Lab Notebook, Team Aalto-Helsinki 2014, May-June

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

20.5. p. 2

Antibiotics were put in the 3rd drawer of the freezer in the gel room. Chloramphenicol (34 mg/ml) was put in the "iGEM" box in the freezer room. 500 ml of LB was used to make plates, of which 200 ml were non-antibiotic plates and 300 ml were chloramphenicol (25 ug/ml). All plates were put in the "iGEM" shelf in the cold room. /LV

21.5. p. 2

An XL1-blue strain grown overnight on a plate was inoculated onto a liquid LB medium with 10 ug/ml of tetracycline. The culture was put in a shaker in a 37 °C incubator. /MS&OV

22.5. p. 2-4

250 ul of the overnight grown liquid culture was pipetted into a sterile conical flask and 25 ml of LB and 50 ul of tetracycline (5 mg/ml) were added. The liquid culture was put in the shaker in a 37 °C incubator. The OD600 was measured after 1 h 40 min and it was 0,12. Meanwhile a TSS buffer was prepared. After 2 h 30 min the OD600 was 0,247.

Heat-shock competent XL1-blue cells were made according to the following protocol.
11 ml of cell culture was pipetted into two 50 ml falcon tubes and centrifuged 3000 rpm at 4 °C for 10 minutes. After that the supernatant was removed. Contrary to the instructions, the cell pellet was resuspended in 2 ml of cold TSS. The cell culture was then divided into small eppendorf tubes, 100 ul in each (done under the hood). Altogether there were about 50 tubes and they were shock freezed with liquid nitrogen and then put in the -80 °C freezer.

Transformation efficiency:
Transformation efficiency kit was taken out of the -20 °C freezer and thawed on ice as were three tubes of competent cells. DNA tube were spinned down and 1 ul of each concentration (0,5 pg/ul, 5 pg/ul, 10 pg/ul, 20 pg/ul, 50 pg/ul) was pipetted into a 2 ml sterile eppendorf tube. 50 ul of competent cells were also pipetted into the tubes. A control was prepared the same way as other samples but with no DNA in it. All six tubes were incubated on ice for 30 minutes, after that they were put on a 42 °C water bath for 60 seconds and then put back on ice for 5 minutes. 200 ul of SOC buffer was added to each tube under the hood and the tubes were put in a 37 °C incubator for two hours. 20 ul of each sample was inoculated onto a plate under the hood and the plates were put in a 37 °C incubator overnight. The tubes containing rest of the samples were put in the +4 °C cold room in case the transformation failed. They can be discarded later./MS,ML&OV

23.5. p. 4-6

All six plates were empty. Possible reasons are:
- competent cells failed
- too low of a cell density (the most obvious reason as the OD was quite low and the cell pellet was suspended in more TSS than adviced)
- wrong antibiotic plate

Today we will do the following:

- a serial dilution of the competent cells (1-1/10 000)
- plate the samples again with a volume of 50 ul (to same plates)
- plate a control and 50 pg/ul sample to LB plates (no antibiotics)

50 ul of each sample (0,5 pg/ul, 5 pg/ul, 10 pg/ul, 20 pg/ul, 50 pg/ul) (stored at 4 °C overnight) was plated on the same plates as yesterday. In addition we pipetted 50 ul of the control and the 50 pg/ul sample on LB plates. All plates were put in a 37 °C incubator.

The serial dilution (1:1, 1:10, 1:100, 1:1000, 1:10000) were made and of each dilution 50 ul was plated on an LB plate. Plates were put in a 37 °C incubator.

We got competent cells and plasmids with ampicillin resistance from Sanni to test our protocols. We made two dilutions of the plasmids: 312 ng/ul and 104 ng/ul. We pipetted 1 ul of both DNA dilutions twice into four 2 ml eppendorfs. 50 ul of our competent cells and 50 ul of Sanni's competent cells were added to two tubes each (both dilutions). Tubes were incubated on ice for 30 minutes, heat-shocked in 42 °C for 60 seconds and put back on ice for 5 minutes after which 200 ul of SOC was added. After this the tubes were incubated at 37 °C for two hours. 100 ul of each sample was plated on an ampicillin plate and all the plates were left on the bench top over the weekend. /MS&OV

