KOMMENTAR

mandag 19. juni 2017 18.54

Gjør som vist på de andre fanene. Sett "Dag X" som overskrift, og bruk samme fane for alt som har skjedd samme dag. For ulik ting som har skjedd i løpet av en dag lag en unik overskrift, sett den i fet og legg til kort beskrivelse av arbeidet to "spaces" under. Pass på at dato er oppgitt i engelsk format.

Dette er for at utskriften når vi konverterer dette til PDF skal se pen og ryddig ut.

Day 1

Wednesday, June 14, 2017 2:32 PM

Annotation of plasmid

Started annotating the MP6 mutagenesis plasmid using the Benchling software.

Day 2

Thursday, June 15, 2017 2:44 PM

Lab set-up

We got equipment (pumps, vessels, temperature regulator, stirrer, pH-meter) today, cleaned it and and started setting up the chemostats in the lab. pH regulator is still missing.

Day 3

fredag 16. juni 2017 14.32

Plasmid design

Designed the new plasmid based on the MP6 plasmid using Benchling.

Day 4

mandag 19. juni 2017 18.38

Equipment testing

We did a simple test of the chemostat equipment.
Everything seemed to work fine (although the tubes were very dirty).

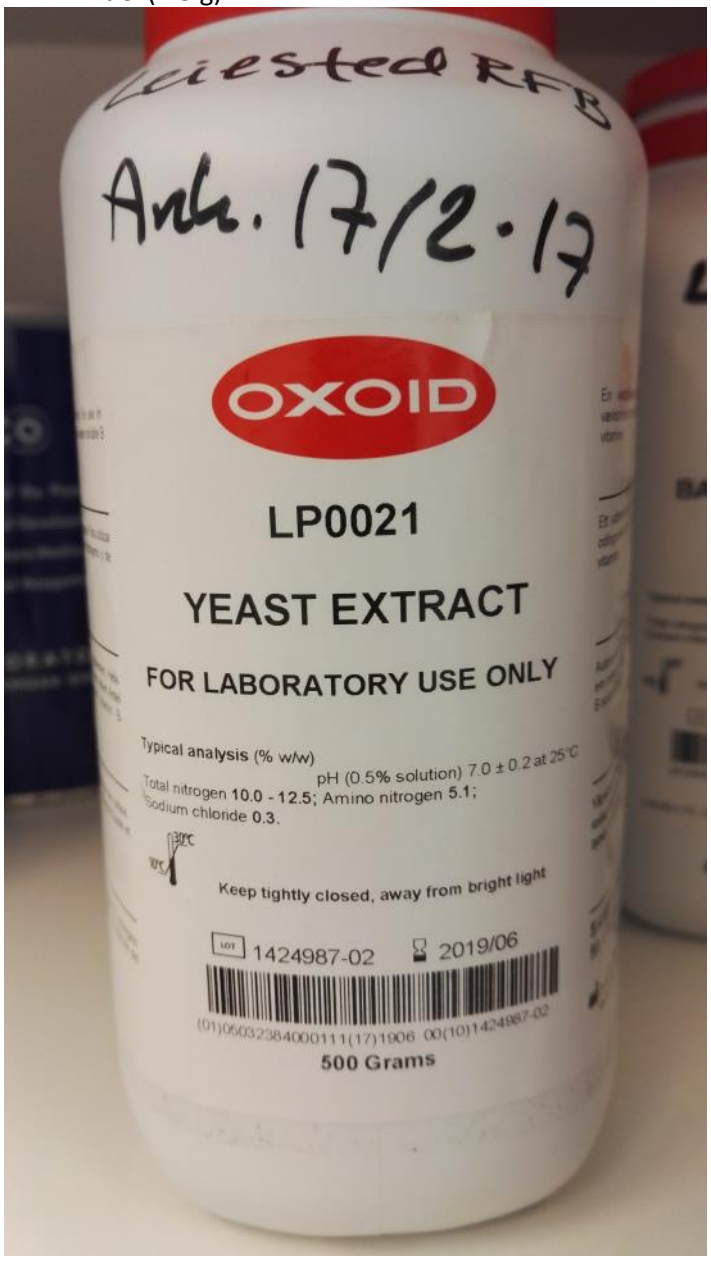
Day 5

torsdag 22. juni 2017 09.58

We ordered the MP6 plasmid, and the 4 gBrick sequences for our edited version of MP6.

We made LB-medium (500 mL) after the following recipe:

-Deionized water (500 mL)
-Tryptone (50 g)
-Yeast extract (2.5 g)
-NaCl (2.5 g)



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OXOID
LP0042
TRYPTONE
IVD
Typical analysis (% w/w) pH (2% solution) 7.3 ± 0.2 at 25°C
Total nitrogen 12.7; Amino nitrogen 3.7;
Sodium chloride 0.4; Tryptophan (present)
Keep tightly closed, away from bright light
LOT 1900868
2021/08
(01)05032384000463(17)2108 00(10)1900868
500 GRAMS
OXOID LTD., BASINGSTOKE, HAMPSHIRE, ENGLAND

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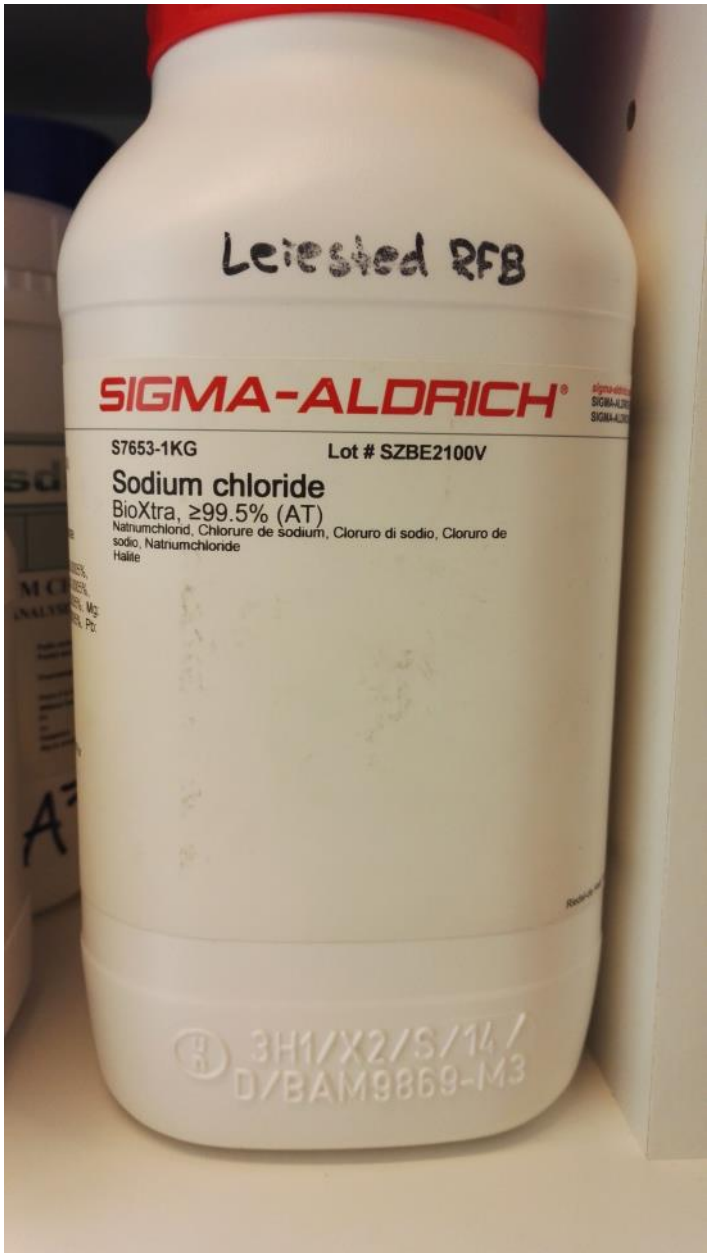

Leitsted RFB
SIGMA-ALDRICH
S7653-1KG
Lot # SZBE2100V
Sodium chloride
BioXtra, ≥99.5% (AT)
Natriumchlorid, Chlorure de sodium, Cloruro di sodio, Cloruro de sodio, Natriumchloride
Halite
3H1/X2/S/14/
D/BAM9869-M3

Day 6

fredag 23. juni 2017 13.48

LB medium was inoculated with E. coli test bacteria.

Day 7

Saturday, June 24, 2017 7:07 PM

Chemostat test

E. coli from Day 6 was transfered into new, diluted (x 0.5) LB medium.

One chemostat was tested with the E. coli in diluted LB and left overnight.
The drip rate was approximately 10 drops pr. minute (30 mL/h).

Day 8

Sunday, June 25, 2017 12:12 PM

Results of chemostat test

The chemostat experiment was finished. No results to discuss.

Day 9

mandag 26. juni 2017 16.09

Soil and water sample collection
Samples form nearby soil and a puddle of water was collected to search for bacteriophages.

- Water sample:

  Innoculated E. coli broth (1 mL) was transferred to LB (50 mL) and water sample (5mL).

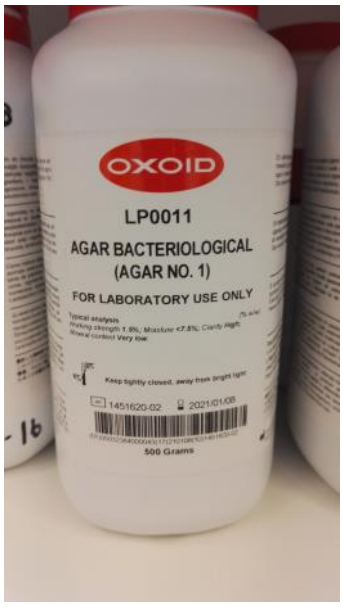
- Soil sample:

  Innoculated E. coli broth (1 mL) was transfered to LB (50 mL) and soil sample (approx. 5 mL)

Both solutions were put in shaker (225 rpm, 37°C) overnight.

Day 10

tirsdag 27. juni 2017 12.35



We made

- 0.5L of top agar using LB medium and 7 g agar bacteriological (agar no. 1)
- 0.5 L agar
- 0.5 L PBS

Phage isolation

Phages from the environmental samples (soil and water) collected on Day 9 was isolated using sentrifugation and filtering according to protocol.

Day 11

onsdag 28. juni 2017 16.25

Phage isolation

Agar plates were inoculated with bacteria and bacteriophage mix from Day 10 and left overnight in 37C.

Characterisation of Lux biobrick

E. coli DH5α was transformed with plasmid from biobrick Bba_K325909 (arabinose induced luminescence from lux operon)

Day 12

30. juni 2017 16:50

29 june

Results from biobrick transfrormation
The transformations of Bba_K325909 from Day 11 was not successful.

The transformation of Bba_K325909 was repeated (see protocol).

Results of phage isolation (water and soil samples)
Could not observere any plaques on the agar plates from Day 11.

The experiment was repeated.

Day 13

30. juni 2017 16:50

Phage isolation (soil and water)

There were still no plaquing from the E. coli inoculated with water and soil samples. The samples were discarded.

Sample collection from wastewater treatment plant

New water samples were collected from the local wastewater treatment plant (WWTP), Trondheim Vannrenseanlegg at Ladehammeren.

The samples came from three sources:

(13,1) Untreated waste water (2x50 mL Falcon tubes)
(13,2) Waste water after sand- and grease (fat) traps (2x50 mL Falcon tubes)
(13,3) Waste water after sedimentation (1x50 mL Falcon tubes)

Phage isolation (WWTP)

5 mL of sample (13,1) was added in 50 mL LB medium and incubated in shaker (37°C, 225 rpm)

5 mL of sample (13,2) was added in 50 mL LB medium and incubated in shaker (37°C, 225 rpm)

Arabinose
1 mL of arabinose stock solution (0,1 mol/L) added in each of 20 eppendorf tubes. The tubes were stored at -20°C.

Characterisation of Lux biobrick

Two transformed colonies containing the Lux biobrick were observed on the plates from Day 12. The two colonies were inoculated in LB broth.

Day 14

Sunday, July 2, 2017 10:03 AM

Isolation of phages
Isolated phages from sewage samples according to protocol.

Day 15

Monday, July 3, 2017 10:04 AM

Luminescence of bacteria

- Both of the transformed DH5α colonies (BBa_K325909) where innoculated in LB with 1/1000 concentration chloramphenicol.
- At OD_600 0.8, samples (5 mL) where transferred and arabinose (0.01 mM) was added.
- 3 hours later the bacteria where checked for luminescence. None was observed.

Sewage sample plating

Purified bacteriophage sewage samples were spotted on agar plates with DH5alpha, and incubated at 37 C overnight.

Day 16

Tuesday, July 4, 2017 3:11 PM

Plated purified phage environmental samples 1 and 2 with DH5alpha on agar. Phage concentrations of 10^-1, -2, -3, -4, -5, -6. Deviation from protocol: Instead of top agar, as described in the protocol, ordinary agar was used in its place.

