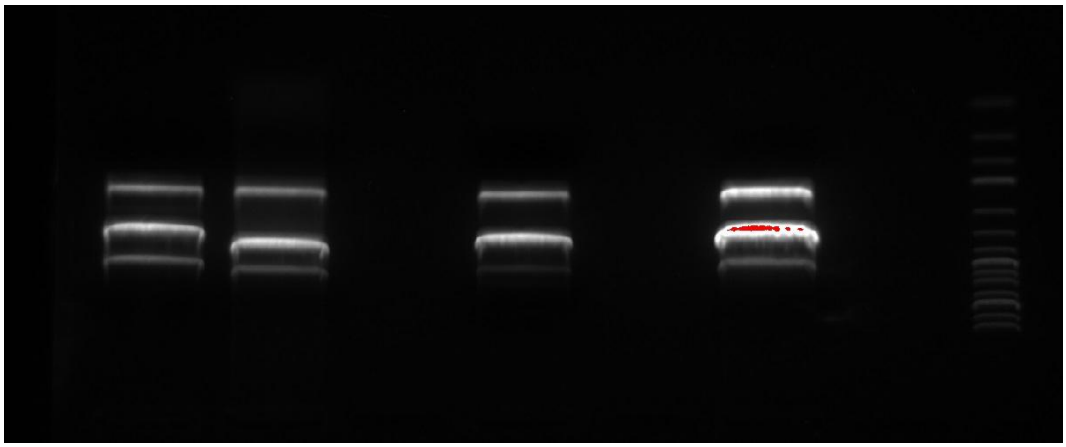
Lab Notebook, Team Aalto-Helsinki 2014, August

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

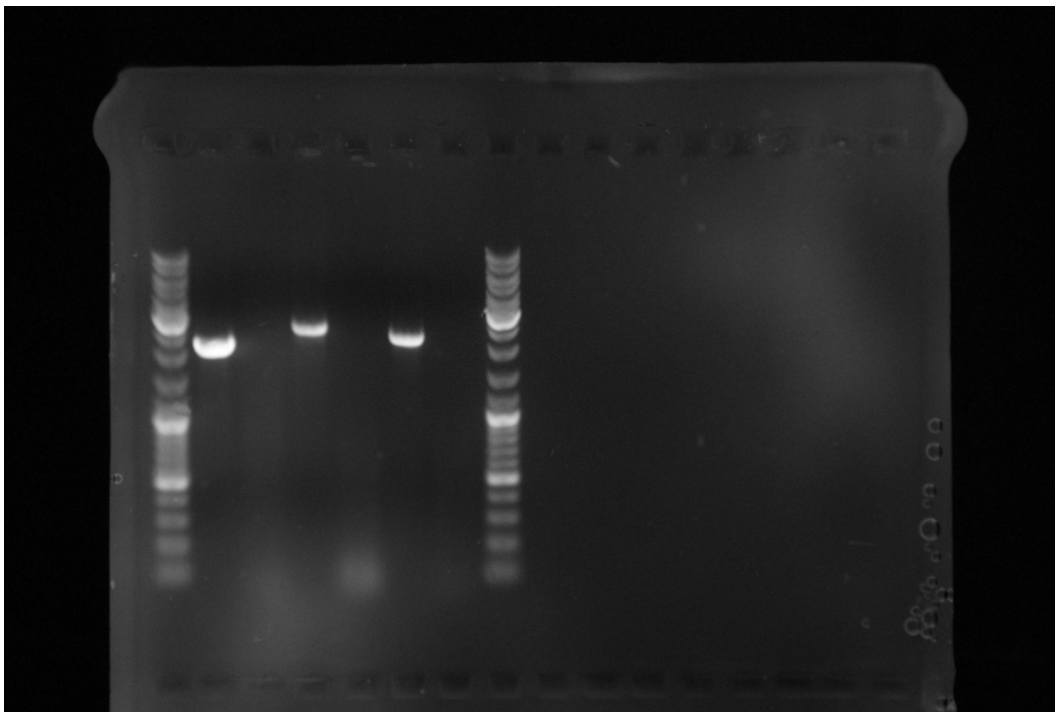
1.8. p. 36

Bartosz run the gel purification of digested backbones at VTT. Using the Gel purification kit the DNA was extracted. AMP, TET and CAM backbones were amplified in PCR using RFP binding primers and annealing temperature of +60 °C. /MI&MS