26.5. p. 6

All the plates that were grown over the weekend had bacterial growth. On control plates there was nothing. From the serial dilution cell density was calculated to be 1,4*10^4 cfu/ul. A new transformation was made with our own competent cells with biobrick BBa_I714031 and Sanni's plasmid (N1). Concentration of both DNAs was about 333 pg/ul and 2ul of each were pipetted to eppendorf tubes. Transformation was made as before. From both cell cultures 3 different concentration plate cultures were made. First 50 ul was pipetted directly on (CAM or AMP) plate, then 5 ul of culture was diluted with 45 ul LB broth and 50 ul of it was plated. The rest of the liquid culture (200 ul) was centrifuged and 150 ul of supernatant was removed. The cells were suspended in the rest of the liquid and 50ul was spread on the plate. Biobrick cells were plated on chloramphenicol plates and N1-plasmid cells on ampicillin plates. The plates were left in 37 °C incubator. More plates were prepared, 200 ml without antibiotics and 300 ml with chloramphenicol. /OV, MI & MS

27.5. p. 8

Biobrick BBa_I714031 had grown on a plate 1:1 (5 colonies) and 4:1 (2 colonies). N1 plasmids had not grown. The reason may be that the plasmids had been left on the table over the weekend, or that the concentration was too low.

Biobricks BBa_K592008, BBa_E0020, BBa_B0015, BBa_J45199 and BBa_B0030 were suspended to 10 ul H2O and moved to eppendorf tubes. From the tubes 2 ul of DNA was pipetted to bigger eppendorf tubes, where 50 ul of competent cells had been pipetted. The transformation was done as before (2 h incubation 13:17 ->). After incubation 125 ul of cells were spread on two plates (CAM) and they were left to be incubated in 37 °C.

The cells (with BBa_I714031) from yesterday were inoculated to LB medium, which had 30 ug/ml chloramphenicol. Tubes were left in the incubator in the shaker in 37 °C. /MI&MS

28.5. p. 8

Plates that had been growing overnight (BBa_K592008, BBa_E0020, BBa_B0015, BBa_J45199 and BBa_B0030) had plenty of colonies. Liquid cultures were made of these (LB+CAM) at 13:10.

From overnight liquid cultures (BBa_I714031) a plasmid extraction was made. Extraction was made according to the instructions of the kit. The OD600 of the culture was about 2,5, so 3,5ml of liquid culture was used. After extraction the concentration of the plasmid solution was measured to be 450-550 ng/ml. /OV, MI&MS

29.5. p. 8

From overnight liquid cultures of biobrick tubes (BBa_K592008, BBa_E0020, BBa_B0015, BBa_J45199 and BBa_B0030) 400 ul was pipetted to cryo tubes, where 100 ul of glycerol was added. The tubes were incubated in room temperature about an hour and put in the -80 °C freezer for future use (glycerol stocks).

From the same overnight cultures 2 ml was pipetted into Eppendorf tubes and centrifuged 1 min, full speed (12 000 x g). The supernatant was removed and the steps were repeated until the whole culture (about 5 ml) was at the bottom of the tube as a pellet. The pellets were stored in -20C. /OV&BG

30.5. p. 10

From pellets the plasmid extraction was made according to the instructions of the kit. The DNA concentrations were measured to be between 240-1070 ng/ul. Tha ampicillin plasmid and red fluorescent protein (BBa_B0033 and BBa_E1010) were transformed to cells. They were left on the bench in RT over the weekend. /MI&OV

2.6. p. 10

There was no visible growth on the plates. From the original XL1-blue culture a new liquid culture was made in LB medium in order to make electrocompetent cells. 10 ug/ml of tetracycline was added in the culture.

3.6. p. 10

Dilutions were made in 3 conical flasks from the overnight grown cells (3x100 ml cultures, 1:100). 10 ug/ml of tetracycline was added in them. The flasks were incubated in 37 °C in a shaker until their OD600 was 0,58-0,61. The cells were made competent according to the [protocol that can be found in our wiki]. The cells were washed with ddH2O and at the end suspended in 10% glycerol. The cells were frozen by putting them directly in the -80 °C freezer, because liquid nitrogen was not available due to the late time. /MI&MS

4.6. p. 10

With electrocompetent cells that were made yesterday an electroporation and transformation was made with the following DNA concentrations: 0,5 pg/ul, 5 pg/ul, 20p g/ul, 50 pg/ul, 333 pg/ul and 1 ng/ul. In addition one control transformation was made with 50 pg/ul DNA and cells given by Georg. Electroporation and transformation was conducted by mixing together 30 ul electrocompetent cells and 2 ul DNA. The tubes were let to stand on ice for a while and then cell-DNA suspension was pipetted to ice cold electroporation cuvettes. The cuvette was put in the machine and an electric shock was given to the cells (2,5 kV). Directly after the shock 970 ul of SOC medium was added to the cuvette and suspended and the cuvettes were put back on ice for

2 min. Cell suspension was poured into Eppendorf tubes and the tubes were incubated in 37 °C for 1 h. After this, 100 ul of the suspension was plated. The rest of the suspension was centrifuged, about 8/9 of the supernatant was removed and the cells were suspended in the rest of the liquid and then plated (volume about 100 ul). The plates were incubated in 37 °C overnight.
In addition, colonies were inoculated in LB broth from “surprise plates” (BBa_B0033 and BBa_E1010) for miniprep. /MS&MI

5.6. p.12

Of overnight plates only AMP plates contained colonies, but they had plenty (Sanni’s plasmid N1. 333 pg/ul, 1 ng/ul). One CAM plate (20 pg/ul, 9:1) had few colonies. All CAM plates were put back in to the 37 °C incubator. AMP backbones and RFP plasmids were miniprepped.