Results from DH5alpha + Lux transformants:

Attempted to observe luminescence from transformed DH5alpha bacteria with 1 mMol concentration arabinose incubated at 20 C. We were not able to observe any luminescence.

Day 17

Wednesday, 5 July 2017 3:11 pm

Results from plated DH5a + purified phages:

- Observered plaques on the agar plates inoculated with E. coli and (filtrated) untreated waste water, and E. coli and (filtrated) waste water after sand- and fat traps. (Plate: phage 1 dilution = 10^-2, phage 1 dilution = 10^-3, phage 2 dilution = 10^-3)

Phage isolation

- Isolated phages from three different (Plate: phage 1 dilution = 10^-2, phage 1 dilution = 10^-3, phage 2 dilution = 10^-3) agar plates. (Deviation from original protocol: used 1 drop of chloroform)

- **Two new parallels of phage plaque were made with two different samples:**
  - Untreated waste water
  - Waste water after sand trap and fat trap
  - Source of Error:
    - Plate 1.2 was left in the hood for solidification, but was not incubated until one hour after. (was left 1 hour in the fume hood).
    - Most likely plate 1.1 or 1.2 has double up of phage mix + top agar.

Lux plasmid isolation and restriction digestion:

- Miniprep for extraction of Lux-plasmid from E.coli cells (Lux1 and Lux 2). Nanodrop were used to measure concentration of lux1 (155.8 ng/uL) and lux2 (151 ng/uL).
- Restriction digestion of the isolated plasmids according to protocol.

Enrichment of bacteriophages from sludge sample:

- 5 mL sludge were inoculated with ca. 25 mL of DH5alpha E. coli

Test of Lux-transformed DH5a:

- 500 uL 0.1 mol/L arabinose was added in 5 mL Lux-transformed E.coli. The bacteria had a weak glow.

Misc:

- New agar plates were made (2 packages)

Day 18

6. juli 2017 13:18

Filtration of phage from sludge sample (3) (Marianna)
10 mL of sludge was centrifuged for 5 min at 2000 rpm, then filtrated in a 0.2 um syringe filter.

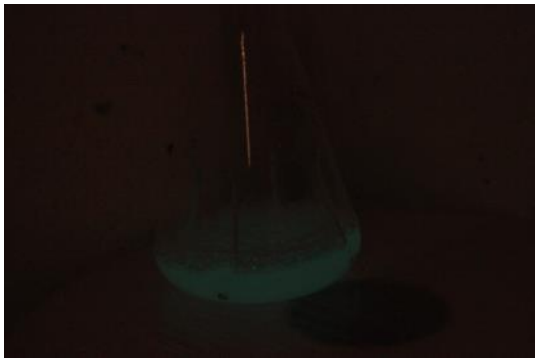
Growth of isolated phages (Marta)
Phages isolated yesterday: 1.3,

- LB medium (25 mL) added to erlendmeyer flasks
- Phage (1 mL) added
- E. coli broth from yesterday (2.5 mL) added

Phage plating and isolation (Marianna and Aslak)

- OD 0.8
- Used phage sample 2 (waste water after sand trap and fat trap) to make these dilutions: -2, -3,-4
- In a new centrifuge tube 0.1 mL phage dilution was mixed with 0.5 mL DH5alpha E. coli.
- Incubated for 10 min (shaking) at 37 C.
- Plated on agar plate with 3 mL top agar.
    - 3 agar plates contained E. coli at OD_{600} = 0.627
    - 3 agar plates contained E. coli at OD_{600} = 0.877

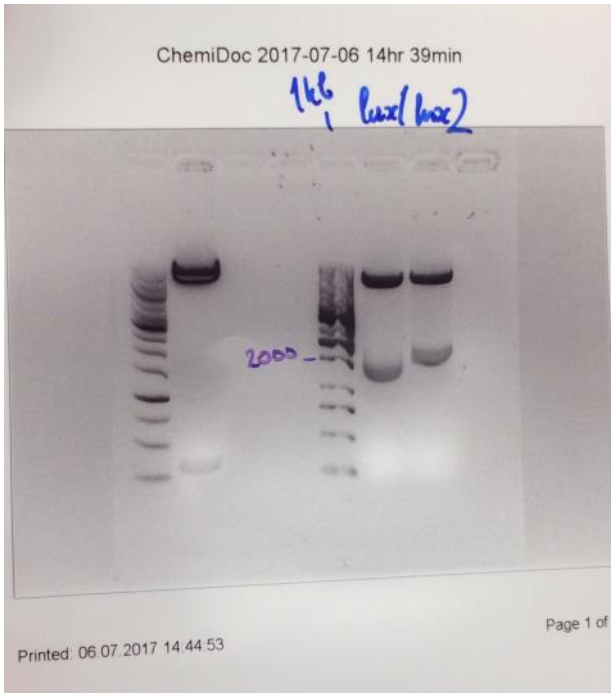
Luminescense (Nikolay and Peter)

- Baffled bottles: arabinose + lux samples
- Lux samples glow after 2 hours incubation
- 

Gel electrophoresis (Nikolay, Jenny, Sheida)

- Gel electrophoresis of Lux plasmid extracts from E. coli cells from yesterday (lux 1 and lux 2).
- Agarose gel (0.8%) was made and samples loaded on the gel. See protocol for details.
- Ladders used:
    - GeneRuler 1 kB DNA from Fermentas NKB (green)
    - GeneRuler 1 kB DNA from Thermo Fisher (red?)
- Loading and running:
    - Ladders (2 uL) loaded into two wells
    - Sample (20 uL) and loading dye (4 uL) mixed and loaded into wells
    - The gel was run for 45 min

Lux1 may have lost some of its backbone - fewer base pairs than Lux2


ChemiDoc 2017-07-06 14hr 39min
1kb
Lux(lux2)
2000
Page 1 of
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Day 19

7. juli 2017 10:13

Plate plaquing OD_{600} = 0.6 vs OD_{600} = 0.8
No difference between OD_{600} of 0.6 and 0.8. Higher bacterial growth observed on plates with higher phage concentration.

Phage plaqueing top agar vs agar (Aslak og Marianna)

- Phage sample from waste water after sand- and fat traps (sample 2)
- Phage dilutions: 10^{-2}, 10^{-3}, 10^{-4}
- Used:
    - 0.1 mL and 0.5 mL DH5alpha E. coli with 3 mL top agar
    - 0.1 mL and 0.5 mL DH5alpha E. coli with 3 mL agar
- OD_{600} = 0.654
- The phage-cell mix were plated on agar plates, and incubated at 37 C for 24 hours.
- After 19 hours incubation there were observed no plaque on the plates with top agar. There were observed few or none plaques on plates with agar (10^-2 and 10^-3), while on 10^-4 there were (many) very small plaques.

Purification/isolation of plaque grown in E.coli (Marta og Peter D)
Isolated plaques grown in E. coli + waste water samples #1 and #2, were purificated (centrifugation/filtration) in concentrations 10^-1, 10^-2 and 10^-3. They are in marked tubes in the fridge.

Lux transformed E. coli (Nikolay)
Induced 24 h DH5a-lux cultures with arabinose (1mM) Replant both for weekend. Result: No luminescence (use exponential instead)

Day 20

10. juli 2017 10:50

Notes on bacteriophage plaqueing (needs to be followed):

The agar temperature can affect bacterial growth, so we have to use an agar temperature of 45C. Transfer agar to 50 mL Falcon tube and put in water bath (45C) for at least 15 minutes.

Shake stock solution of bacteriophages and bacteriophage dilutions.

Use the thin falcon tubes for bacteriophage- and cell mixing.

Use E.coli control (without bacteriophage), plate on the first agar plate.

Bacteriophage dilution series must be made beforehand (10^-1 to 10^-10 dilutions)

E.coli DH5a needs to be at OD600=0.8

Characterization of biobrick (Nikolay)
Initiated Lux 1 and lux 2 25 mL cultures 1/10x in the morning. Used 50 uL chloramphenicol, leading to no growth of E. coli with lux. Diluted with 25 mL LB-medium around 12:45.

Induce and record fluorscence of Lux with Tecan plate measurer at OD600 (lux 2 OD600=0.584) with different arabinose concentrations (0, 10^-2, 10^-3, 10^-4, 10^-5, 10^-6 M). 270 uL cell culture per well + 30 uL 10x arabinose. 3 Replica of the different concentrations. Measure fluorescence in the interval 450 - 540 nm.

Tecan machine had a temperature of 28.2 Celsius degrees. The machine is a little bit warmer than expected

Plate plaquing of phage from waste water sample after sand- and fat traps (sample #2)
Initiated DH5alpha E. coli at 10:15.
Plated phage dilutions: 10^-2, 10^-3, 10^-4, 10^-6, 10^-8, 10^-10 with 0.7 % agar. Incubate 37 C for 24 h.
OD600=0.84

Dilution serie of sludge sample (sample #3)
Phage dilutions: 10^-1 to 10^-6

Testing temperature in 3 connected chemostats.(Aslak)

Autoclavated 3D-printed plastic part
Worked, but with minor deformalities.

Day 21

Tuesday, July 11, 2017 11:39 AM

Results from day 20 – phage isolation:
(phage sample #2 in standard top agar (0.7% agar))

Phage sample dilution:

10^-2: only bacterial colonies
10^-3: only bacterial colonies
10^-4: many densely grown plaques + big area of bacterial growth
10^-6: 1 big plaque (app. 5 cm diameter) + 3 smaller plaques (1 cm diameter)
10^-8: one barely visible plaque
10^-10: no plaques, even bacterial growth on the entire plate

Today: One plaque from the 10^-6 dilution plate was isolated (piece cut out from agar + 1 drop of chloroform + LB medium) and put in the fridge. However the plaque is a bit big and possibly contains several different phages.

Results from day 20 - **characterisation of biobrick:**
(bioluminescence from lux transformants)

No results due to software error. New attempt today:

Initiated Lux 1 and 2 in 25 mL cultures 1/10x concentration after lunch (12). Used 25 uL chloramphenicol, leading to no growth of E. coli with lux, diluted with 25 mL LB-medium around 12.35.

Induce and record fluorscence of Lux with Tecan plate measurer at OD600 (lux 2 OD600= 0.584) with different arabinose concentrations (0, 10^-2, 10^-3, 10^-4, 10^-5, 10^-6 M). 270 uL cell culture per well + 30 uL 10x arabinose. 3 Replica of the different concentrations. Measure fluorescence in the interval 450 - 540 nm.

Temperature: 28. 15:20.

Plate plaquing of phage from waste water sample after sand- and fat traps (sample #2) - third attempt
0.3 % agar vs 0.7 % agar
Initiated DH5alpha E. coli at 10:15.
Plated phage dilutions: 10^-5, 10^-6, 10^-7, 10^-8, with 0.7 % and 0.3 % top-agar. OD600=0.801
Incubate 37 C or 3 h, then stored in the refrigerator.
After 3 hours this was observed:

Phage Dilution	**0.3 % Agar (# plaque)**	**0.7 % Agar (# plaque)**
10^{-5}	38 small plaqued D* = 2-3 mm	41 small plaques D = 2-3 mm
10^{-6}	9 small plaques D = 2mm	5 small plaques D = 1 mm
10^{-7}	1 smal plaque D = 2 mm	0 plaque

10^{-8}	0 plaque	1 plaque 3-4 mm
Conrol (without phage)	Bacteria	Bacteria

*D = Diameter

Made 6 agar plates with glucose and chloramphenicol for the mutagenic bacteria (containing MP6 plasmid). See protocols for making agar plates with glucose.

Day 22

Wednesday, July 12, 2017 9:15 AM

Growth of isolated phages (Marianna)
Phages isolated yesterday: sample #2 10^{-6} (waste water sample after sand and fat traps)

- LB medium (25 mL) added to erlendmeyer flasks.
- E. coli broth from yesterday (2.5 mL) added
- Phage (1 mL) added when OD600=0.8
- Incubated at 37 C 226 rpm

Growth of isolated phages from 7. July (Aslak)
Phages isolated from 7. July were grown in LB with DH5a.

- 6 erlenmeyer flasks with 25 mL LB and 2.5 mL DH5a prepared.
- When OD600=0.8, added 1 mL phage in each flask.
- Incubated at 37 C 226 rpm.

Plaque count of plaques from 11. July (Marianna)

Plate no.	Plaques per plate	Dilution factor	Volume plated (mL)	Titer calculation (Number of plaques) (DF)/Volume plated (mL)	Titer: Plaque-forming units (PFU) per mL
10^{-5} 0.3 % agar	38	10^{-5}	0,1 mL phage	$(38*10^5)/0.1$	38 000 000
10^{-6} 0.3 % agar	9	10^{-6}	0.1		90 000 000
10^{-7} 0.3 % agar	1	10^{-7}	0.1		100 000 000
10^{-5} 0.7 % agar	41	10^{-5}	0.1		41 000 000
10^{-6} 0.7 % agar	5	10^{-6}	0.1		50 000 000
10^{-8} 0.7 % agar	1	10^{-8}	0.1		1 000 000 000

Isolation of phage plaques from agar plates (Marianna)
Two plaques from each of the agar plates 0.3 % and 0.7 % with phage dilution 10^{-5} were isolated and added in two separate eppendorf tubes containing 1 mL LB and 1 drop chloroform. Kept in refrigerator.