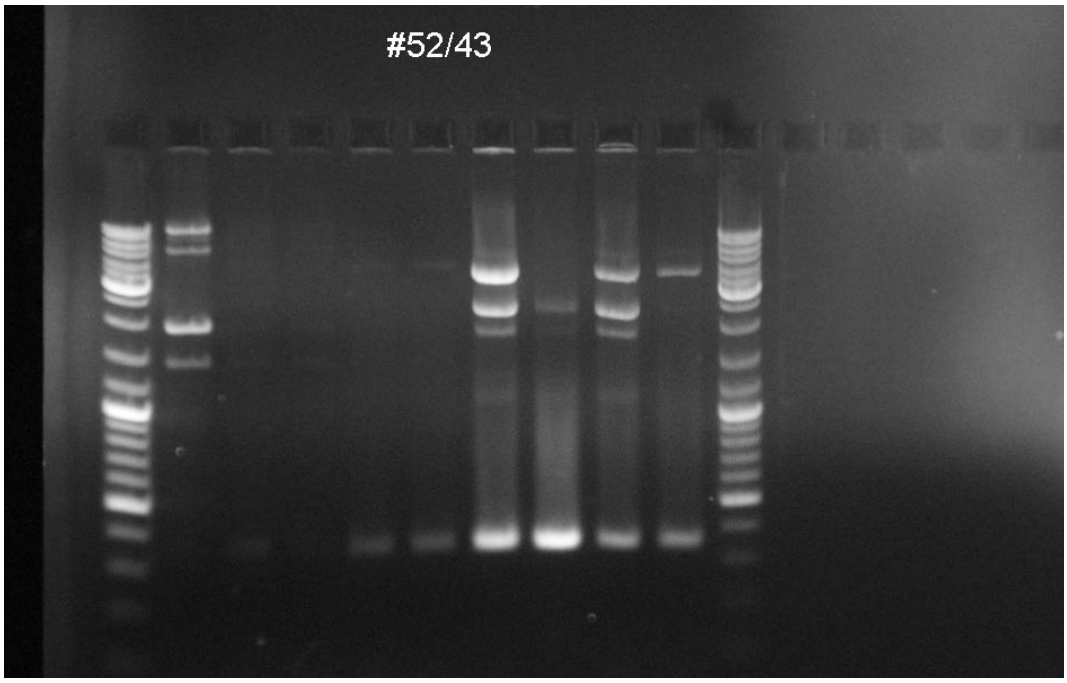
4.8. p. 36

The DNA concentration of the backbones purified and amplified last week were measured. BBa_R0040 and amplified backbones were digested and backbones were run on a gel. It showed that the samples didn't contain what they should (empty gel, but high concentrations on nanodrop). BBa_R0040 and BBa_B0034 were ligated to TET backbone (gel purified) and transformed to Top10 (60 ul cells + 0,8 ul DNA). 100 ul of cell culture was plated. For Demo Day three plates were prepared containing bacteria from a phone touch screen, washed hands and saliva. /MI&MS



5.8. p. 36

Ligation control plate was empty, so the ligation was assumed to be succesful. Nine colonies were chosen to colony PCR and gel. Also backups from colonies were made inoculating cells to 20 ul water (on ice). One sample looked good and the backup was suspended in 3 ml LB + 6 ul TET. The liquid culture was put to +37 °C overnight. /MS&LL



6.8. p. 36

The liquid culture of BBa_R0040/BBa_B0034 was miniprepped. Only 10 ng/ul (50ul) of plasmid was extracted, so it should be retransformed to prevent the sample from running out. /MI&MS