RBS (BBa_B0030) part was digested from the AMP backbone with restriction enzymes. To 1 ml eppendorf tube 5 ul NEBuffer2, 28,5 ul plasmids, 14,5 ul H2O and 1 ul of PstI and EcoRI were pipetted (Vtot=50ul). NOTE! Enzymes are always pipetted the last. The tube was incubated in 37 °C for 30 min. Then 1 ul of XbaI and SpeI were added and the tube was again incubated for 30 min. Finally, the enzymes were inactivated by incubating the tube in 80 °C for 20 min. The tube was stored in -20 °C. /MI&MS

6.6. p.12

Digested AMP backbone (RBS taken out) was purified with PCR purification kit. Biobricks BBa_K592008, BBa_E0020, BBa_B0015, BBa_J45199 ja BBa_B0030 were digested and ligated the following way: BBa_K592008 + BBa_E0020, BBa_K592008 + BBa_J45199, BBa_E0020 + BBa_B0015, BBa_E0020 + BBa_J45199. From restriction digestion tubes 4 ul of DNA were pipetted into appropriate ligation tubes. 1,43 ul AMP backbone, 2 ul T4 ligase buffer, 7,57 ul H2O and 1 ul T4 ligase were added (Vtot= 20 ul). NOTE! The enzyme was added the last! The tubes were incubated 1,5h in 16 °C, then inactivation in 80 °C for 20 min. The tubes were stored in -20 °C /MS&MI

9.6. p. 14

Ligated plasmids were transformed by electroporation to XL1-blue cells (30 ul comp. cells + 2 ul ligation mix). 970 ul SOC media was added. Tubes were incubated for 1h in 37 °C and then 10 ul, 100 ul and the rest of each tube were pipetted to the plates. In addition 4 new biobricks (BBa_K395602, BBa_K517002, BBa_K517003 and BBa_I742111) were transformed. /MI&MS

10.6. p. 14

All plates had plenty of colonies, even 10 ul of cell suspension plated was a bit too much. Liquid cultures were made of the new biobricks and ligated constructs for miniprep. Also a control ligation with backbone only was made by pipetting water instead of restricted biobrikcs. After ligation, plasmids were transformed to XL1-blue and incubated in 37 °C.

New odorless E. coli strain YYC912 arrived on a filter plate. The filter plate was put on an LB plate, and 170 ul of LB was pipetted on it. Gently suspending the cells, 20 ul of suspension was taken and plated on a chloramphenicol plate, followed by 70 ul of LB. Plates were incubated in 37 °C