NB! There are some chlorofom (small falcon tube) in the refrigerator for plaque isolation.

Plating of E.coli with MP6 plasmid
E.coli NEB Turbo containing the MP6 plasmid was plated on two agar petri dishes and incubated at 37 °C for 24 hours (no growth after 3 hours).

Chemostat
Stable magnetic stirrers.

Lux luminescens by Tecada

Autoclavated container with glas equipments.

Day 23

Thursday, July 13, 2017 10:15 AM

Transformation of MP6

E.coli NEB Turbo MP6-transformants from plating on agar on Day 22, was isolated and grown in 25 mL LB, 35 mM glucose, and 2,5 mL CM. Two flasks were inoculated with 1 colony each. The flasks were grown in overnight in 37 °C.

(Made batch of 125 mL LB + 0.78 g glucose + 125 microliter CM – glucose and CM was added to sterile LB medium, but we assume that there will be no contamination due to the CM. Remaining medium stored for later.)

Luminescence measurement of LUX cultures with Tecan
Replant Lux 1 and Lux 2 with 25 mL LB-medium, 0.025 mL Chloramphenicol and 2.5 mL overnight Lux 1 and Lux 2 cultures.
Tecan machine and computer moved to the cold storage room (4 C) to keep the machine at 20 C.

Induce and record fluorscence of Lux with Tecan plate measurer at OD600 (lux 2 OD600=0.577) with different arabinose concentrations (0, 10^-2, 10^-3, 10^-4, 10^-5, 10^-6 M). 270 uL cell culture per well + 30 uL 10x arabinose. 3 Replica of the different concentrations. Measure fluorescence in the interval 450 - 540 nm. Measure without lid on 96-well plate.

Phage 2 plaquing (Marianna)
Replant E. coli DH5a with 25 mL LB-medium and 2.5 mL overnight culture.
Phage #2 (from waste water after sand- and fat traps) dilutions 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} (0.1 mL) and an E. coli DH5a control were plated on agar plates with LB-medium and 0.7 % top agarose. The plates were in the sterile hood with open lid until the agarose had solidified. Incubated at 13:15 in 37 C for 3.5 h.

E. coli DH5a had OD600=0.832.

See protocol for isolation of bacteriophages.

Plaque count:

Dilution factor	Plaques per plate	Titer: Plaque-forming units (PFU) per mL
10^{-4}	>200	-
10^{-5}	92	9.9×10^7
10^{-6}	5	5×10^7
10^{-7}	0	-
10^{-8}	0	-

The control showed continuous growth of E. coli.

Phage filtration (Peter H)
Phage #1 (untreated waste water) and #2 (waste water after sand and fat traps) grown overnight with E. coli DH5a (7 erlenmeyer flasks). 10 mL of each of the flasks were transferred to separate centrifuge tubes, and centrifuges at 2000 RPM for 5 min. The supernatant contain phages. Then the supernatants were filtrated with a 0.2 μm micron syringe filter and collected in three different falcon

tubes: one for phage #2 grown once with E. coli, one for phage #1 grown twice with E. coli, and another for phage #2 grown twice with E. coli. The tubes are in the refrigerator 4 ^{o}C.

Day 24

Friday, July 14, 2017 1:58 PM

Isolation and transformation of mutagenesis inducing plasmid
MP6 plasmid was isolated from NEB Turbo E.coli cells from Day 23, using Monarch Plasmid Miniprep Kit.

Concentrations obtained:

Sample 1: 246,6 ng/uL
Sample 2: 157,6 ng/uL

Plasmid from both samples were transformed into competent DH5alpha cells and plated on Kan + LB. Put in fridge (4 °C) over week-end.

Remaining plasmid in freezer.

Chemostat lid
Made prototype lid for the chemostat using 3D printing.

Lux. Started Lux2 on 20C, 0, -2 - -6. Gain 255.

Mutagenesis control plasmid
pLA230 plasmid was plated on LA + Kan and put in fridge over the weekend.

Day 25

Monday, July 17, 2017 10:10 AM

Transformation of DH5alpha with plasmid MP6

The transformation from Day 24 went wrong, so we tried again today:

Plasmid from two samples (Sample 1: 246,6 ng/uL, Sample 2: 157,6 ng/uL) were transformed into competent DH5alpha cells and plated on CM + Glc + LA and incubated in 37C.

Isolation of pLA230 plasmid

No growth on the plates that were put in the 4°C fridge over the week-end. The plates were put in 37°C to see if we can get any growth.

As there were no visible growth after a couple of hours, two additional plates with Kan + LA were plated out and put in the 37°C incubator.

Chemostat lid
Tested lid printout. Made some slight modifications and another prototype with 3D-printer (Ultimaker)

Day 26

Tuesday, 18 July 2017 9:18 am

Chemostat lid
Tested second version of chemostat lid. Made some more modifications. Dropped pH-meter and pH-regulatory system for now.
Started print of third version of lid (non-prototype) in resin (Formlabs).

Supercompetent DH5alpha with MP6
The protocoll for creation of supercompetent DH5α with MP6 was initiated. As we did not have a media with said cells, 10 mL og IPS media was inoculated with said bacterai.

E.coli with pLA230
The bacteria from AddGene had begun to grow on the agar + KAN plates. Two Erlendmeyer flasks with LB-media were each inoculated with an isolated bacterial colony

Day 27

Wednesday, July 19, 2017 2:12 PM

pLA230
The plasmid was isolated by Monarch Plasmid miniprep in two parallels.

Concentrations of plasmid:
Sample 1: 159,0 ng/uL
Sample 2: 188,4 ng/uL.

The two parallells of pLA230 were transformed into DH5α E.coli.

MP6
The MP6 plasmid was isolated again

Lux plasmid
The Lux plasmid was transformed into DH5α in two parallells to create stock.

Chemostat lid
Resin print failed. Made another attempt.

Broth lid
Designed lid for chemostat broth container.

Day 28

Thursday, 20 July 2017 3:14 pm

Restriction digestion of pLA230 and MP6

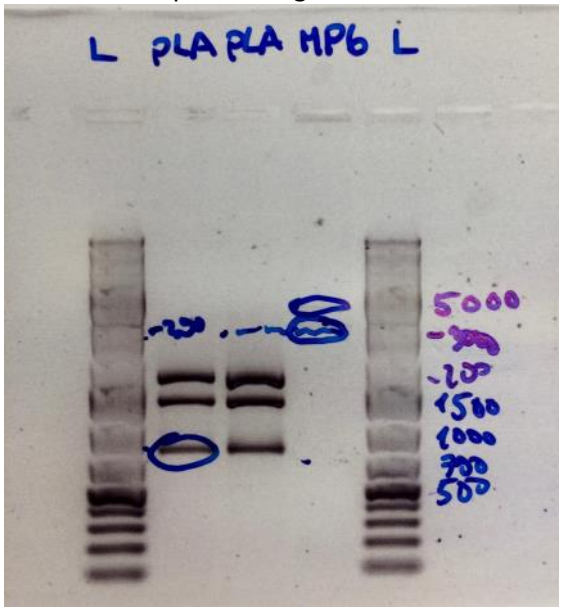
The plasmids pLA230 and MP6 were restriction digested (protocol) with the restriction enzyme BspHI:

- 0.5 ug plasmid, 2 uL buffer, 1 uL enzyme

After digestion, the samples were run on gel electrophoresis:

- Gel: 0.8% agarose + GelRed
- 2 uL ladder (GeneRuler 1kb DNA Ladder Plus)
- 4 uL loading dye + 20 uL samples
- 80 V for 45 min

Enzyme BspHI was expected to cut plasmid MP6 into 2900 + 3700 bp, and plasmid pLA230 into 769 + 1679 + 1235 bp. Gel image:



Chemostat lid

Resin print failed again. Printer non-functional. Made some sloight modifications on 3D-model and started 3D-print in PLA (Ultimaker).

Broth lid

Made some fixes and modifications.

Day 29

Friday, July 21, 2017 1:24 PM

Plasmid isolation

The MP6 and Lux plasmids were isolated using ZR Plasmid MiniPrep – Classic. Lux were isolated in two parallels. The concentrations were measured using NanoDrop Microvolume Spectrophotometer:

- MP6: 53,1 ng/uL
- Lux1: 20,1 ng/uL
- Lux2: 50,7 ng/uL

Supercompetent DH5alpha cells with pLA230

Supercompetent cells with plasmid pLA230 were made following protocol 6, however some mistakes were made:

- Double amount of TFB 1 and 2
- Centrifugation at 20 °C instead of 4 °C
- Pellets not completely resuspended (due to a broken pipette)
- Transformation buffers (TFB) stored at room temperature instead of 4 °C

Light sensor case

Started designing 3D-model for light sensor case.

Day 30

Monday, 24 July 2017 10:12 am

Supercompetent DH5alpha cells with pLA230
Supercompetent cells with plasmid pLA230 were made following protocol 6, however some mistakes were made:

- The first centrifugation at 11 °C instead of 4 °C. Second centrifugation was correct.
- After resuspension with TFB1 the solution was incubated for 10 minutes instead of 5 minutes.

Before start DH5alpha cells with pLA230 had OD_{600}=0.440.
10 aliquotes were made.

Transformation of MP6
Plasmid MP6 from Friday 21 July was transformed into:

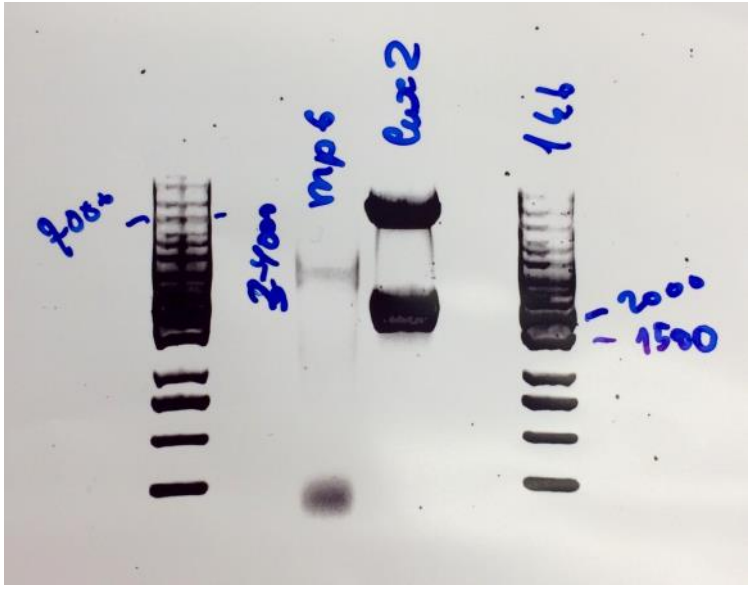
- Supercompetent DH5**α** with pLA230 from today and from Friday 21 July
- Ordinary competent DH5**α** cells

1 uL MP6 was added, however 2 uL should have been added due to low MP6 concentration.

Glycerol stock of DH5α with Lux 2 plasmid
There was made 5 tubes of glycerol stock of DH5α with Lux 2
(1 mL in each tube) - stored in –80 deg freezer.

Restriction Digestion and gel electrophoresis
Isolated MP6 and Lux plasmid from Day 29 was digested with either the enzymes XbaI and SpeI (Lux) or BspHI (MP6) and run in gel electrophoresis.

Results:



The Lux plasmid was expected to be cut in two fragments of 7645 and 2062 bp. This was confirmed by the gel image.

The MP6 plasmid was expected to be cut in two fragments of 2948 and 3733 bp. The gel image from this well was very blurry and it seemed like the plasmid was not cut properly. However there was a weak band in the 3500 region. (see image)

Light sensor case
Finished designing 3D model for Light sensor case. Printed some minor parts in PLA (black).

Chemostat lid
Made some fixes so it should be easier to print in PLA with Ultimaker.

