K098991 and BBa_C0051 were miniprepped and the DNA concentration was measured. Also the DNA concentration of gel purified plasmid backbones was measured.

Biobricks BBa_B0034 and BBa_K592016, BBa_K592006 and BBa_K082003 were restricted, and with previously restricted parts the following combinations were assembled and ligated: BBa_K592016/BBa_B0015, BBa_K082003/BBa_B0015, BBa_K592006/BBa_B0034, BBa_K823012/BBa_E0240, and BBa_K823005/BBa_E0240 was changed to pSB1C3 backbone. Also control ligations of CAM, TET and AMP were made. In the ligations purified backbones were used, and the ligation calculator was modified to take purified backbone into account.

Ligated plasmids were transformed to Top10 by electroporation. In the transformation a ratio of 0,7 ul of DNA and 48 ul cells was mostly used. For BBa_K082003+BBa_B0015 a ratio of 0,45 ul of DNA and 65 ul of cells was used to get the ion concentration low enough. The cells were plated and put to +37 °C incubator.

3 x 100 ml of SOB medium and 1 M $MgCl_2$ were prepared for autoclavation. /MI&MS

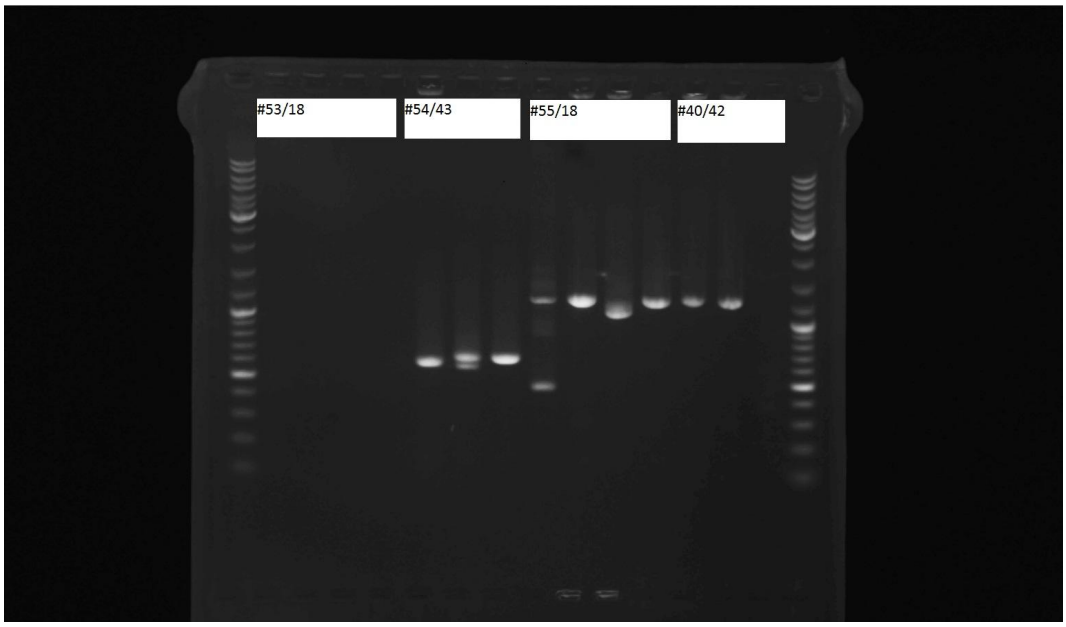
16.7. p. 30

The cells plated yesterday had again grown very slowly and the colour difference wasn't evident, so the colony PCR was postponed to the following day. The effectiveness of YYC912 was tested

by transforming the ligations made yesterday (excluding the ones with pSB1C3 backbone) to the YYC912. /MI, MS & LV

17.7. p. 30

Colony PCR was run of the ligations (Top10), and the samples were run on a gel. About 50 pcs à 100 ul of heat shock competent cells of the YYC912 strain were prepared. Liquid cultures were made of samples BBa_K082003/BBa_B0015, BBa_K823012/BBa_E0240 (both successful), BBa_K592016/BBa_B0015 (disappeared on the gel), BBa_K592006/BBa_B0034 (moved in a strange way). /MI&MS