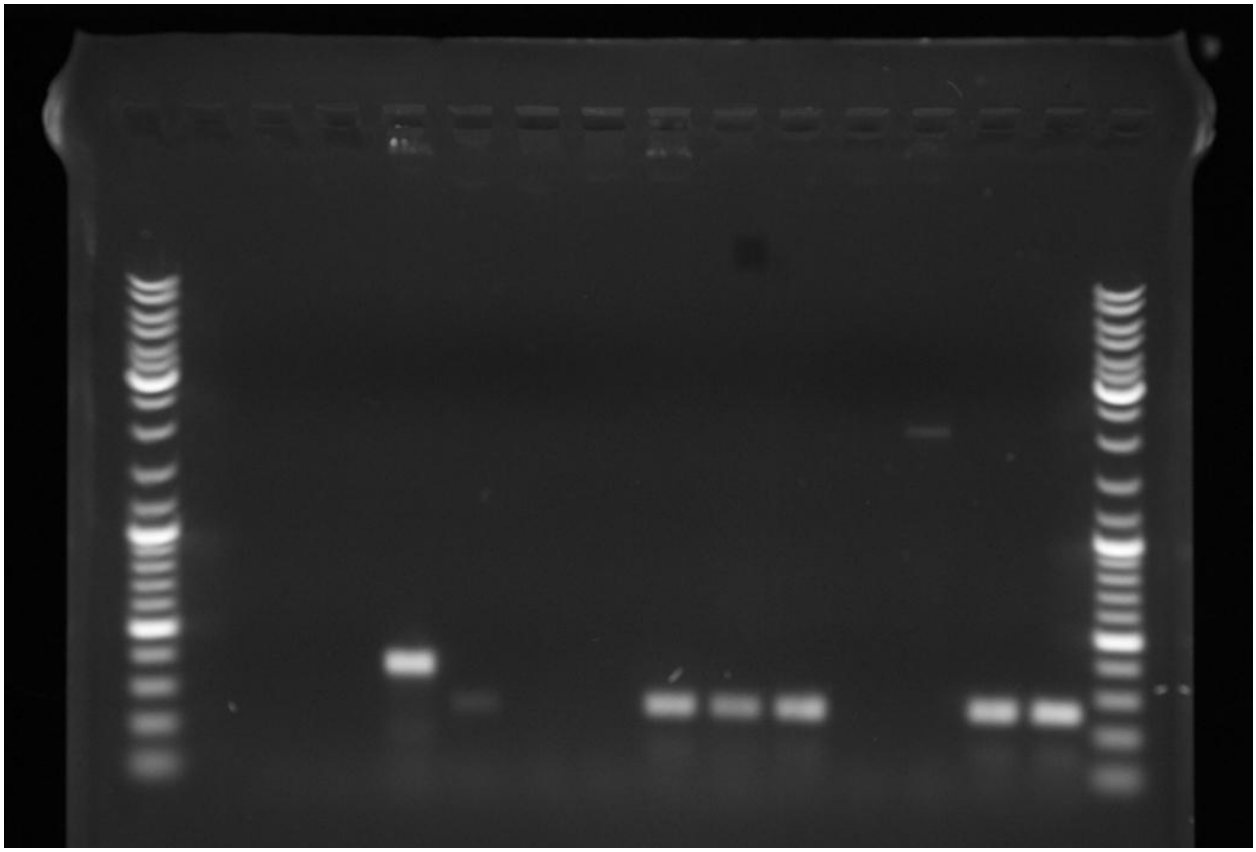
11.6. p. 14

Samples were taken from plates BBa_K592008/BBa_0030, BBa_E0020/BBa_B0015, BBa_I742111 (backbone control) and AMP backbone to make colony PCR. Gel electrophoresis was run and no right insert could be seen. Ligated plasmids and cells containing them were thrown away as useless.

Liquid cultures of Biobricks BBa_K395602, BBa_K517002, BBa_K517003 and BBa_I742111 were put in +4 °C.

Linearized plasmid backbones were put in PCR. The thermocycler was set to store the samples in +4 °C until next morning after the PCR run was finished.

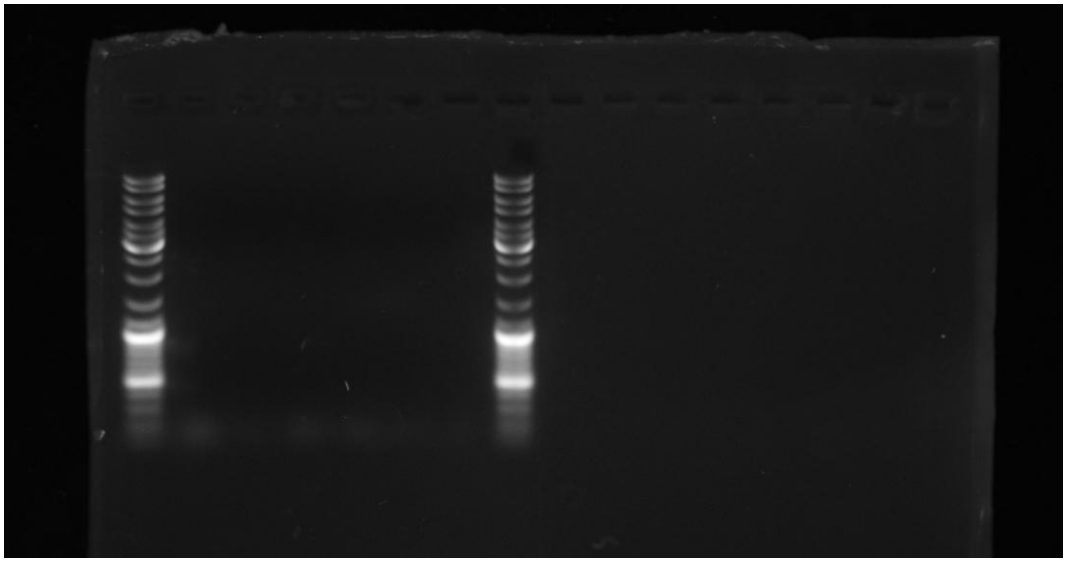
New *E. coli* strain had grown well on both plates. A colony was replated on a new CAM plate and incubated in +37 °C.