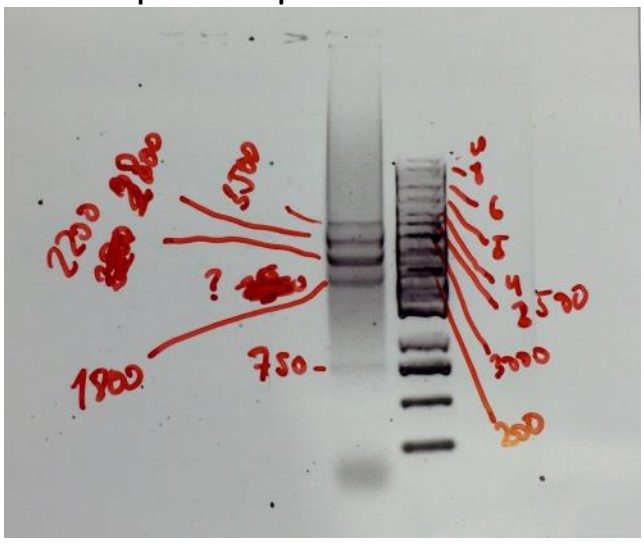
Day 31

Tuesday, July 25, 2017 11:11 AM

Miniprep of pLA230 from DH5alpha E. coli
The pLA230 plasmid were isolated using ZR Plasmid MiniPrep – Classic. Did not measure concentration (Nanodrop).

Colonies with MP6 and pLA230 grown in liquid culture.
Colonies (containing MP6 and pLA230) on three agar plates with LB, kanamycin and chloramphenicol were detected. One colony from each of the agar plates were inoculated in three different Erlenmeyer flasks containing LB medium, kanamycin and chloramphenicol.

Gel electrophoresis of plasmid MP6



Glyserol stock of MP6, pLA230 and MP6+pLA230

Light sensor case
Printed main body.

Broth lid
Redesigned to be both easier to print and to fit our new setup with digital weights. Made holder for 0,2 µm filter.

Day 32

26. juli 2017 13:12

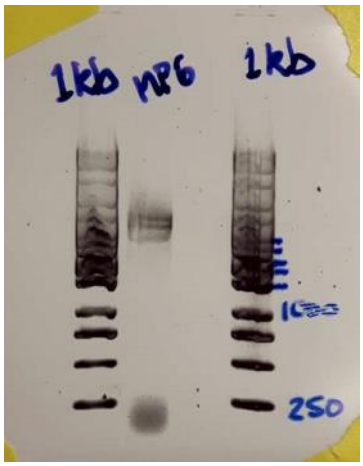
DNA purifiction (MiniPrep) of MP6 and pLA230
1 mL of each culture. 16000 rpm for all centrifugation steps.

Restriction digestion and gel electrophoresis of plasmid MP6
The concentration of plasmid was measured with NanoDrop Microvolume Spectrophotometer:

- MP6: 46.1 ng/uL

Restriction digestion of MP6 was made with Bbs1 and incubated for 2.5 hours.

Gel electrophoresis was run at 90 V for 45 min. 2 uL ladder, 20 uL sample + 4 uL loading dye.



Bacterial stock solutions
Five of each of the glyserol stock solutions were made:

- DH5alpha E. coli with pLA230
- DH5alpha E. coli with MP6
- DH5alpha E. coli with pLA230+MP6

Stored in –80 C fridge.

Luminescence measurement with Tecan measurement
Induce and record fluorscence of Lux with Tecan plate measurer at OD600 (lux 2 OD600=0.603) with different arabinose concentrations (0, 10^-2, 10^-3, 10^-4, 10^-5, 10^-6 M). 270 uL cell culture per well + 30 uL 10x arabinose. 3 Replica of the different concentrations.

Measure fluorescence in the interval 450 - 540 nm and without lid on 96-well plate. The temperature of the machine was 28 C

Broth lid
Printed out broth lid. Did not fit. Redesigned based on former lid.

Day 33

Thursday, July 27, 2017 9:26 AM

Luminescence measurement with Tecan measurement
Induced and recorded fluorscence of Lux with Tecan plate measurer at OD600 (lux 2 OD600=0.603) with different arabinose concentrations (0, 10^-2, 10^-3, 10^-4, 10^-5, 10^-6 M). 270 uL cell culture per well + 30 uL 10x arabinose. 3 Replica of the different concentrations.

Measured fluorescence in the interval 450 - 540 nm and without lid on 96-well plate. The temperature of the machine was 30 C

Broth lid
Printed out new version. It fit.

Day 34

28. juli 2017 09:51

Luminescence measurement with Tecan measurement
Induce and record fluorscence of Lux with Tecan plate measurer at OD600 (lux 2 OD600=0.603) with different arabinose concentrations (0, 10^-2, 10^-3, 10^-4, 10^-5, 10^-6 M). 270 uL cell culture per well + 30 uL 10x arabinose. 3 Replica of the different concentrations.

Measure fluorescence in the interval 450 - 540 nm and without lid on 96-well plate. The temperature of the machine was 30 C

Light sensor cover
Made some adjustments to the light sensor cover for easier use and printed aout new lids.

Day 35

1. august 2017 09:00

31. July 2017

Plate plaquing of phage from waste water sample after sand- and fat traps (sample #2)

- Plaques from waste water sample after sand- and fat traps (#2) that was isolated from day 18 and then grown twice with E. coli DH5a (to get more concentrated phage culture) were plated on agar plates with E. coli DH5a and 0.7 % top agar. See protocol 3.
- Plated phage dilutions: 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and control (with only DH5a).
- E. coli DH5a had OD_{600} = 0.831
- No plaques were observed after 3 hours incubation in 37 C. After 15 hours incubations in 37 C only bacteria were observed on the agar plates.

Media

- 1 L LB-medium was made + autoclavated

3D print

- Printed out broth lid for second chemostat setup.

Day 36

1. august 2017 09:00

Made new media and agar

- 1 L LB-medium 5 g/L NaCl (According to protocol in the "cooking book" at lab)
- 1 L LB-medium with 10 g/L NaCl (According to protocol on OneNote)
- 1 L LB-medium with 15 g/L agar

Finished autoclavating the media

Day 37

2. august 2017 09:06

Plating of phages

- Phages from waste water sample #2 were plated on agar plates with E. coli DH5a and 0.7 % top agar. See protocol 3.
- Plated phage dilutions: 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and control (with only DH5a).
- E. coli DH5a had OD_{600} = 0.78.
- After 3 hours incubation in 37 C there were observed no plaques. The plates were left for incubation overnight in 37 C. No plaques were observed on the agar plates.

Media

- LB medium with agar was autoclaved and used for phage plating. Additionally, new agar plates with LB were made.
- Glucose 35 mM (6.3 g/L) was added to an autoclaved LB medium - should have been added before autoclaving

Day 38

Thursday, 3 August 2017 1:02 pm

Results from plating of phages
After incubation overnight in 37 C no plaques were observed on the plates.

Media
- LB medium (500 mL) and 35 mM glucose (3.15 g) was mixed and autoclaved
- All cultures were transfered to new media and inoculated at 37 C

Phage isolation
- Waste water sample #2 (after sand and fat trap; 40 mL) was added to LB medium (5 mL) and E. coli broth in exponential phase (1 mL) and inoculated at 37 C and 225 rpm for growth overnight.
- Waste water sample # (after sand and fat traps; 5 mL) was added to LB medium (50 mL). Incubated at 37 C and 225 rpm for growth overnight. (Phage from this sample marked with *).

3D-printing
Printet chemostat lid + additional parts in PLA.

Day 39

Friday, August 4, 2017 10:47 AM

Isolation of bacteriophages

Two overnight cultures (' , *) of waste water #2 after sand and fat traps (10 mL of each) were centrifuged at 2000 rpm for 5 minutes, and then filtrated with 0.2 um syringe filter. A serial dilution were made of both of the phage samples. When E. coli DH5a had OD600=0.837, the different phage dilutions were inoculated with Dh5a, then plated with 0.7 % top agar on agar plates containing LB medium. The plates were incubated at 37 C for 3 hours. Small phage plaques (19) were observed on 10^-5 from waste water sample *. The plaques were too small to be isolated. Mistake that the agar is the cause of poor plaques. New attempt on monday.

Phage dilutions' plated: 10^{-3}, 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8}
Phage dilutions* plated: 10^{-5} , 10^{-6} , 10^{-7}

' Waste water sample #2 (after sand and fat trap; 40 mL) was added to LB medium (5 mL) and E. coli broth in exponential phase (1 mL) and inoculated at 37 C and 225 rpm for growth overnight.
* Waste water sample # (after sand and fat traps; 5 mL) was added to LB medium (50 mL). Incubated at 37 C and 225 rpm for growth overnight. (Phage from this sample marked with *).

Growth of MP6 and pLA230

- LB medium with agar was mixed and autoclaved.
- Ampicillin was added to the LA and agar plates were made
- When OD_{600} = 0.3, DH 5a + MP6 + pLA230 was mixed with arabinose:
    - 5 mM
    - 20 mM
- The bacteria were plated and incubated in 37 C overnight

Day 40

7. august 2017 09:22

Test of MP6 mutation effectivity
Bacterial growth observed on all plates from Friday:

- DH5a + pLA230
- DH5a + pLA230 + MP6

These plates all had Ampicillin, so this was not as expected: there should not be any growth on the plates with DH5a + pLA230 without MP6. We need to redo the experiment. Hypothesis: Amp stock was old and might have been stored improperly.

New Amp stock was made.

LA medium was mixed and autoclaved.

Bacteria were transferred to new Erlenmeyer flasks for growth

- DH5a
- Lux
- MP6+pLA230
- pLA230

3D-printing
Printet additional broth lid in PLA.

Day 41

Tuesday, August 8, 2017 10:06 AM

Plates
Plates with LA and LA + Amp were made to repeat experiment from Day 39.

Media
1 L LB medium were made and autoclavated.

Stock solution of 0.1 M arabinose
Four falcon tubes (15 mL) containing 0.1 M arabinose are stored in the freezer at –20 C.

Growth of bacteria in new erlenmeyer flasks
- Stock MP6 + pLA230
- Stock MP6 + pLA230
- Stock pLA230
- DH5a
- Lux
- pLA230
- MP6 + pLA230

Bacteriophage plating
Phage #2 from waste water sample after sand- and fat traps were plated on agar plates with LB (according to protocol 3). Phage dilutions: 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were plated, together with a control (only DH5a). DH5a had OD_{600}=0.794. The outside of the plated were washed with bleach (2 % sodium hydrochloride) before incubation at 37 C. After 3 hours incubation there were no plaques on the plated, due to unsolidified top agarose. Until next time, there should be made new top agarose.

3D-printing
Printet light sensor parts in PLA.

Day 42

9. august 2017 09:00

Testing for DH5a with MP6 + pLA230
Bacteria were transferred into new Erlendmeyer flasks for growth overnight (before plating at Ampicillin tomorrow):

- Stock MP6 + pLA230 + Glc
- Stock MP6 + pLA230 + Ara (20 mM at OD_{600} = 0.3)
- Stock pLA230

Bacterial glyserol stocks

- At OD_{600} = 0.45 and 0.57, DH5a + MP6 + pLA230 (500 uL) was added to 15 microfuge tubes
- 50% glyserol (500 uL) was added to the tubes
- The tubes were stored at -80 degrees

Incubation of environmental samples

- Water samples were collected in 50 mL Falcon tubes from the waste water treatment plant at Lade
- One of the samples (waste water after sand and fat traps; 40 mL) was incubated with E. coli (5 mL) and LB medium (5 mL) at 225 rpm and 37 degrees overnight

3D-printing
Printet more light sensor parts in PLA.
Printet chemostat lid + additional parts in PLA.

Day 43

10. august 2017 09:26

Plasmid mutation rate
The following overnight cultures of bacteria with plasmids and activators/repressors were plated out on LA + Amp and LA (control) plates:

- DH5a
- DH5a + pLA230
- DH5a + pLA230 + MP6 + Glc (repressor)
- DH5a + pLA230 + MP6 + Ara (activator)

All plates were incubated at 37 C.

Phage plaquing (1939-protocol)
5 agar plates containing LB medium were dried with filter paper overnight in 37 C incubation.

Phage plaquing (Alex protocol)
There were not enough agar plates, so 500 mL agar with LB medium was made.

10 mL x 2 of sewage-bacteria-bacteriophage culture were centrufuged and filtered with 0.2 um syringe filter. Kept in 4 C fridge.

Day 44

Friday, August 11, 2017 8:32 AM

New LA agar plates were made.

Result from "plasmid mutation rate" plating from day 43

Medium	Plasmid	Result
LA	DH5a	Massive growth of bacteria
LA	pLA230	Growth of bacteria
LA+Amp	DH5a	Growth of bacteria
LA+Amp	pLA230	Growth of bacteria
LA+Glc	PLA230+MP6	Growth of bacteria
LA+Amp+Glc	PLA230+Mp6	Some colonies
LA+Ara	PLA230+Mp6	Many colonies
LA+Amp+Ara	PLA230+Mp6	Many colonies

Need further dilution to get a quantitative comparison.

Phage plating according to Alex's protocol
Phage dilution 10^-1 to 10^-8 was made from phage solution filtrated from waste water after sand and fat traps. In seven empty eppendorf tubes (15 mL) labeled 10^-2 to 10^-8, 0.9 mL overnight DH5a E. coli was added to each of them. Then 0.9 mL of the corresponding phage dilution was added to the tubes. The bacteria-phage mix was mixed by inverting. 3 mL of top agar (50 C) was added to the mixture and mixed before plating on LA agar plates. The plates were incubated overnight at 37 C.