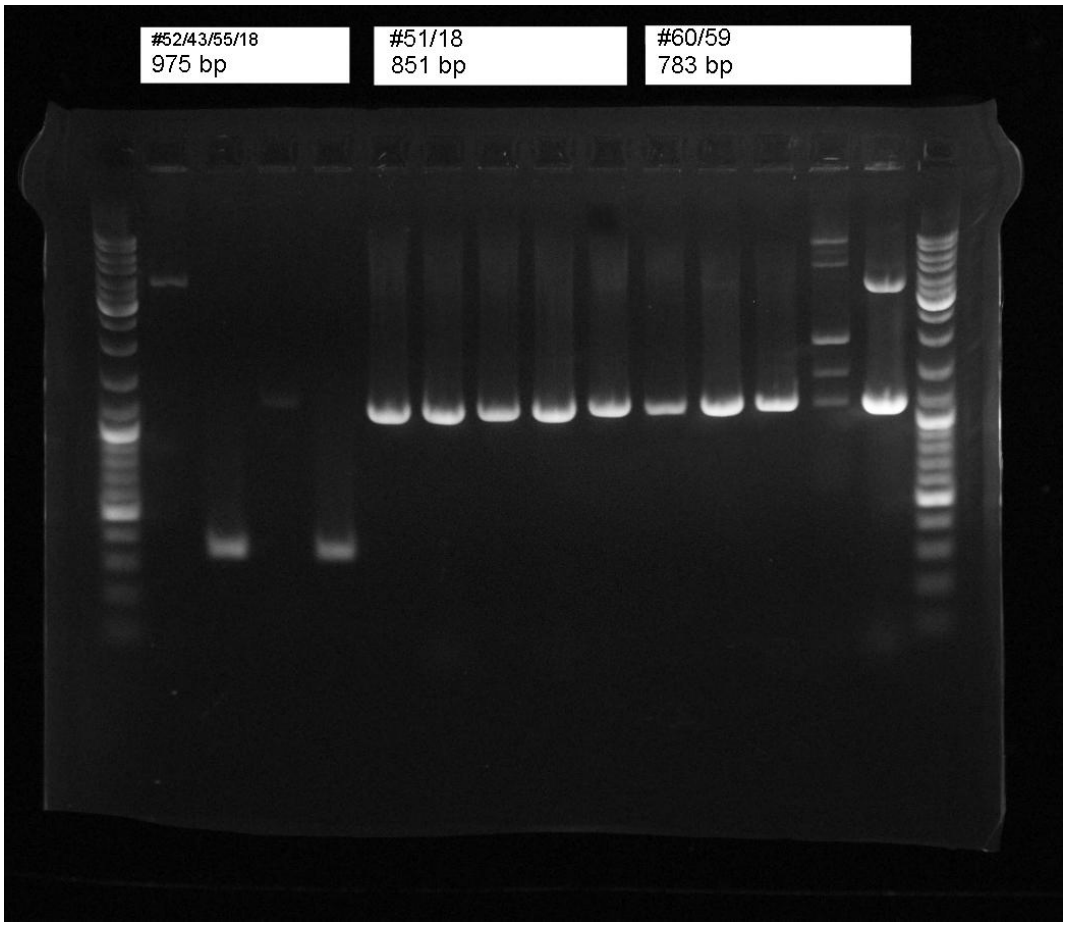
7.8. p. 36

Bricks BBa_R0040/BBa_B0034, BBa_C0040, BBa_B0032 and BBa_E0032 were restricted. As the concentration of BBa_R0040/BBa_B0034 was only 10 ng/ul, its concentration in the restriction mix was only 0,5x. This was compensated in the ligation mix where 8 ul of restriction mix was pipetted (instead of 4ul, the normal amount).
Biobricks BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 (pSB1C3), BBa_E0032/BBa_B0032 (pSB1A3) and BBa_C0040/BBa_B0015 (pSB1A3) were ligated and transformed to Top10. To make sure we wouldn't run out of miniprepped ligation of BBa_R0040/BBa_B0034, it was also transformed to Top10 (twice). /MI&MS

8.8. p. 38

A colony PCR of yesterday's ligations was made. According to the gel, the ligations might have been successful (the bands were pretty close to where they should've been). Liquid cultures were made of the good samples (colonies 3, 6 and 11) and they were put in the + 37 °C incubator overnight. According to the sequencing results BBa_K823008/BBa_K592016/BBa_B0015 and BBa_K823005/BBa_E0240 weren't right so we started building them again.
BBa_K823008, BBa_K592016, BBa_B0015, BBa_K823005 and BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 were digested. BBa_K823005 +

BBa_E0240 (in pSB1A3), BBa_K823008 + BBa_K592016 (in pSB1A3) and BBa_B0015 + BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 (in pSB1A3) were ligated together. All ligations were transformed to Top10 with the ratio of 60 ul of cells and 0,70 ul of DNA in electroporations (BBa_B0015 + BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 made an exception and we had to use 65 ul of cells and 0,50 ul of DNA).
BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 was also transformed again since we were about to run out of the miniprepped plasmid. A liquid culture of BBa_R0040/BBa_B0034 was made because the concentration of the previously miniprepped sample was so low and we were about to run out of it. /MI&MS

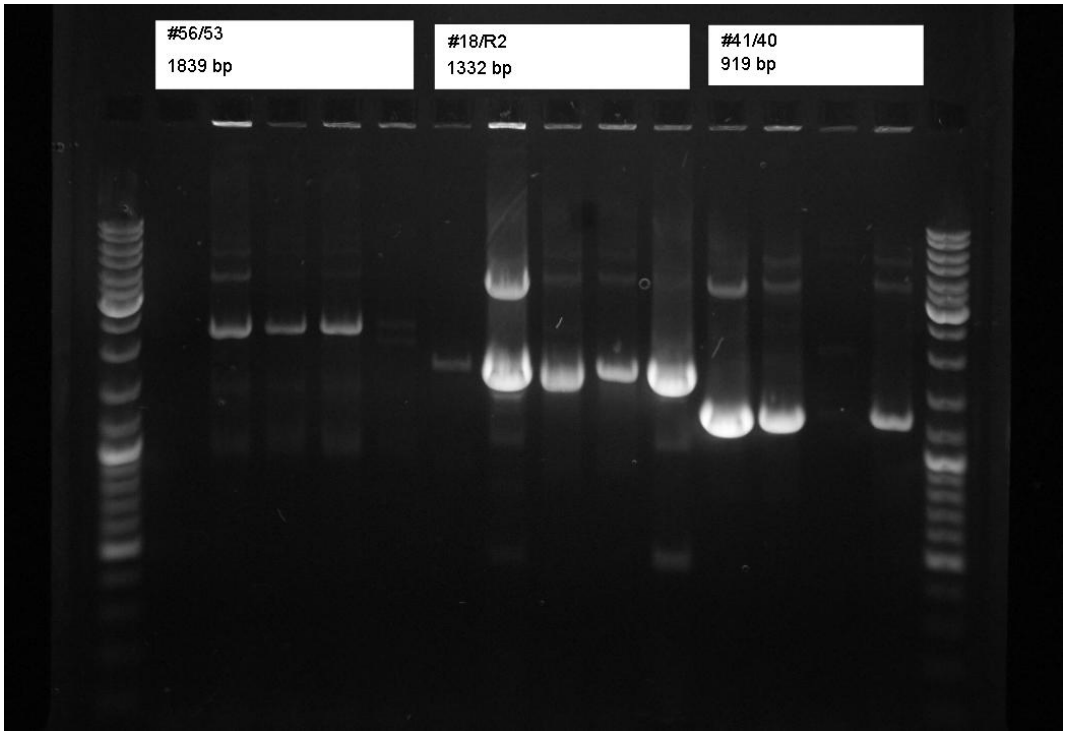


9.8. p. 38

Plates and liquid cultures grown overnight were put in the cold room (+4 °C). Ligation control plate was empty and the plate that had the BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 ligation had a bacterial lawn. /MS&PR