21.7. p. 30

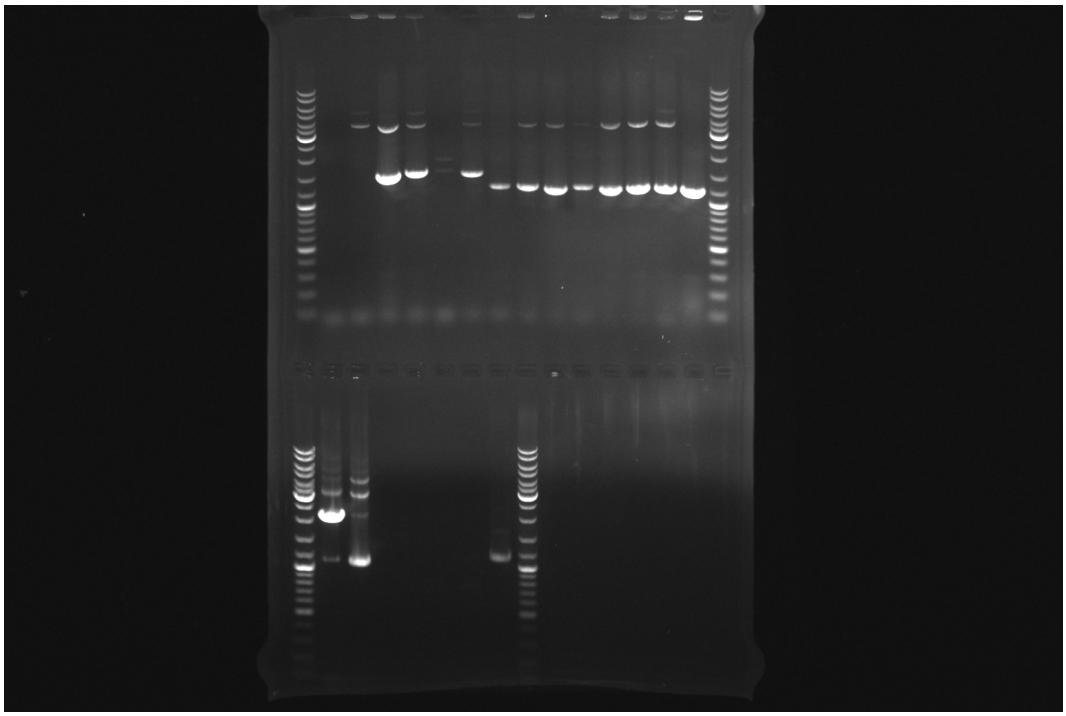
Ligated bricks BBa_K592016/BBa_B0015 and BBa_K592006/BBa_B0034 (colonies 4 and 5) were miniprepped. Bricks BBa_K592016/BBa_B0015, BBa_K592006/BBa_B0034, BBa_K823008 and BBa_K082003/BBa_B0015 and also BBa_K823012/BBa_E0240 and BBa_K823005/BBa_E0240 were restricted. Following ligations were made: BBa_K592016/BBa_B0015/BBa_K823008 (pSB1T3), BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 (pSB1C3), BBa_K823005/BBa_E0240 (pSB1C3) and BBa_K823012/BBa_E0240 (pSB1C3). The ligated plasmids were transformed to heat shock competent cells (YYC912 and XL1-blue). 150 ul of cell suspension was plated and the plates were put to the +37 °C incubator. /MI&MS

22.7. p. 32

New primers were suspended in water. Their functionality was tested with following samples: Prefix-R/Suffix-F: BBa_E0020 (CFP), AMP and TET plasmids, for RFP binding primers: all backbone plasmids. Also colony PCR was made of ligations of the previous day.

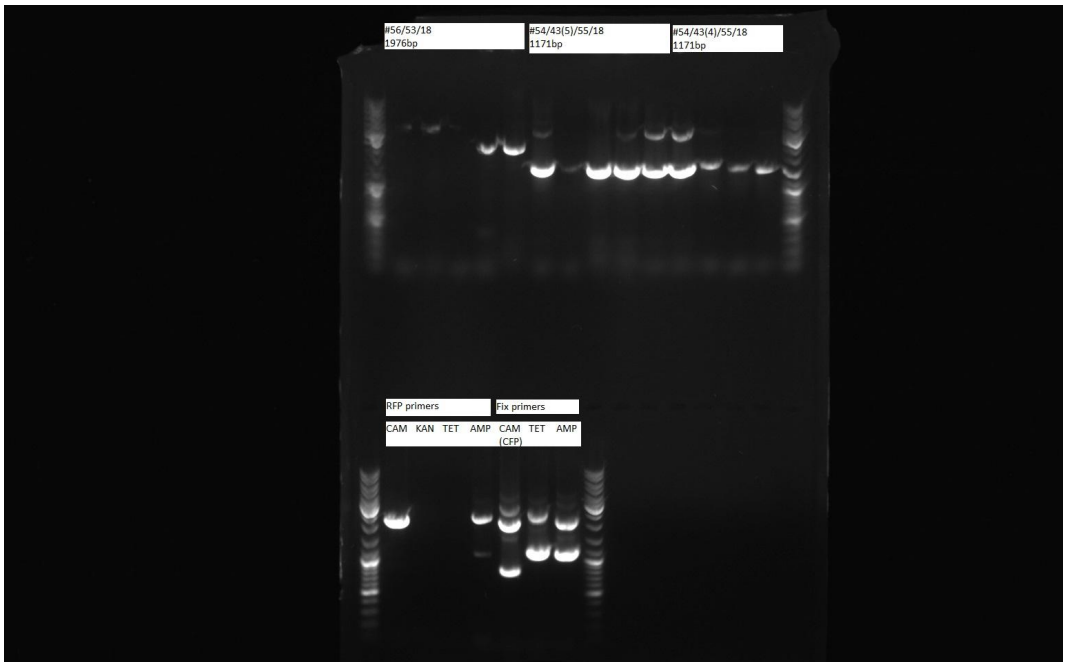
To test the efficiency of PCR-cleanup kit in deionization, the ligation mixes of the previous day were purified with the kit and transformed to Top10 by electroporation. The results were slightly