After 3 hours incubation in 37 C there were observed some small plaques on some of the plates.

After 10 hours incubation in 37 C there were observed small plaques on 10^-4, 10^6, 10^-7 and 10 ^-8.

After 24 hours incubation in 37 C there were observed one very small plaque on the following plates: 10^-4, 10^-6 and 10^-7. They were too small to isolate.

Day 45

12. august 2017 12:44

"Plasmid mutation rate" - new attempt
Initiated growth of DH5a+pLA230 stock and DH5a+MP6+pLA230 stock and incubated overnight in 37 C shaking.

Day 46

Sunday, August 13, 2017 3:05 PM

Continue of "Plasmid mutation rate"
Overnight stock bacterial cultures were transferred into new Erlendmeyer flasks for growth overnight (before plating at Ampicillin tomorrow):

- Stock MP6 + pLA230 + Glc
- Stock MP6 + pLA230 + Ara (20 mM at OD_{600} = 0.3)
- Stock pLA230

Day 47

Monday, August 14, 2017 9:23 AM

Phage plating
The following phage dilutions of waste water after sand and fat traps were made:
10^{-1} to 10^{-8} . 0.1 mL of phage dilutions 10^{-4} to 10^{-8} were incubated for 10 minutes shaking in 37 C with 0.5 mL DH5a E. coli (OD600=0.879). Then 3 mL of top agar were added to each tube, before plating on LA agar plates. The agar plates were incubated inverted overnight in 37 C.

After 2.5 hours there were observed some (small) phage plaques on all the agar plates.

Plasmid plating
The table below shows the different plasmids, plates and corresponding dilutions of the bacteria that were plates, and incubated overnight in 37 C.

Medium	Plasmid	10^-6	10-8
LA+Amp	PLA230 Pla230+MP6+GLc (start) Pla230+MP6+Ara (OD = 0.3)		
LA	Pla230 Pla230+MP6+Glc Pla230+MP6+Ara		

DH5a+pLA230 glyseol stock solution
- At OD_{600} = , DH5a+pLA230 (500 uL) was added to microfuge tubes
- 50% glyserol (500 uL) was added to the tubes
- The tubes were stored at -80 degrees

LB medium for chemostat
2 L LB were made and autoclavated.

Chemostat lid
Made new verion of chemostat lid with centered senor-fitting for better distance sampling. 3D-printed the new verion in PLA.

Day 48

15. august 2017 14:29

Results from yesterday's plasmid plating

Medium	Plasmid	10^-6	10-8
LA+Amp	PLA230 Pla230+MP6+GLc (start) Pla230+MP6+Ara (OD = 0.3)	0 0 0	0 0 0
LA	Pla230 Pla230+MP6+Glc Pla230+MP6+Ara	201 86 13	4 1 0

Results from yesterday's phage plating

After 3 and and 6 hours there were observed phage plaques on the agar plates. After incubation overnight the plaques were overgrown with bacteria.

Phage plating

Phage from waste water after sand and fat traps were used to grow plaques. Phage dilutions 10^-1 to 10^-8 were made. 0.1 mL of phage dilutions 10^-4 to 10^-8 were incubated with 0.5 mL DH5a E. coli (OD600=0.9) for 10 minutes in 37 C 226 rpm shaking. Then 3 mL top agar (45 C) were added to each phage-bacteria mixture and mixed by inverting, and then plated on LA agar plates. The plates were incubated in 37 C for 3 hours.

Plaque count

Dilution factor	Plaques per plate	PFU
10^{-4}	27	$2.7*10^{6}$
10^{-5}	9	$9*10^{6}$
10^{-6}	1	$1*10^{7}$
10^{-7}	10	$1*10^{9}$
10^{-8}	3	$3*10^{9}$

Plaque isolation and propagation via lysate plate

One phage plaque from the plates labeled phage 10^{-4} and phage 10^{-7} were added in two separate eppendorf tubes (1.5 mL) with 1 mL of SM buffer. The solution were mixed by vortex and centrifuged at 4000 x g for 5 minutes.

1 mL of the isolated phage solution were added in two seperate erlenmeyer flasks (125 mL) containing 10 mL of DH5a E. coli (OD_{600}=0.59), and incubated in 37 C 226 rpm shaking overnight (??).

Light sensor

Assembled cyvets for us in the light sensor, by melting holes in the bottom and gluing printet parts at te top and bottom sealing it with only to small openings.

Day 49

Wednesday, August 16, 2017 3:52 PM

Phage isolation
Phages were isolated from overnight culture with DH5a by centrifuging the content at 4000 x g for 5 minutes and filtering the supernatant with a 20 μm syringe filter.

Phage plating
Phage from the phage isolation were used to grow plaques. Phage dilutions 10^-1 to 10^-8 were made. 0.1 mL of phage dilutions 10^-4 to 10^-8 were incubated with 0.5 mL DH5a E. coli (OD600= 0.9) for 10 minutes in 37 C 226 rpm shaking. Then 3 mL top agar (45 C) were added to each phage-bacteria mixture and mixed by inverting, and then plated on LA agar plates. The plates were incubated in 37 C for 3 hours.

Plaque count

Dilution factor	Plaques per plate	PFU
10^{-4}	1	$1*10^5$
10^{-5}	5	$5*10^6$
10^{-6}	1	$1*10^7$
10^{-7}	-	-
10^{-8}	8	$8*10^9$

The plate with phage dilution 10^{-7} contained bits of top agar that had become solid during plating and therefore interfered with plaque counting.

PCR – prepping for Gibson assembly
Ran a PCR of the following templates using the corresponding primers with overlapping sequences for use in Gibson assembly. Used the standard procedure for Roche's Expand High Fidelity PCR System. Se protocols for more details.

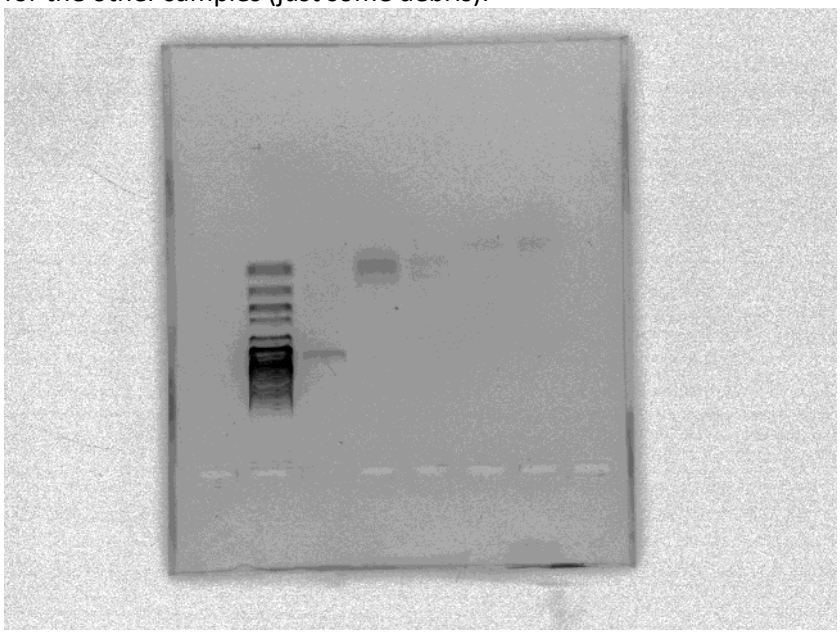
Nr.	**Template**	**Upstream primer**	**Downstream primer**
1	pSB1A3 (backbone)	pSB1A3_FW	pSB1A3_RV
2	Mono1	MUT1_FW	MUT1_mono_RW
3	Mono1	MUT1_FW	MUT1_semi_RW
4	Mono2	MUT2_mono_FW	MUT2_RW
5	Semi2	MUT2_semi_FW	MUT2_RW

Making of agar-plates
Made 10 normal LA-plates, 7 plates with LA + glucose, and 6 with LA + glucose + Ampicillin.

Day 50

Thursday, August 17, 2017 4:56 PM

Gel electrophoresis – checking the PCR
Gel electrophoresis of products from yesterdays PCR showed good yield of sample nr. 1, but none for the other samples (just some debris):



From left to right: 1k DNA ladder, PCR sample nr. 1, 2, 3, 4 and 5.

PCR – wash
Cleaned the PCR products from sample nr. 1 with Zymo Research's "DNA Clean & Consentrator™-25" (Catalog No: D4005).

PCR – preparation for Gibson assembly
Tried repeating PCR of sample nr. 2-5 from yesterdays PCR due to bad yield. Followed the same procedure, but increased amount of template DNA from approximately 10 ng to 30 ng, making sure the stock was thoroughly mixed before use.

Culture transfer
Transferred cultures from earlier of the week consisting of DH5a both with pLA230 and pLA230 + MP6 to identical new media consisting of LB + Kan and LB + Glc + Kan + CM respectively. In addition transferred normal DH5a from yesterdays culture to new LB.

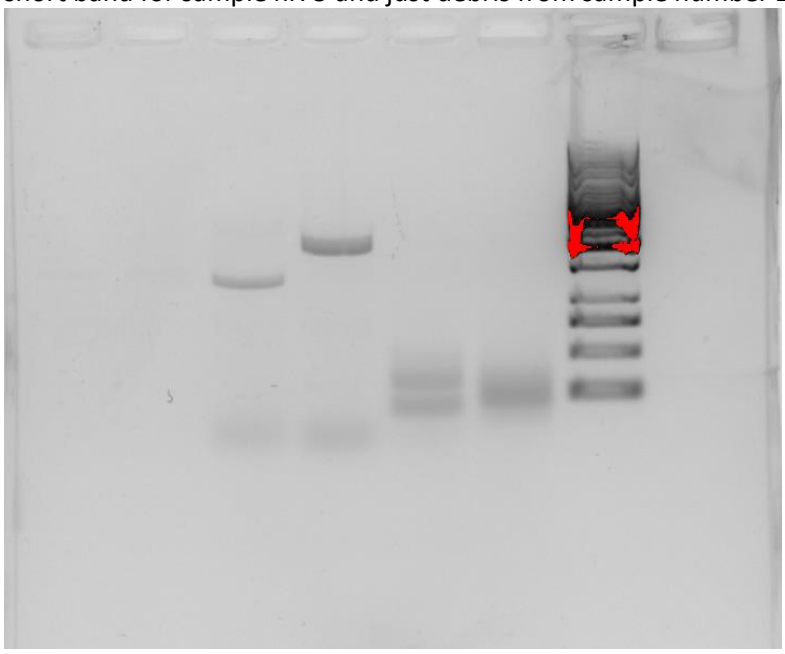
Chemostat
Additional tweaking was done to the chemostat system.

Day 51

Friday, August 18, 2017 4:24 PM

Gel electrophoresis – checking the PCR

Gel electrophoresis of products from yesterday's PCR showed good yield of sample number 4, a too short band for sample nr. 5 and just debris from sample number 2 and 3:



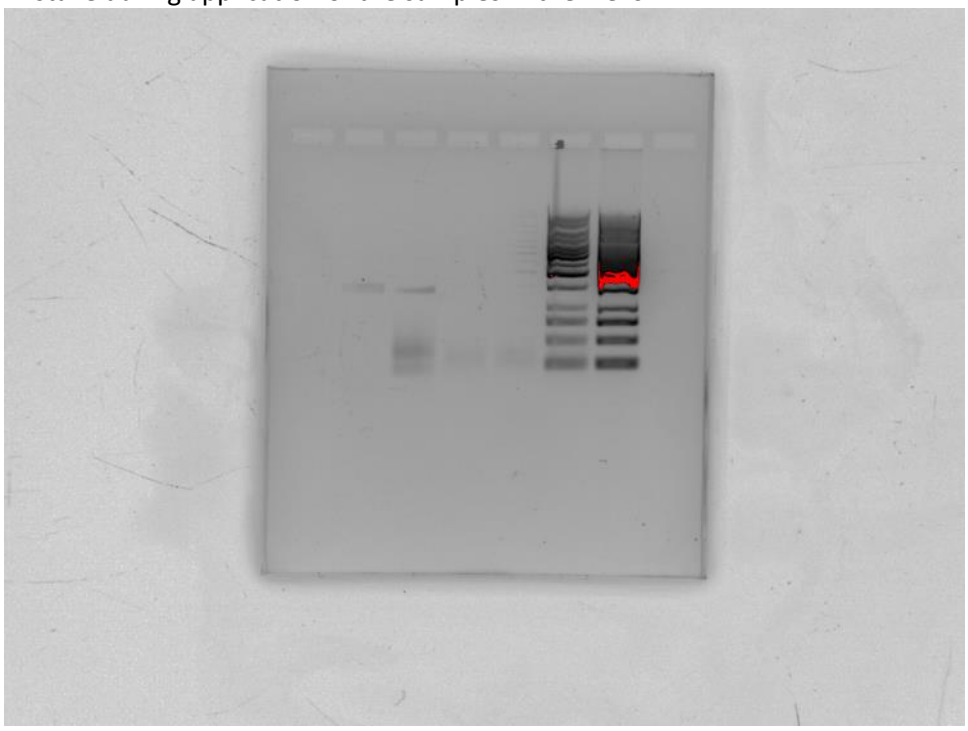
From right to left: 1k DNA ladder, PCR sample nr. 2, 3, 4 and 5.