12.6. p.18

From the overnight PCR a gel was run. The gel didn't contain any visible bands, so apparently the PCR hadn't worked. The primer sequences were checked, they bind to the Biobrick prefix and suffix so the backbone shouldn't matter. The annealing temperature was suspected to be too high so a test PCR was made using a temperature gradient. Ten samples were made from each, BBa_E0020 and BBa_B0033, and annealing temperature varied between +50-70 °C.

From the new odorless *E. coli* strain YYC912 a liquid culture was made in LB with CAM and put in the +37 °C incubator. /MI&MS



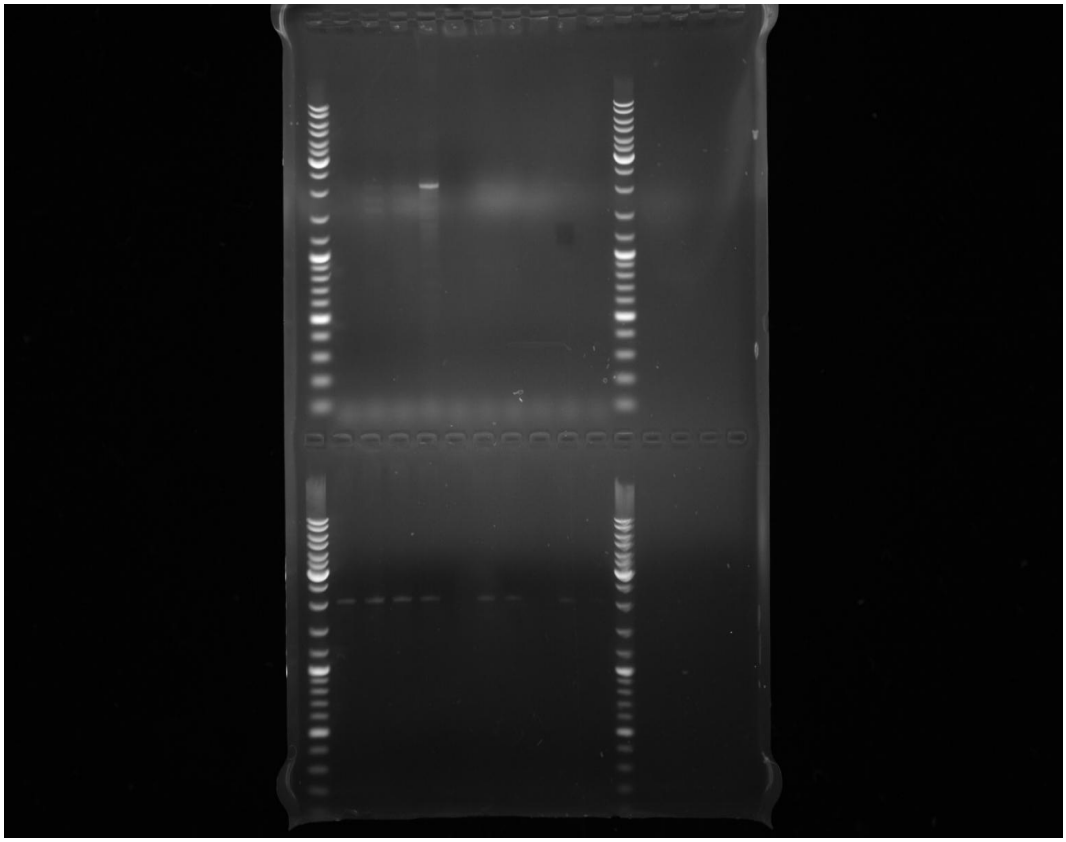
13.6. p. 18

(Friday the thirteenth! D:)

From the overnight YYC912 culture a glycerol stock was made in -80 °C. In a cryo tube 400ul of liquid culture and 100ul of 85% glycerol were mixed. The tube was incubated in RT for 1 h before putting it to the freezer. A gel was run of the PCR samples. The best result was given by the sample A3, which contained AMP backbone (BBa_B0033) and the annealing temperature was +54 °C. With nucleotides, polymerase and buffer provided by Georg, and enhanced PCR program (annealing temperature +55 °C, extension time 2 min, 30 cycles) a test-PCR (BBa_E0020, BBa_B0033) was conducted.

New biobricks (BBa_J04450 in pSB1A3, pSB1C3, pSB1T3 and pSBK3) were transformed in order to use them as plasmid backbones. They have RFP coding device as the insert, so they can easily be screened from succesful ligations as background (red colonies). The plates were left on the bench to be incubated over the weekend. /MI&MS

NOTE!
The antibiotics were given a colour code to prevent errors (a line on the side of the plate)
Chloramphenicol
Ampicillin
Tetracycline
Kanamycin



16.6. p. 20

The tetracycline plate had a bacterial lawn, so the antibiotic screening had not worked. On kanamycin plate there were no recognizable colonies, but that one, the ampicillin and chloramphenicol plates were put in the +37 °C incubator.