10.8. p. 38

A colony PCR of the ligations BBa_K823005/BBa_E0240, BBa_K823008/BBa_K592016 and BBa_B0015/BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 was made and a gel run of the samples. Liquid cultures were made of successful ligations and the rest were put in the cold room, just in case we needed them later. The liquid cultures made on Friday were miniprepped and CAM plates were prepared./MS&LV



11.8. p. 38

Ligated Bricks BBa_K823008/BBa_K592016, BBa_E0032/BBa_B0032, BBa_C0040/BBa_B0015, BBa_B0015/BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 and BBa_K823005/BBa_E0240 were restricted. BBa_K823008/BBa_K592016 + BBa_B0015 (in pSB1T3), BBa_E0032/BBa_B0032 + BBa_C0040/BBa_B0015 (in pSB1C3), BBa_K823008/BBa_K592016 + BBa_B0015/BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 (in pSB1C3) and BBa_K823005/BBa_E0240 (changing backbone to pSB1C3) were ligated together. Control ligations of the CAM and TET backbone were also made. All ligations were transformed to Top10 (60 ul cells + 0,65 ul of DNA in electroporations) and plated.
BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 was also retransformed since the last attempt failed. A stock solution of 10 mg/ml of tetracycline was prepared and put in the -20 °C freezer.

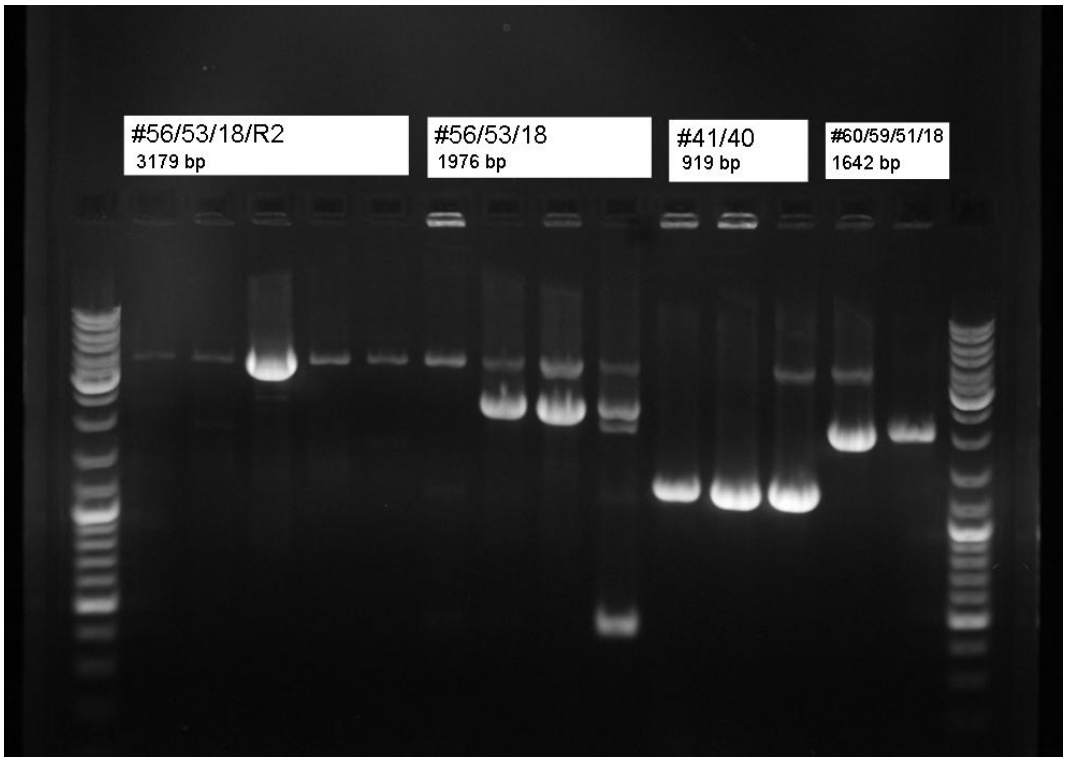
12.8. p. 38-40

Ligation control plates were empty but the plate that had the BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 ligation still had a bacterial lawn. To prepare competent cells the liquid culture of Top10 was diluted 1:100 (3 x 100 ml) and put in the shaker in the +37 °C incubator. After 2 h 40 min the OD600 was 0,6-0,7 and the cultures were put on ice. A colony PCR of yesterday's ligations was made.
Competent cells were made according to [the protocol that can be found in our wiki].
BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 was transformed again but this time the DNA sample was diluted by 1:50 before electroporation and the cell culture was diluted 1:100, 1:10 and 1:1 before plating. /MS, MI, LV&OL

13.8. p. 40

A gel run was made of yesterday's colony PCR. All plates had had at least one colony that had the correct plasmid. Yay! Liquid cultures were made of the successful ligations.