PCR – preparation for Gibson assembly

Tried repeating PCR of samples 2, 3 and 5 from yesterday's PCR due to bad yield. Followed mostly the same procedure, but made sure to make new dilutions of primers from stock and selecting a new annealing temperature of 50 ^{o}C and elongation temperature of 68 ^{o}C for all samples. Also increased number of repeats from 20 to 25.

Gel electrophoresis – checking the PCR

Gel electrophoresis of products from today's PCR showed somewhat unclear results due to a mistake during application of the samples in the wells:



From right to left: 1kb DNA ladder, 1kb DNA ladder, PCR sample nr. 2, none, PCR sample nr. 3 and 5.

Culture transfer

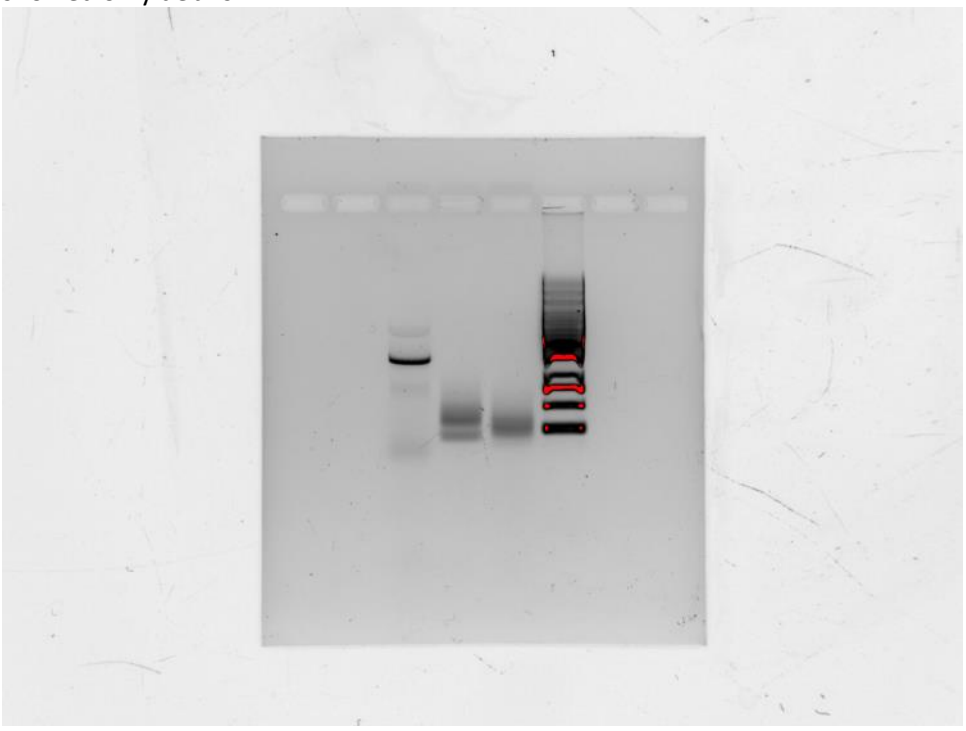
Transferred cultures from yesterday consisting of DH5a both with pLA230 and pLA230 + MP6 to identical new media consisting of LB + Kan and LB + Glc + Kan + CM respectively. In addition transferred normal DH5a from yesterday's culture to new LB. All were applied with 1:100 dilution rate to last the weekend.

Day 52

Monday, August 21, 2017 5:07 PM

Gel electrophoresis – checking the PCR 2nd time

Ran a gel electrophoresis of the PCR-products from day 51. This time it turned out that sample 5 had some major bi-product too short to be the sequence of interest, however some small amount of a strand with approximately right size seemed to have been synthesised as well. Samples 2 and 3 showed only debris:



From right to left: 1kb DNA ladder, PCR sample nr. 2, 3, and 5.

PCR – preparation for Gibson assembly

Tried repeating PCR of sample 2, 3 and 5 due to bad yield. Followed mostly the same procedure, selecting a new annealing temperature of 51 ^{o}C (and elongation temperature of 68 ^{o}C) for all samples.

PCR – wash

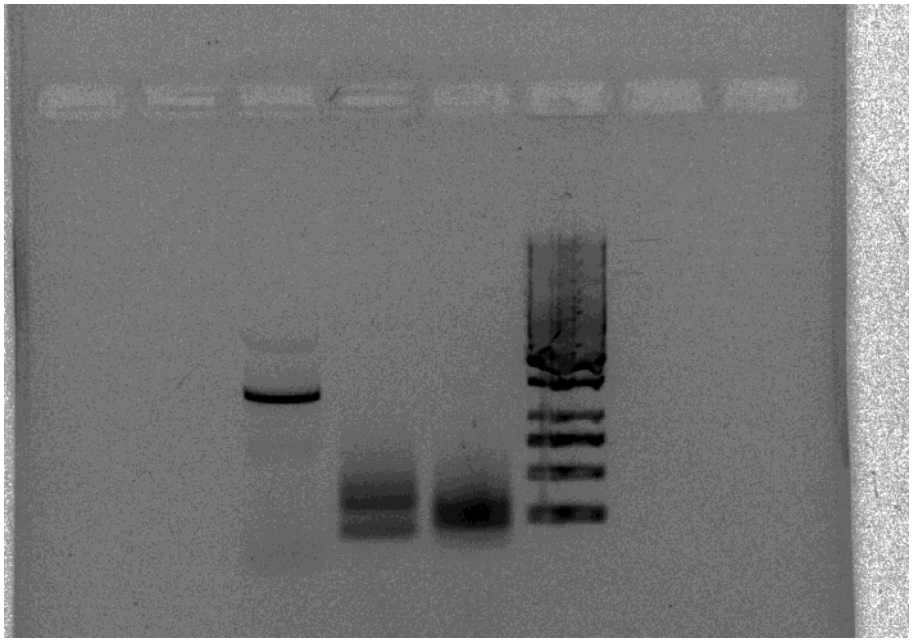
Cleaned the PCR products from sample 4 from PCR on day 50 with Zymo Research's "DNA Clean & Consentrator^{TM-}25" (Catalog No: D4005).

Day 53

Thursday, August 24, 2017 1:10 PM

Gel electrophoresis – checking the PCR

Ran a gel electrophoresis of the PCR-products from day 52. It showed more or less the same results as the PCR from day 51: Sample 5 had some major bi-product too short to be the sequence of interest, but with some small amount of a strand with approximately right size seemed to have been synthesised as well. Samples 2 and 3 showed only debris:



From right to left: 1kb DNA ladder, PCR sample nr. 2, 3, and 5.

Day 54

Friday, August 25, 2017 12:16 PM

PCR – preparation for Gibson assembly

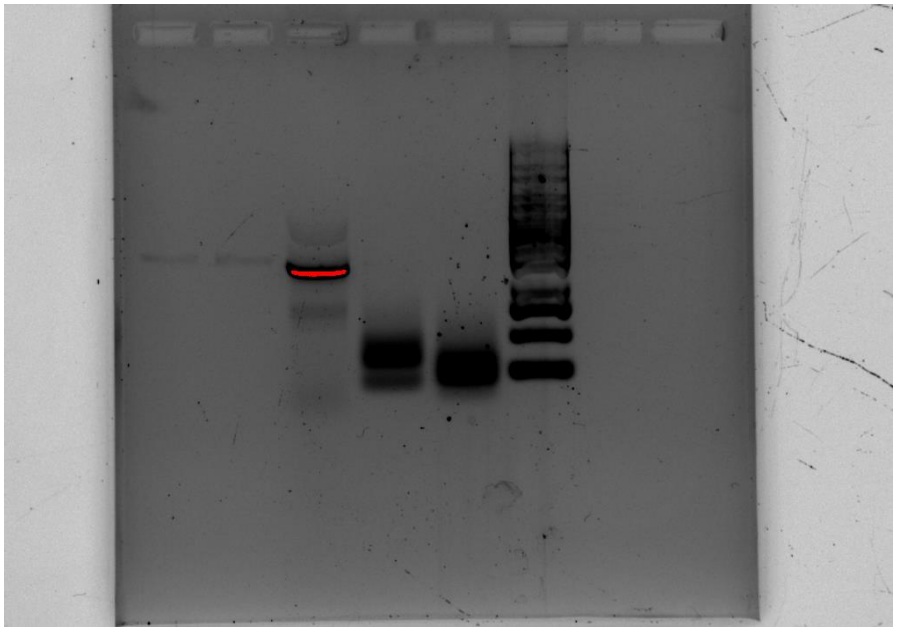
Tried repeating PCR of samples 2, 3 and 5 due to bad yield, but this time with 5 % DMSO in all samples. Also added additional annealing step of 69 °C (30 sek.) in hopes of better yield of sample 3 (with one of the primers having a melting point of 69 °C) before an annealing step with temperature gradient, 50.5 °C for samples 2 and 3 and 54 °C for sample 5 (30 sek.). Added additional cycles to a total of 25.

Day 55

Saturday, August 26, 2017 4:18 PM

Gel electrophoresis – checking the PCR

Ran a gel electrophoresis of the PCR-products from day 54. It showed more or less the same results as the PCR from day 52: Sample 5 had some major bi-product too short to be the sequence of interest, but with some small amount of a strand with approximately right size seemed to have been synthesised as well. Samples 2 and 3 showed only debris:



From right to left: 1kb DNA ladder, PCR sample nr. 2, 3, and 5.

Day 56

Monday, September 11, 2017 6:48 PM

Chemostat
Made medium and sterilized equipment for first chemostat run.

Day 57

Wednesday, September 13, 2017 12:24 PM

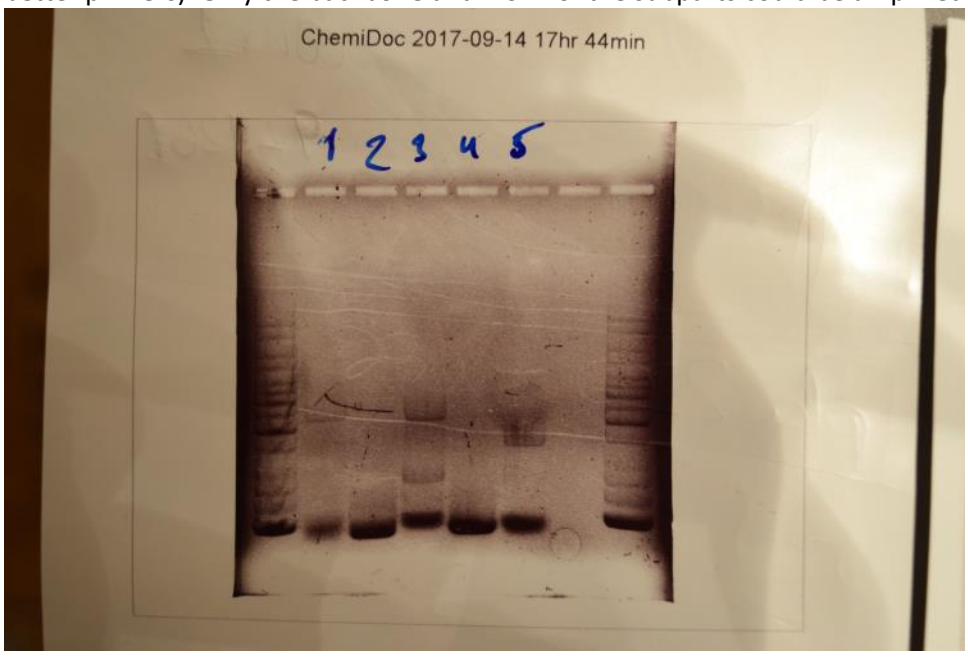
Chemostat
Chemostat experiment ended. Sterilization seemed fine, but ultrasound sensor failed due to condensation of medium. Load cells ordered to replace ultrasound sensors.