better than without purification (a bit more DNA to a bit smaller volume of cells). The PCRed samples were run on a gel. /MI&MS



23.7. p. 32

Bacteria transformed to Top10 had grown very well and colony PCR was made of them. The new primers were tested again, because the gel made yesterday showed that we had amplified backbone in only one sample/well. The gel was made of different agarose powder, and the solidifying took about an hour [the gel was later found out to be low-temp melting]. PCR samples were run on the gel. /MI&LV



24.7. p. 32
Liquid cultures were made. /MI&LV

25.7. p. 32
New biobricks BBa_B0032, BBa_E0032, BBa_E0022 and BBa_K592009 were transformed to Top10 electrocompetens cells (1 ul DNA, 50 ul cells). Some samples were miniprepped for sequencing. 3 out of 4 backbone liquid cultures (TET, KAN and AMP) were miniprepped for gel purifying at VTT. The liquid culture of CAM backbone wasn't red so it was discarded. /MI, LV&OV

27.7. p.32
Liquid cultures were made of the new biobricks BBa_B0032, BBa_E0032, BBa_E0022 and BBa_K592009 with 3 ml LB and 2,6 ul CAM. The cultures were put to the +37 °C incubator at 11.32. /LV&OV

28.7. p. 34
Bricks BBa_B0032, BBa_E0032, BBa_E0022 and BBa_K592009 were miniprepped. Biobrick BBa_R0040 was digested and BBa_R0040/BBa_B0034 (in pSB1T3) ligation was made. A ligation control was also made.

Sequencing samples were run through the PCR purification kit and the DNA concentration was measured. To all 16 (2x8) tubes primers, water and samples were pipetted according to the instructions given by the sequencing lab. The samples were wrapped in bubble wrap and enclosed in an envelope. The envelope was put in the fridge and in the morning it would be sent.

BBa_R0040/BBa_B0034 ligation and the control was run through the PCR Cleanup kit and transformed to Top10 strain. The cells were plated on tetracycline plates at 18:20. /LV&MS

29.7. p. 34
The ligation control plate contained many colonies (all white!). The BBa_R0040/BBa_B0034 plate also had plenty of colonies (of which few were red). Thus, something mysterious had happened in the ligation. The ligation was re-made using BBa_R0040 digested yesterday. Also, by accident, the non-gel purified backbone had been used yesterday. In the new ligation the purified backbone was used. The transformation was successful when 60 ul of cells and 0,70 ul DNA was used (deionized and purified DNA). 150 ul cells were plated.
CAM backbone liquid culture was made for gel purification. /MS&LV