An inventory was made in the cold room and -20 °C freezer and old, useless plates and tubes were thrown away.
Liquid cultures were made of the finally successful transformations of BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015, BBa_K823008/BBa_K592016/BBa_B0015 (ligated on 11.8.), BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015, BBa_K823008/BBa_K592016/BBa_B0015/BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015 and BBa_K823005/BBa_E0240 (in CAM). /MI&MS



14.8. p. 40

BBa_K823008/BBa_K592016/BBa_B0015 had not grown in the LB but all the other liquid cultures had growth in them. Colony number 7 and 8 (of the ligation BBa_K823008/BBa_K592016/BBa_B0015) were reinoculated onto two plates, one with old tetracycline and one with the new stock since we thought the new stock of TET might be the problem. The rest of the liquid cultures were miniprepped.
BBa_I20260, BBa_E0032/BBa_B0032, BBa_C0040/BBa_B0015 and BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 were deionized by using the PCR purification kit (elution with water). Water was also used in elution when miniprepping. Samples sent to be sequenced were prepared. BBa_I20260 and BBa_K823012/BBa_E0240 were retransformed to Top10 to make sure that we had the miniprepped sample from the same colony and could send it for sequencing. A liquid culture of BBa_I20260 was also made because the concentration of the previous miniprep was so low (~ 10 ng/ul).
Previously reinoculated colony 7 (from BBa_K823008/BBa_K592016/BBa_B0015) was inoculated to two more liquid cultures with old and new tetracycline. /MI&MS

15.8. p. 42

On the plate with new tetracycline there were colonies but the liquid culture with new TET was clear. BBa_I20260 and BBa_K823008/BBa_K592016/BBa_B0015 were miniprepped and the samples were incubated in +70 °C for 5 minutes with before elution with water. DNA concentrations were measured.
Rest of the sequencing samples were prepared and all the samples were stored in the cold room. /MI&LV

19.8. p. 42

The synthesized genes arrived. The tubes were centrifuged and the dry DNA was suspended in 20 ul of water. The parts were named S1 and S2. S1, S2 and BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 were transformed to Top10 and the plates were put in the +37 °C incubator.
A liquid culture of our test unit (BBa_K823008/BBa_K592016/BBa_B0015/BBa_K592006/BBa_B0034/BBa_K082003/BBa_B0015) and BBa_K823005/BBa_E0240 was made in 3 ml LB and it was put to grow in 37 °C under constant light overnight. BBa_K823005/BBa_E0240 and the test unit were reinoculated on new plates. /MI&MS

20.8. p. 42

At 11.01 1,5 ml of the test unit liquid culture was put into another tube and it was covered with foil so that no light could penetrate it. Both tubes (light and dark cultures) were put back in the incubator. There was no visible difference in GFP under UV light at 14.30.
BBa_K823008/BBa_K592016/BBa_B0015, BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015, S1, S2, BBa_E0022 and BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 were restricted.
BBa_K823008/BBa_K592016/BBa_B0015 + BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 (in pSB1K3), S1 + BBa_E0022 (in pSB1T3) and S2 + BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 (in pSB1T3) were ligated together and transformed to Top10. Control of TET and KAN backbones were made.
Liquid cultures of BBa_K823008/BBa_K592016/BBa_B0015 (about to run out), BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015, S1 and S2 were made. /MI&MS