Day 58

Monday, October 30, 2017 3:13 PM

14/9/17

Tried PCR-amplifying different synthesized subparts of two improved MP6 biobricks using new (and better primers). Only the backbone and 1 of 4 of the subparts could be amplified:

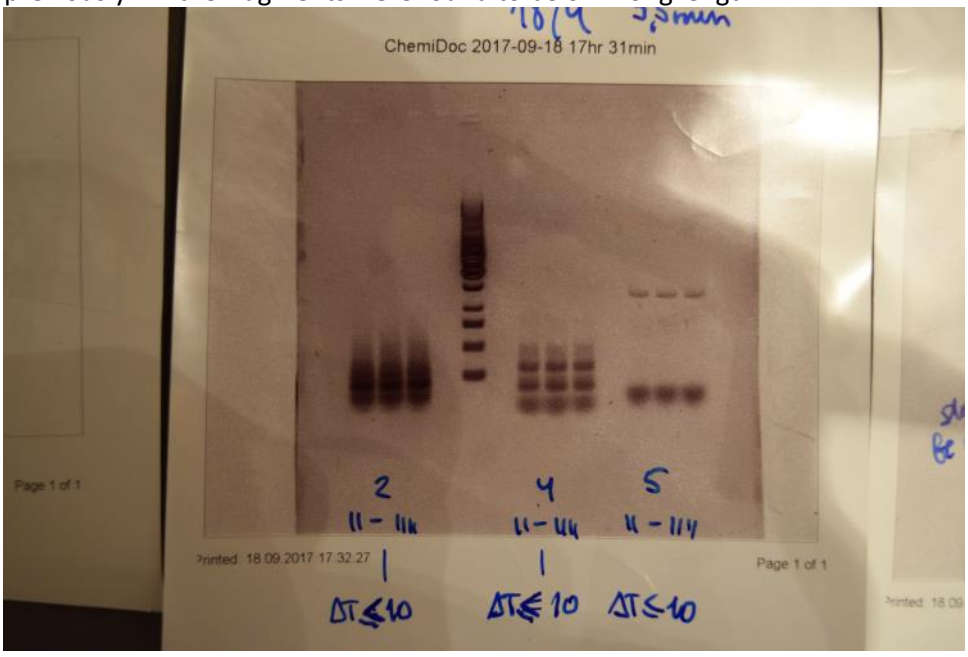


Day 59

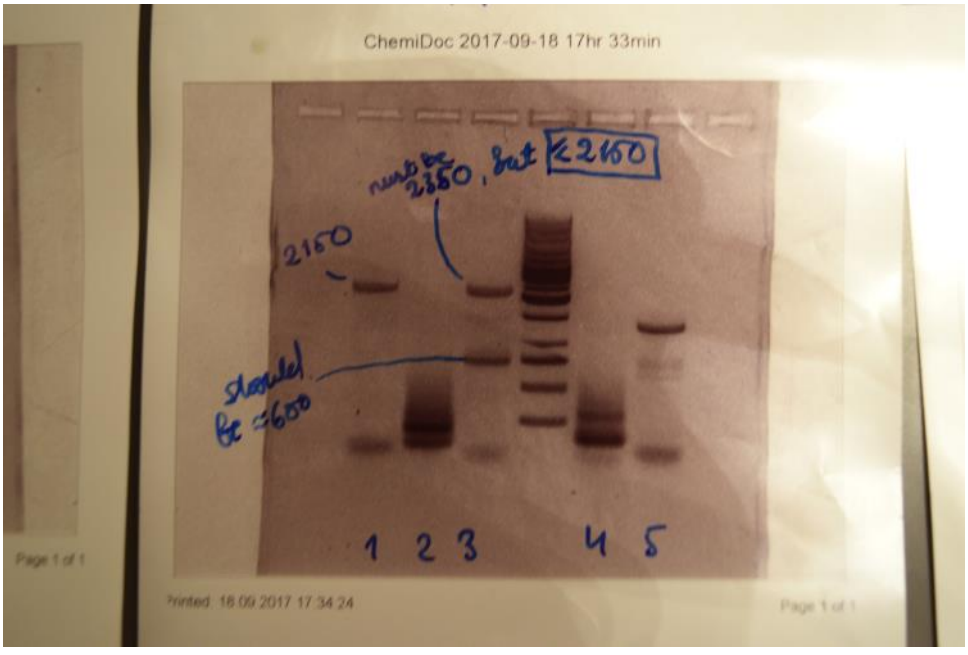
Monday, October 30, 2017 3:17 PM

18/9/17

Tried 10 degrees temperature gradients on the 3 subparts that could not be PCR amplified previously. All the fragments were found to be of wrong length:



Redoing the amplification from 14/9 with a different protocol did not yield any better resluts..

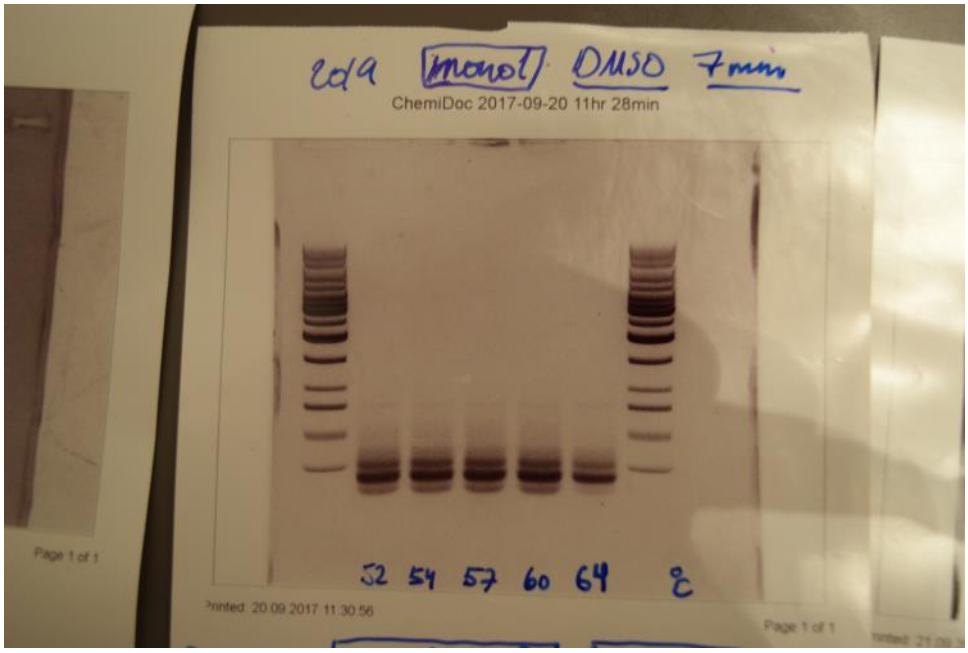


Day 60

Monday, October 30, 2017 3:22 PM

20/9/17

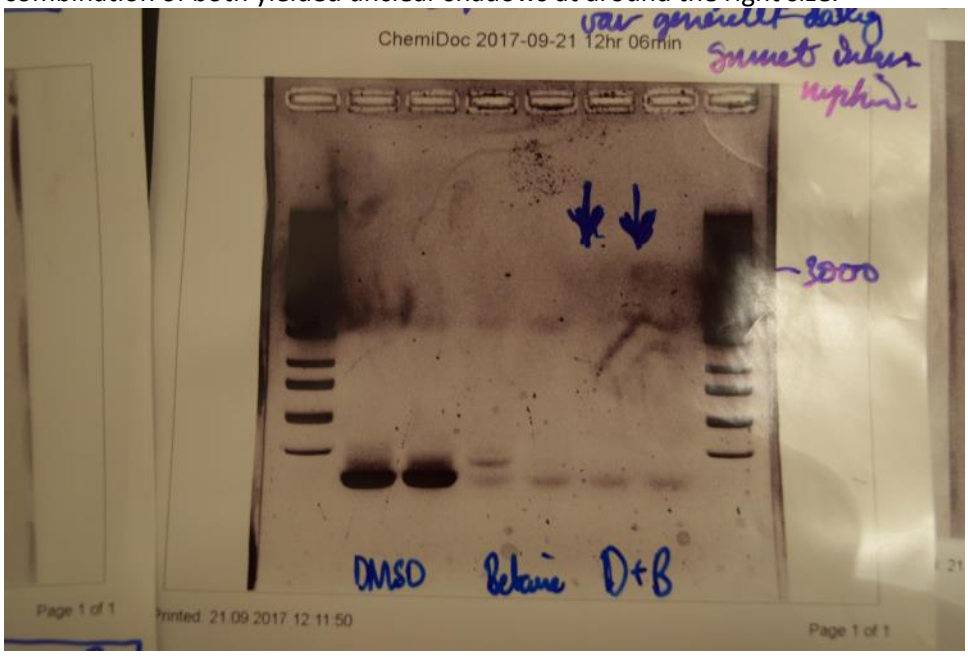
Tried an extended annealing temperature gradient with one of the subparts with addition of 1.5 % DMSO. All amplified fragments smaller than the expected 3000. Bur somewhat longer fragments appeared.



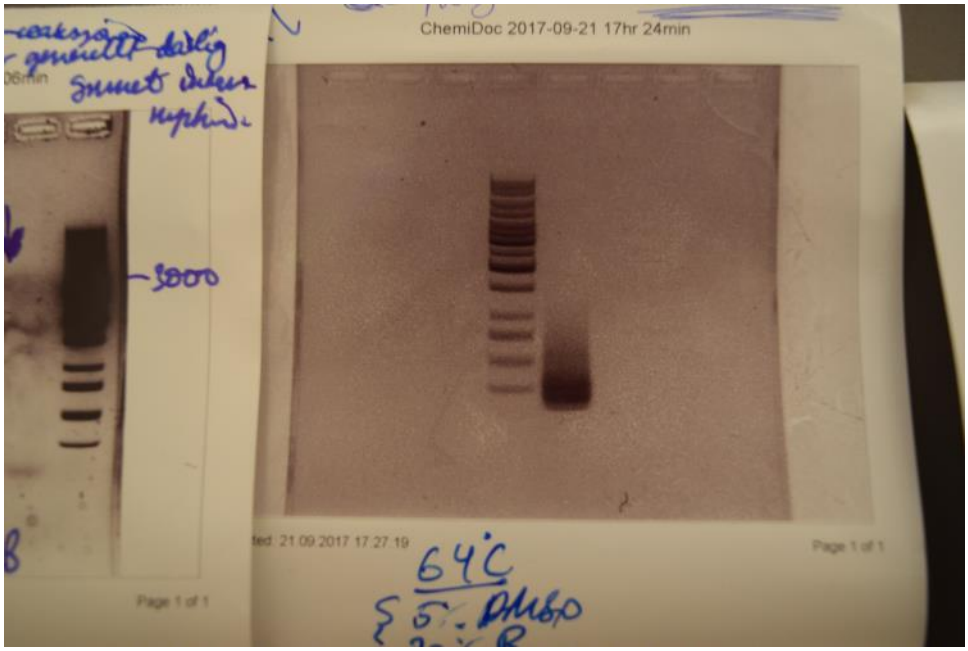
Day 61

Monday, October 30, 2017 3:24 PM

21/9/17
Tried amplifying the same subpart adding combinations of 5% DMSO and 20% Betaine. The combination of both yielded unclear shadows at around the right size.



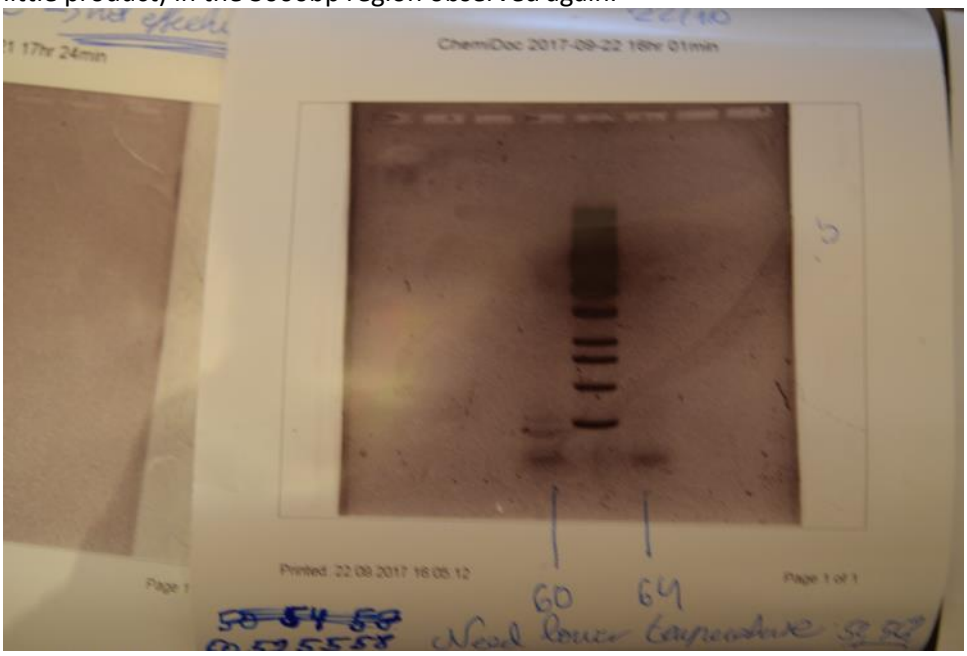
Tried the good conditions with another polymerase (Q5), with much worse results. (No bands)



Day 62

Friday, September 22, 2017 12:29 PM

22/9/17
Retried the optimal conditions with the old polymerase (Taq based). Weak shades (generally very little product) in the 3000bp region observed again.