A gel was run of the PCR test samples, which showed that still no backbones had been successfully amplified. From the other plasmid a product of 700-800 bp had been amplified. Also the restricted and ligated biobricks were analyzed on the gel. All but RBS (BBa_B0030) had been successfully digested. BBa_B0030 and the ligated plasmids had been moving slowly on the gel because of the circular shape.

A note to self: XL1-blue already has tetracycline resistance, so TET backbones cannot be grown in it. Liquid cultures were made of the colonies from the plates incubated during the day (also KAN plate had colonies) and left in +37 °C to be incubated.

A liquid culture was made of the strain YYC912 in chloramphenicol and put in +37 °C to be incubated.

17.6. p. 20

3 x 100ml cultures were prepared of YYC912 strain and put in the shaker in +37 °C in order to make competent cells.

PCR looked like the primers had been wrong (even though we are sure that we had used the right ones). A new PCR test was made so that BBa_E0020 (original), BBa_J45199 (original), BBa_E0020 (miniprepped) and BBa_J45199 (miniprepped) were amplified with both primers (A=VF2&VR, B= suffix-F&prefix-R).

Biobricks BBa_J04450 in pSB1A3,BBa_J04450 in pSB1C3 and BBa_J04450 in pSB1K3 were miniprepped (miniprepped plasmids marked with a “+” sign on the tube).

When YYC912 had grown in the broth about 3,5h and OD600 was about 1,0, the preparation of electrocompetent cells was begun (the protocol can be found in our wiki). In the freezing step liquid nitrogen was used.

Parts BBa_E0020, BBa_J45199 were digested and ligated together with pSB1A3 (from BBa_J04450). Also a control ligation was made without insert parts. /MS, MI & OL

18.6. p. 22

LB plates with 10ug/ml of tetracycline were prepared.

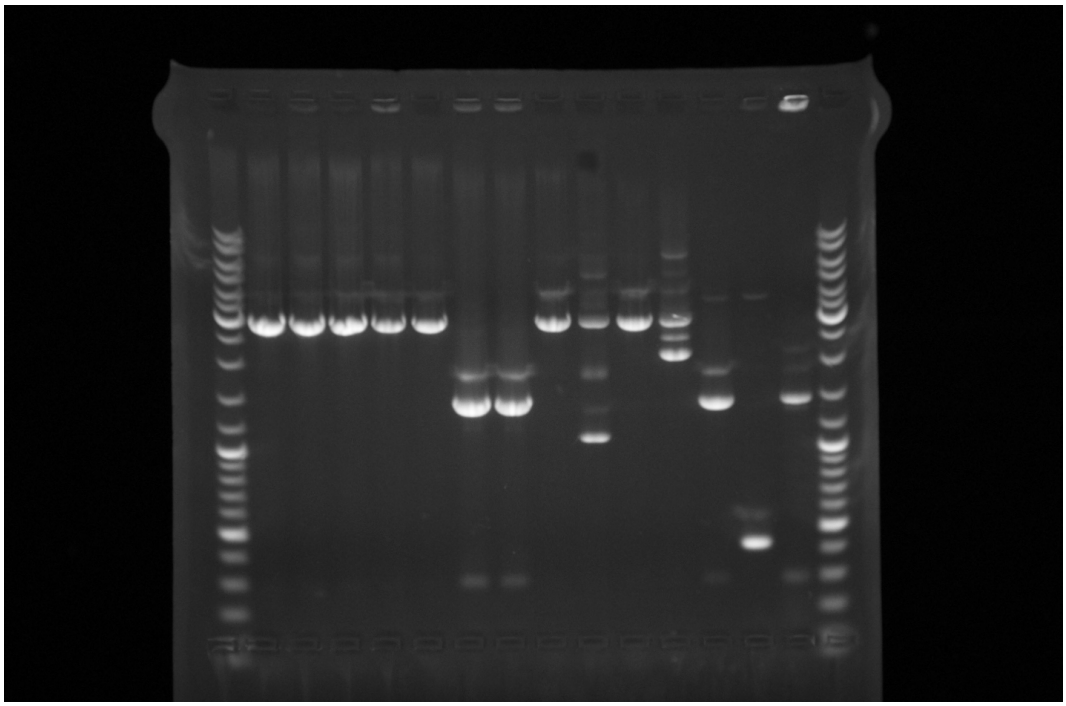
The RFP plasmid with tetracycline resistance (BBa_J04450) was transformed to YYC912 strain, and ligated BBa_J45199/BBa_E0020 and insertless control to both YYC912 and XL1-blue.