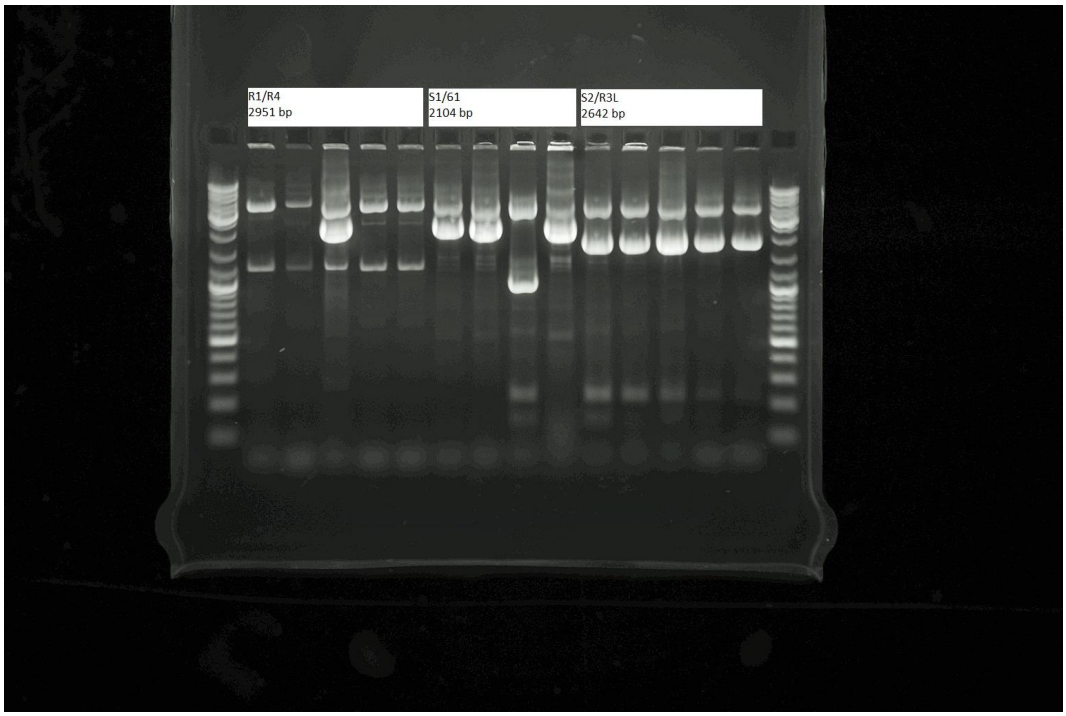
21.8. p. 42-44

The ligation controls were empty but on all other plates the colonies were still so small that they were put back in the incubator and colony PCR was postponed to the following day. The liquid culture of BBa_K823008/BBa_K592016/BBa_B0015 had also grown slowly so it was also put back in the incubator. Rest of the liquid cultures were ok and they were miniprepped. As a precaution if the promoter in BBa_K823008/BBa_K592016/BBa_B0015 were to be too weak, a ligation of BBa_K823005 + BBa_K592016/BBa_B0015 was made and transformed to Top10.
As the transformations done yesterday had so little colonies, BBa_K823008/BBa_K592016/BBa_B0015/BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 and S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 were retransformed in Top10 and all cells were plated by centrifuging the cells down./MI&MS

22.8. p. 44

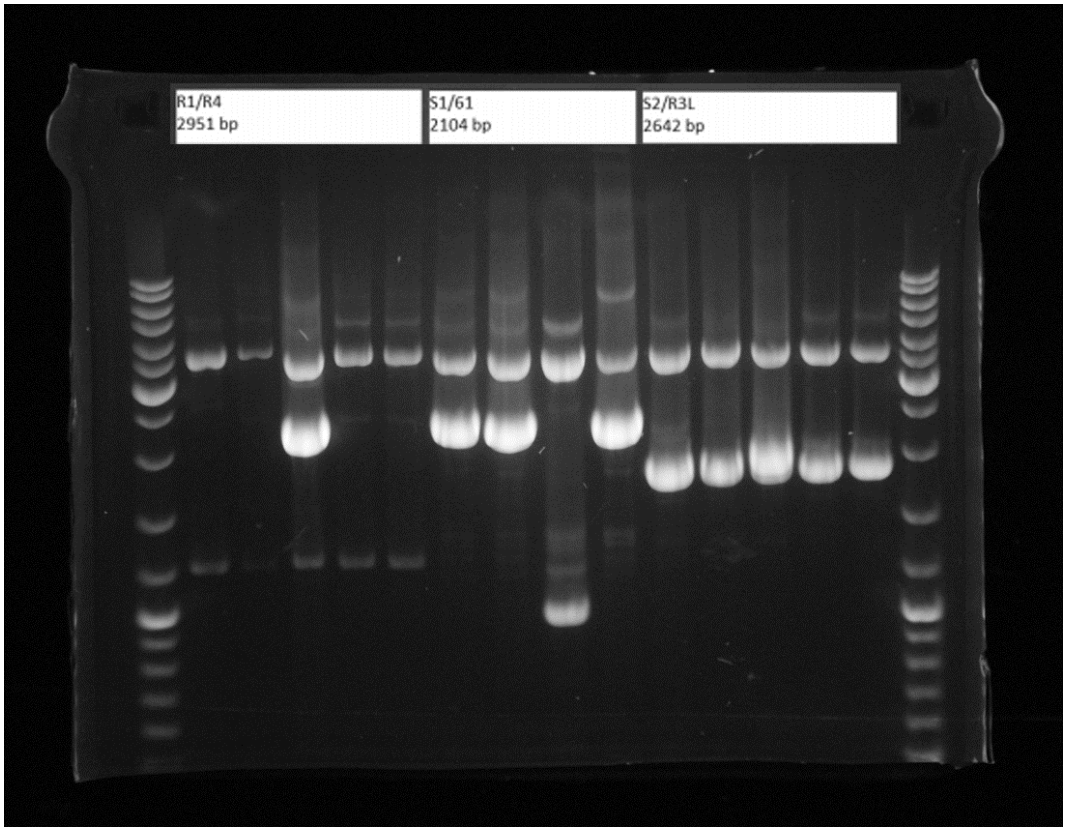
A colony PCR was made of the ligations of the day before yesterday and a gel was run. We also got an opportunity to test a programmable microscope that can detect GFP. After incubating for two days BBa_K823008/BBa_K592016/BBa_B0015 had grown enough and it was miniprepped.