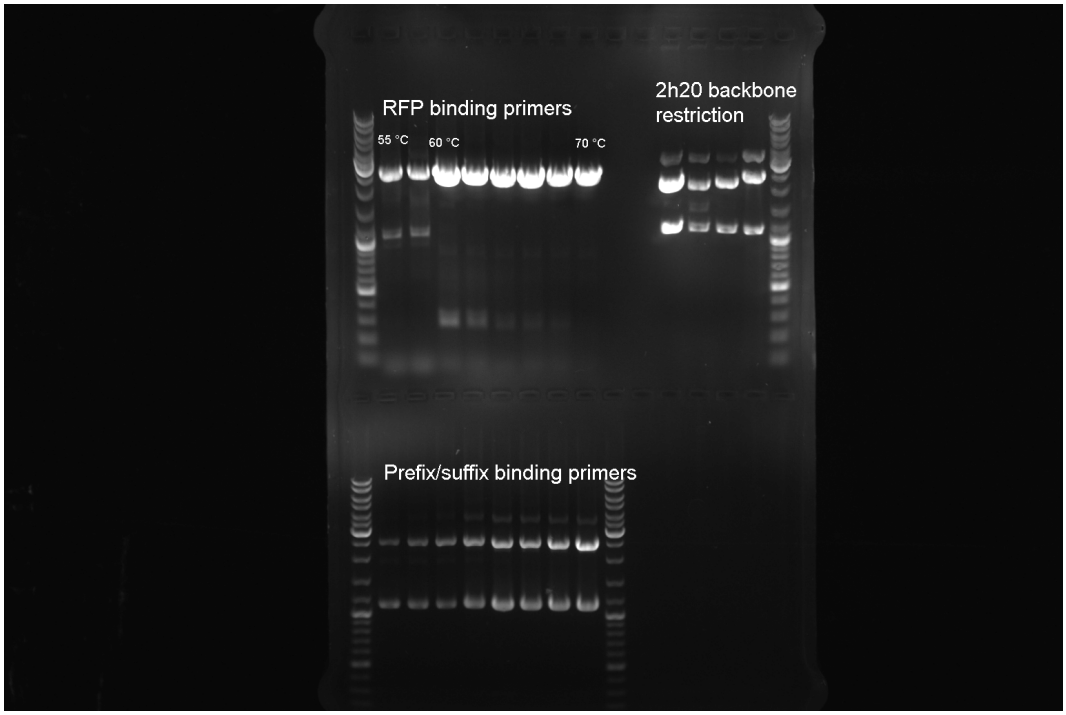
30.7. p. 34
The ligation control still had plenty of white colonies just like the ligation plate (this time no red colonies). We decided to make a colony PCR of colonies from today and yesterday in case some of them were good. Also two colonies from control plate were taken for colony PCR.

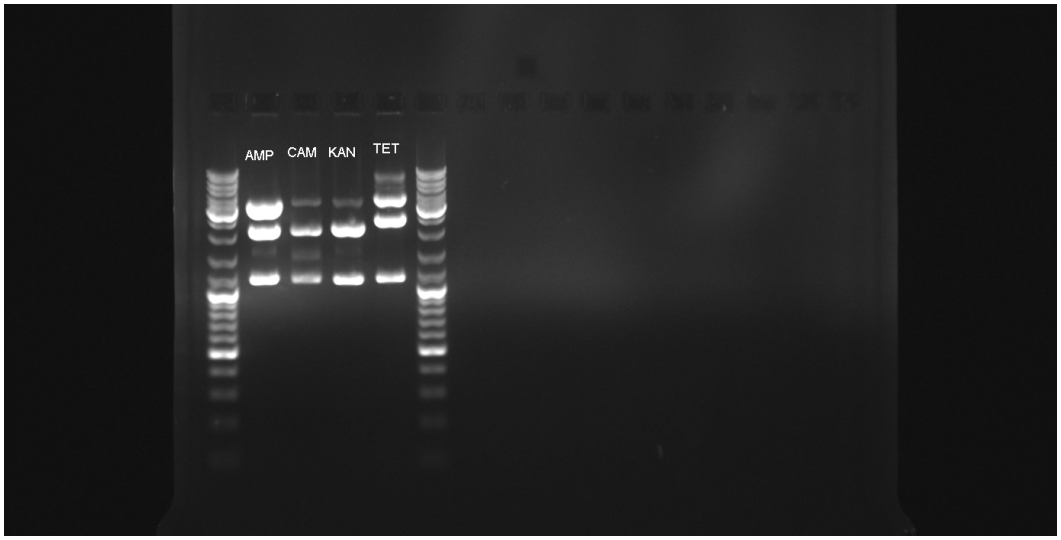
The CAM backbone was miniprepped. The liquid culture was light yellow (instead of red) but presumably right.

According to the gel run of the colony PCR, the TET backbone had some problems. Colony PCR samples and water culture backups were stored in the freezer, to be thrown away when the gel is fully analyzed. /LV&MS

31.7. p. 36

New primers were tested in the temperature gradient +55-70 °C (pSB1T3 backbone as the template). Miniprepped backbone plasmids were digested 3h 45min in +37 °C. The gel was run twice, after 30 min and after 2 h 20 min of digestion. /MI&MS




AMP
CAM
KAN
TET