A gel was run of the PCR samples. The primers (for amplifying the backbone) seemed to be wrong). /MI, MS, OV, LV

23.6. p. 22

All plates had growth: on ligation plates 50% background (red), controls had red colonies. YYC912 grows much faster and forms bigger colonies. Also TET backbones had grown well. Of 14 colonies (YYC912 and XL1-blue white and control red colonies, also 1 white from control plate) a colony PCR was made and later a gel was run. According to the gel all white colonies were successful ligations but two of XL1-blue colonies were something wrong. XL1-blue didn't show the colour so clearly at this point, so it was difficult to see the difference between white and to-be red colonies.

Liquid cultures were made of biobricks (BBa_K395602, BBa_K517002, BBa_K517003, BBa_I742111 and all four backbones (BBa_J04450). /MI&MS

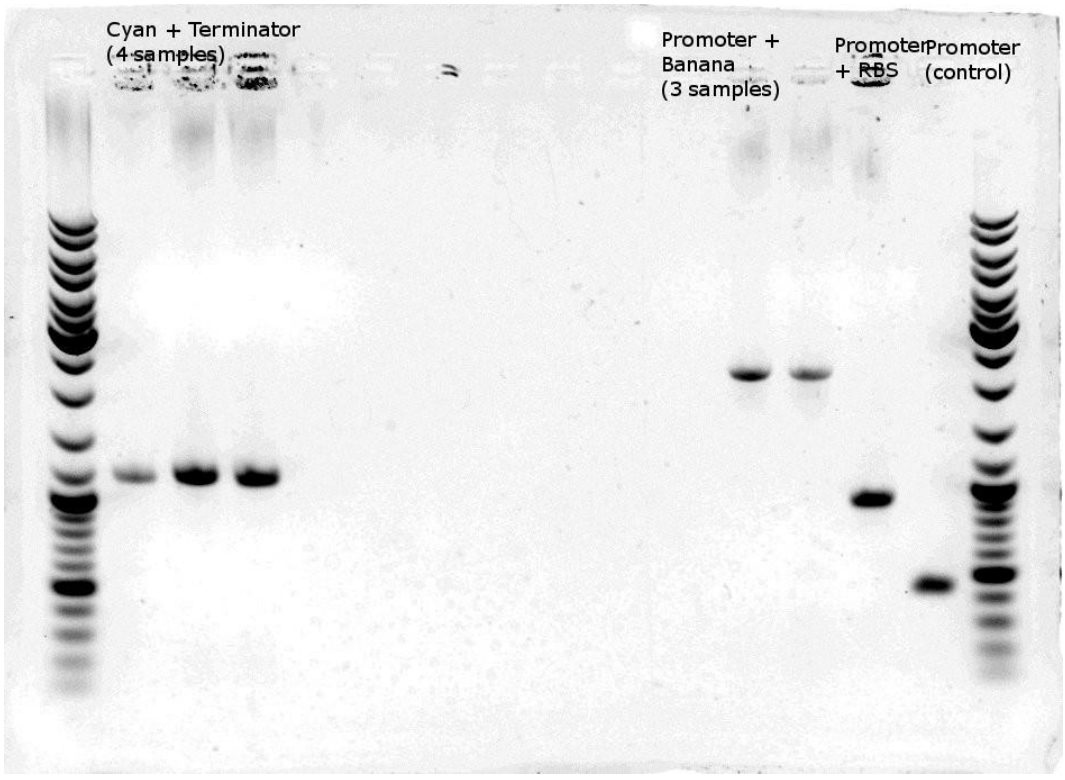


24.6. p.22

A restriction digestion was made of biobricks BBa_K592008, BBa_E0020, BBa_B0015, BBa_J45199, BBa_B0030 and BBa_J04450 in pSB1A3. The digestion was conducted as before, but in BBa_B0030 only one enzyme was added first, then incubated in +37 °C 30 min, inactivated in +80 °C 20 min and only then added the other enzyme. The second incubation and inactivation were conducted as before (+37C 30 min, +80 °C 20 min). The liquid cultures (BBa_K395602, BBa_K517002, BBa_K517003 and BBa_I742111 + all backbones) were miniprepped. After the restriction BBa_K592008/BBa_B0030, BBa_K592008/BBa_J45199 and BBa_E0020/BBa_B0015 were ligated together. Ligated plasmids were transformed (BBa_K592008/BBa_J45199 ligation mix had to be diluted 1:10, because otherwise the electroporation didn't work). /MI&MS