The gel that had been run today was hard to interpret as it wasn't run long enough and it was decided to be run again on Monday. /MI&MS



25.8. p. 44

A new gel run with longer running time was done. It could be seen that the ligation of S2 + BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 hadn't succeeded. The rest seemed ok. Liquid cultures of BBa_K823008/BBa_K592016/BBa_B0015/BBa_R0040/BBa_B0034/BBa_K082003/BBa_B0015 and S1/BBa_E0022 were made for miniprepping. For testing our construct, a liquid cultures of the test unit were made and they were put in the +37 °C incubator in light and in dark (covered with foil). As controls liquid cultures of BBa_K592009 (negative) and BBa_K823005/BBa_E0240 (positive) were made. Also liquid cultures of the Measurement Interlabs Study devices BBa_I20260, BBa_K823005/BBa_E0240 and BBa_K823012/BBa_E0240 were prepared. /MI&MS



26.8. p. 44

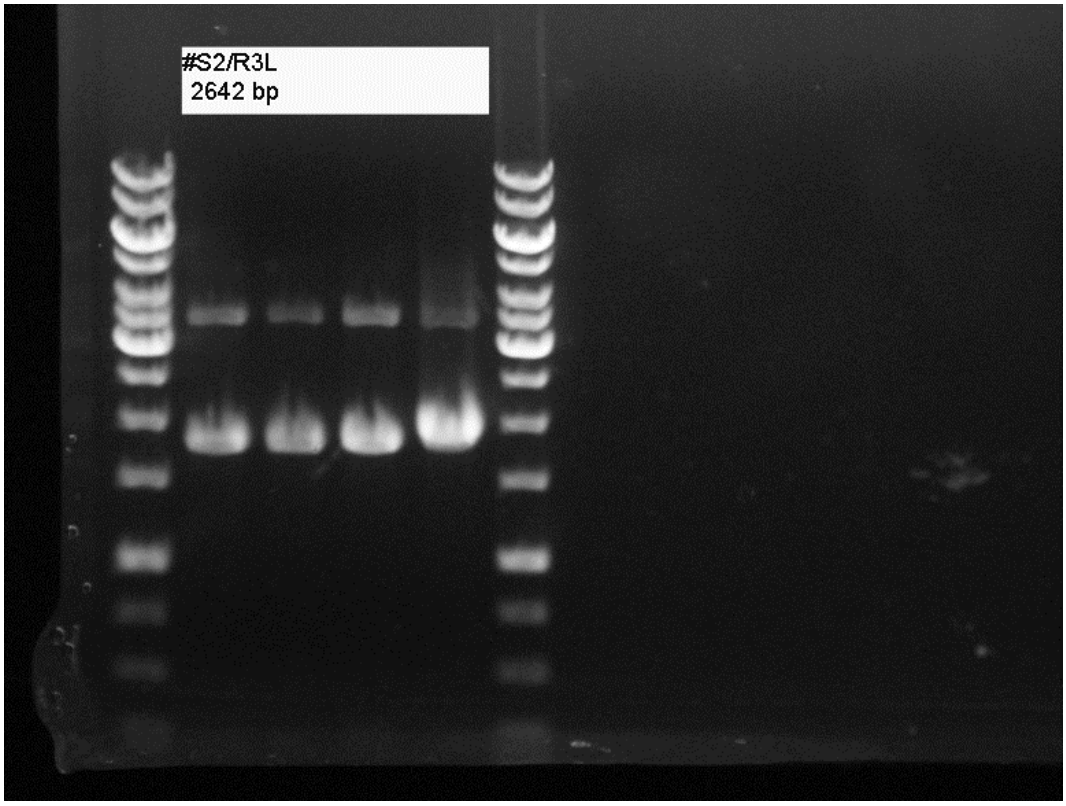
Miniprep of liquid cultures. Fluorescence measurements at VTT. Sequencing samples were prepared. LEDrig was tested: incubation 11-14.30, fluorescence measurement at 15.00, 15.15 back in the incubator. /MI, MS, OV&PR

27.8. p. 44

Second measurement of the LEDrig cultures (23 h) at VTT. Religation of S2 + BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 and transformation to Top10 was made, 150 ul and the rest (of the cells) were plated. More liquid cultures (BBa_K823005/BBa_E0240, test unit and BBa_K592009) were made for LEDrig testing purposes. A liquid culture of BBa_I20260 was made for getting the backbone (pSB3K3).
The OD600 was measured of the testing liquid cultures but they were too low and they were decided to be left in the incubator overnight. Minimal media was prepared to be autoclaved. /MI&MS

28.8. p. 44-46

Minimal media was prepared to be used by adding glucose, MgSO4 and Na2HPO4. The OD600s of the testing liquid cultures were measured and they were diluted in minimal media (LB was removed by centrifuging) so that the OD600 was 0,5 in all samples. 5 x 100 ul of each sample was pipetted in the microtiter plate. Each sample was incubated in 5 different light intensities and the fluorescence was measured every hour.
A colony PCR was made of S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 and the samples were run on a 0,7% gel (a new ladder that defines larger plasmids better was also used). New Biobricks BBa_E0430, BBa_E0420 and BBa_K823004 were transformed to Top10. /MI&MS