25.6. p. 22

All plates contained white colonies, but BBa_K592008+BBa_B0030 plate had only one white colony. The control was OK. Colony PCR was made of 8 colonies (all white, 4xBBa_E0020/BBa_B0015, 3xBBa_K592008/BBa_J45199, 1xBBa_K592008/BBa_B0030) and also from the earlier transformed promoter Brick (BBa_K592008). The gel was run of all samples. From BBa_K592008/BBa_B0030 transformation an extra plating was made, because there was only one colony. /MI&MS



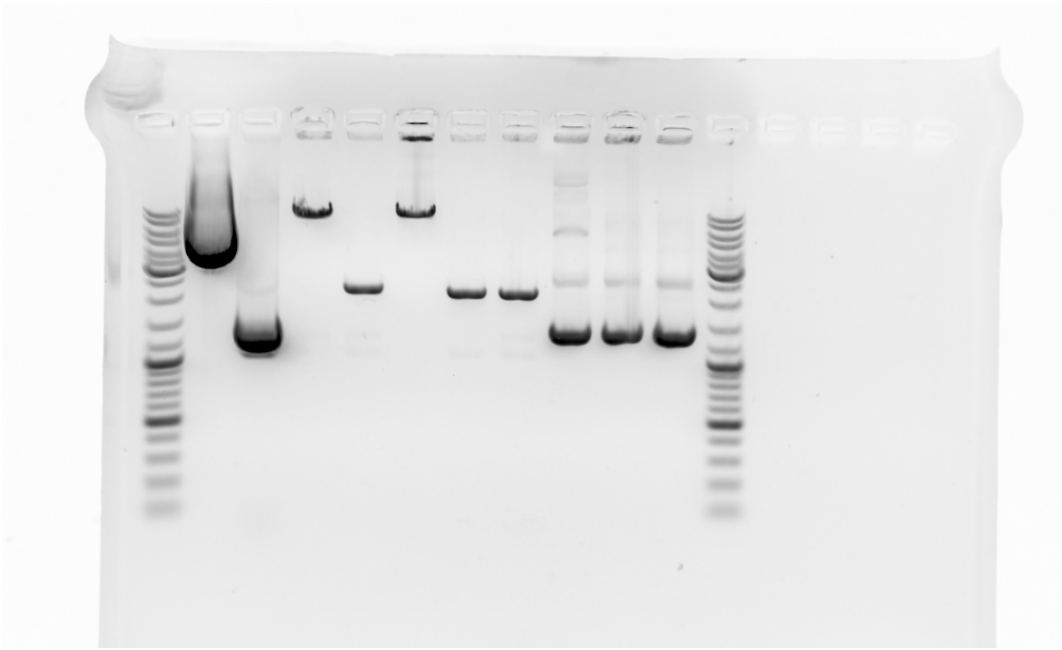
26.6. p. 24

As the ligation of the BBa_B0030 to the BBa_K592008 didn't work, a new restriction-ligation was made with old RBS (BBa_B0030) and a new RBS (BBa_B0033). Restrictions were conducted one enzyme at a time. With the new RBS also a sample was made so that both restriction enzymes were added at once. Also KAN plasmid backbone was restricted.

The successful ligations (BBa_E0020/BBa_B0015 & BBa_K592008/BBa_J45199) were miniprepped.

After restriction the promoter (BBa_K592008) was ligated together with different RBSs (->KAN). To figure out why the ligation hadn't worked before, samples were taken before restriction, after 1 enzyme (the enzymes at different times), after the restriction and after ligation. The samples were run on a gel later (//EDIT later on turned out that BBa_B0030 from plate 3 well 21A was inconsistent and that's why we couldn't digest or ligate it!)

New biobricks BBa_I20260 and BBa_B0034 and the ligated samples and the control were transformed and plated. The plates were put in +37 °C to be incubated. /MI&MS



27.6. p. 24

SOB medium was prepared to be autoclaved. Also 1 M glucose solution was made. Ligation plates only contained one red colony (background) so on Monday a retransformation should be done. /MI&MS

30.6. p. 24

To the SOB medium MgCl2 MUST NOT be added before autoclavation (->precipitates…)! SOC was made from SOB provided by Sanni by adding MgCl2 and 1 M glucose. Biobricks BBa_E0240, BBa_K823005 and BBa_K823012, BBa_B0030, BBa_K819017 and BBa_K880005 were transformed to XL1-blue and previously ligated BBa_K592008/BBa_B0033 and control to both XL1-blue and YYC912. Of biobrick transformations the plating volumes were 10 ul and 100 ul and of ligated samples to XL1-blue 10 ul and 100 ul and to YYC912 100 ul and the rest.
TET and CAM plasmid backbones were restricted for future use. /MI&MS