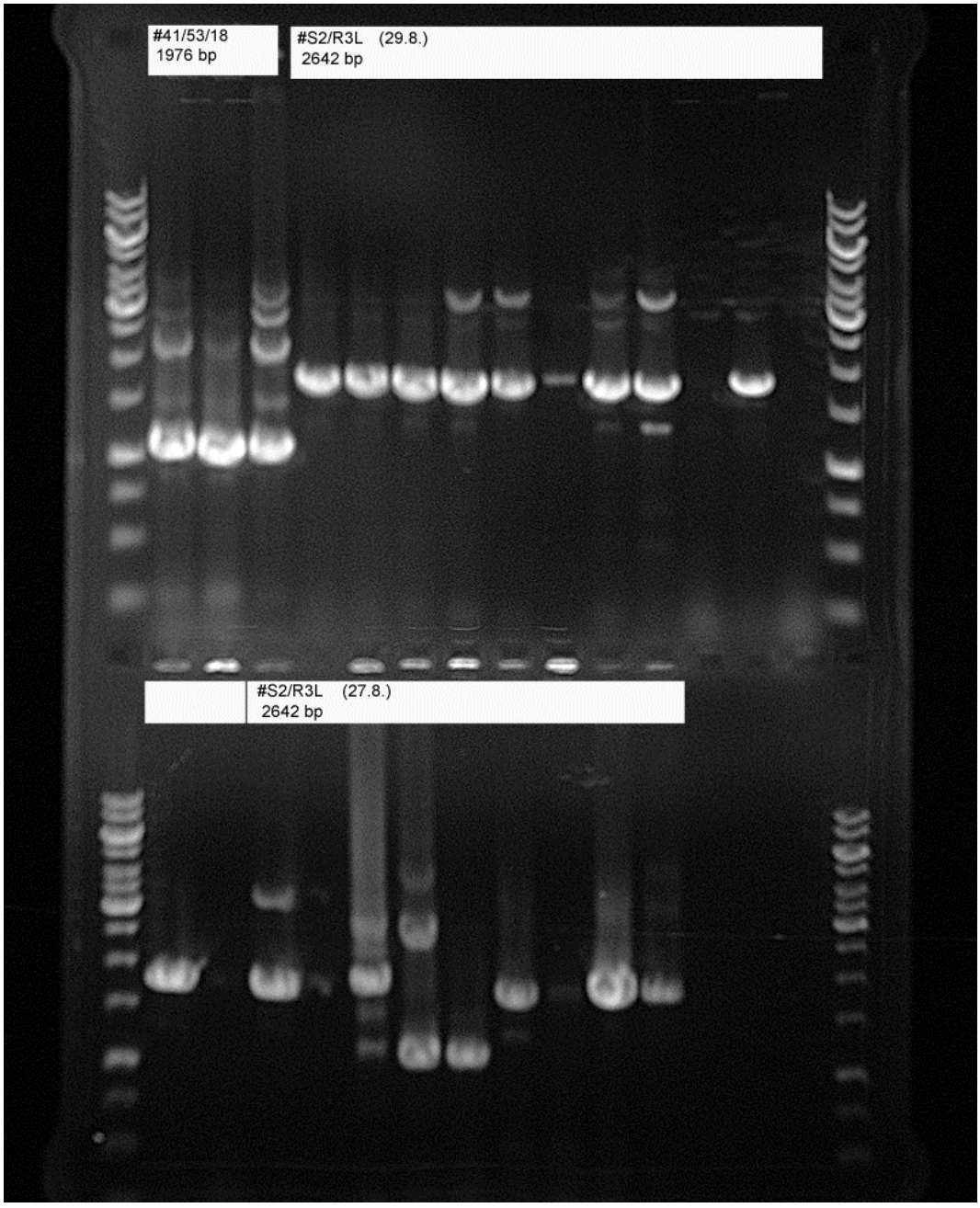


29.8. p. 46

S2 and BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 were restricted again and then ligated together in pSB1T3 backbone. The ligation was transformed in Top10 and 150 ul and the rest of the cells were plated. On the control plate all cells were plated. pSB3K3 (from BBa_I20260) was restricted and then gel purified at VTT. Liquid cultures of BBa_E0430, BBa_E0420 and BBa_K823004 were made. At VTT a Quikchange mutation kit was also run to remove some of the LVA tags in S1 [none of these mutated parts were used in the end]. The sample and control were incubated for 1 h 30 min and inactivation was done in + 80 °C for 5 minutes. The rest was done according to the Quikchange protocol (no transformation was done yet). /MI, MS&OV

30.8. p. 46

Liquid cultures of BBa_E0430, BBa_E0420 and BBa_K823004 were miniprepped. pSB3K3 backbone was extracted from the purifying gel. BBa_E0430, BBa_E0420 and BBa_K823004 were restricted. BBa_K823004 + BBa_E0430 and BBa_K823004 + BBa_E0420 were both ligated in the normal pSB1K3 and pSB3K3 backbones. All ligations were transformed in Top10. A colony PCR was done of BBa_K823005/BBa_K592016/BBa_B0015 (done 21.8.) and two different S2/BBa_E0032/BBa_B0032/BBa_C0040/BBa_B0015 ligation batches (done 27.8. and 29.8.). A 0,7% gel was run with the new ladder (1 kb #SM0313). One of the wells (second row, 5th from left) on the gel was left empty as some of the sample from a well next to it leaked into it. /MS&LV


#41/53/18
1976 bp
#S2/R3L (29.8.)
2642 bp
#S2/R3L (27.8.)
2642 bp