Mutagenesis (round 1)
Plasmid pLA230 inoculated with/without arabinose

Plates prepared:

- LA + Amp + Glc
- LA + Glc
- LA + Kan + Glc
- LA + CM + Kan + Glc (LCKG)

Day 63

Saturday, September 23, 2017 12:15 PM

Mutagenesis (round 1)
Plasmid pLA230 plated on LA + Glc + Amp and control with LA + Glc.
Concentrations: 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}

Day 64

Sunday, 24 September 2017 10:21 am

Mutagenesis (round 1)

Counted colonies on plates with pLA230 - **13:00**

Plasmid	**Dilution**	**Colonies (LA + Glc + Amp)**	**Colonies (LA + Glc)**
pLA230	10^{-9}	0	> 300
pLA230	10^{-7}	0	> 300
pLA230	10^{-5}	0	> 300
pLA230	10^{-3}	0	> 300

Mutagenesis (round 2)

Transformed pLA230 (number 1) and MP6 into DH5alpha

- Used 1 uL of each plasmid
- Thermal bath 42.5 degrees
- Incubator shaker 20 degrees when incubated, heated to 37 degrees during incubation. Therefore incubated for 5 minutes longer than usual (65 minutes).
- Plated on LA + CM + Kan + Glc (LCKG)
- Incubated at 37 degrees - **15:30**

Day 65

Monday, 25 September 2017 10:39 am

Mutagenesis (round 2)

- Bacterial growth observed on the plate from yesterday.
- Inoculated one colony of DH5alpha + MP6 + pLA230 in LB + Kan + CM + Glc (20 mM)
- Erlendmeyer flask in incubator shaker in the other room due to booking of the one in our room.

PCR (Nikolay)

Tried different temperatures. Did not go well.

Day 66

Tuesday, 26 September 2017 8:05 pm

Transformed new PLA230 into DH5alpha
Innoculated a solution of LB + GLC + KAN + CM, and one with LB + ARA + KAN + CM with MP6 + PLA230 from yesterday.

Day 67

Thursday, September 28, 2017 10:51 AM

Mutagenesis
Counted colonies on plates with pLA230, pla230+MP6 induced, pla230+MP6 uninduced - **11:00**

Transformed the following plasmids into DH5alpha, and inoculated the bacteria in flasks instead of plating them:

- MP6+pLA230 (LA+Glc+Kan+Cm)
- pLA230 (La+Glc+Kan)

Controls (plate with LA + glucose):

Plasmid	**Dilution**	**Colonies**
pLA230	10^{-8}	53
PLA230 + MP6-u	10^{-8}	14
PLA230 + MP6-i	10^{-8}	0

Results (plates with LA + glucose + AMP):

Plasmid	**Dilution**	**Colonies**
pLA230	10^{-2}	8
PLA230	10^{-4}	1
PLA230	10^{-6}	0
PLA230 + MP6-u	10^{-4}	0
PLA230 + MP6-u	10^{-6}	0
PLA230 + MP6-u	10^{-8}	0
PLA230 + MP6-i	10^{-4}	0
PLA230 + MP6-i	10^{-6}	0
PLA230 + MP6-i	10^{-8}	0

The controls imply that the total concentration of living MP6 is too small for such strong dilutions (10^{-4} to 10^{-8}). We therefore propose diluting the controls of MP6-u to 10^{-7} next time and MP6-I to 10^{-4} and 10^{-6}. Similarly pla230 in AMP should be diluted 10^{-1}. MP6-I and MP6-u should be diluted to 10^{-2} and 10^{-1}.

Day 68

Friday, 29 September 2017 12:22 pm

Mutagenesis
Plates prepared:

- LA+Glc
- LA+Glc+Amp

Cultures transferred to new media:

- MP6+pLA230
    - --> LA+Glc+Kan+Cm
    - --> LA+Ara+Kan+Cm
- pLA230
    - --> LA+Glc+Kan

Weight sensors
Nikolay worked with this.

Day 69

Saturday, September 30, 2017 2:47 PM

Plating of DH5a with plasmids and sugars:
Plated out DH5a (overnight cultures) with the following plasmids and sugars in LA+ Amp+Glc and La+Glc (control):

LA + Amp + Glc:

PLA230 (dilution: 10^-1)
PLA230 + MP6 + Glc (10^-1 and 10^-2)
PLA230 + MP6 + Ara (10^-0, 10^-1, 10^-2)

Control: LA + Glc

PLA230 (10^-7, 10^-8)
PLA230 + MP6 + Glc (10^-7, 10^-8)
PLA230 + MP6 + Ara (10^-4, 10^-6)

All put in 37C incubator

Day 70

Sunday, October 1, 2017 3:24 PM

Counted plates from yesterday:

LA + Glc + Amp:

Plasmid	Concentration	Number of colonies
PLA+MP6 + Ara	Undiluted	too many colonies to count
''	10^-1	83
''	10^-2	6
PLA+MP6 + Glc	10^-1	11
''	10^-2	3
pLA	10^-1	1

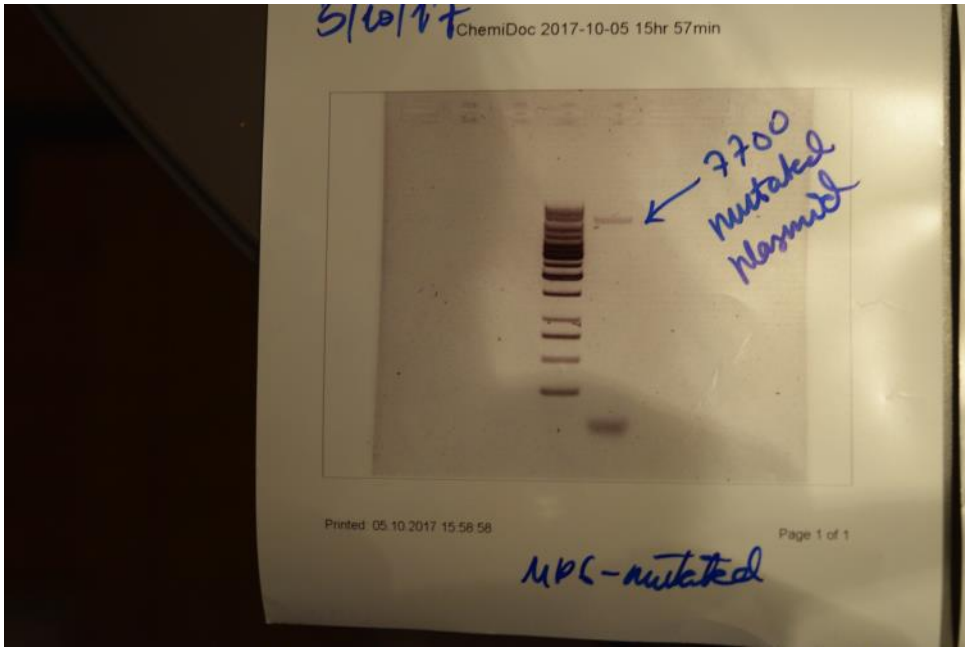
La + Glc (control): in general much bigger colonies

Plasmid	Concentration	Number of colonies
PLA+MP6+Ara	10^-4	Too many to count
''	10^-6	26 (but very very big)
PLA+MP6+Glc	10^-7	5
''	10^-8	8
pLA	10^-7	26, but many have grown together, so probably much more in reality
''	10^-8	2

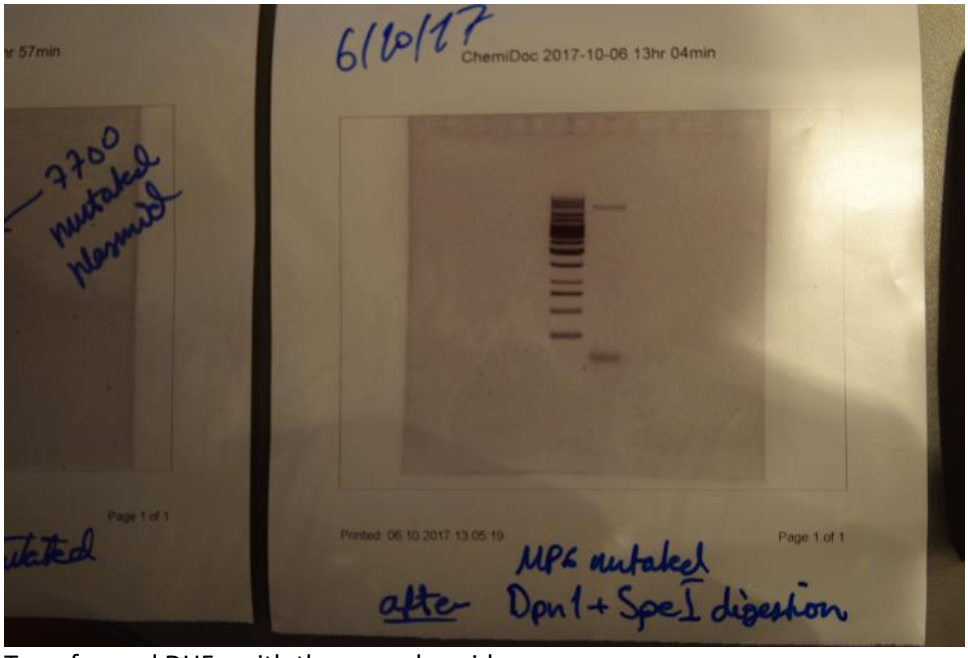
Day 71

Friday, 6 October 2017 3:37 pm

Used mutagenic primers to remove a SpeI site from the original MP6 in order to make it biobrick-friendly. The fragment that was gotten was the right size straight away!



After transfection, miniprep and digestion with DpnI and SpeI only one fragment was detected, meaning that the site was removed successfully. The fragment was the right size.



Transformed DH5a with the new plasmid.

Day 72

lørdag 7. oktober 2017 13.43

Inoculated mutated MP6 (DH5a) into 4 liquid media cultures with LB, glucose and CM.

Day 73

Sunday, October 8, 2017 12:41 PM

MiniPrep
Mutated MP6 in DH5alpha

NanoDrop of extracted DNAs
1) 68.3 ng/uL
2) 117.3 ng/uL (A260/A230 below acceptable limit). Second test: 148.0 and 162.1 ng/uL
3) 187.6 ng/uL (A260/A230 below acceptable limit, bubbles detected)
4) 100.9 ng/uL (A260/A230 below acceptable limit, 1.75)

Stored in a white box in the -20 freezer, labelled with number (1-4) and date (8/10)

Restriction digestion

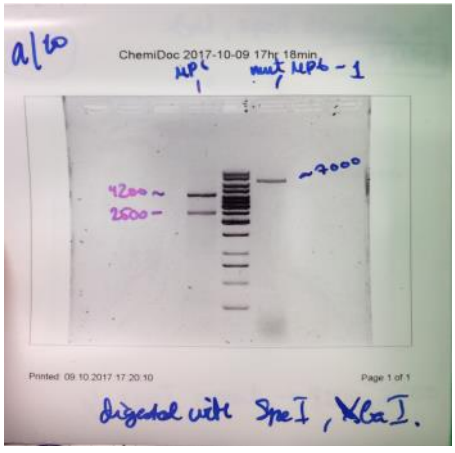
- 0.5 uL of enzyme XbaI and SpeI
- 2 uL CutSmart buffer
- 100 ng of DNA:
    1) 1.464 uL
    2) 0.852 uL
    3) 0.534 uL
    4) 0.991 uL
- Up to 20 uL distilled water
- Incubated in 37 C water bath overnight 10:40 am

Day 74

Monday, October 9, 2017 12:43 PM

Restriction digestion
Mutated MP6 number 1 and 4 from restriction digestion yesterday were run on the same gel. Both samples showed bands on 7000 bp.

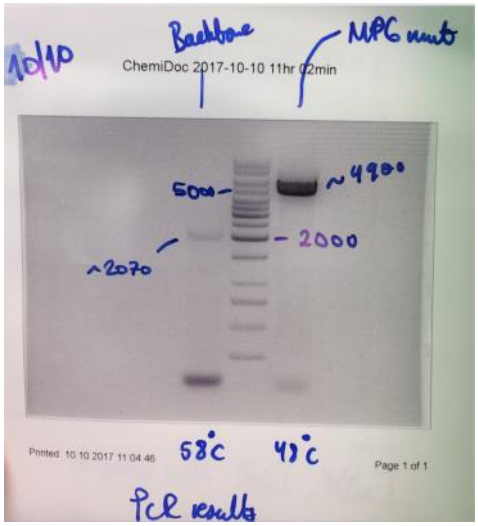
If speI is present, bands wil appear at 4200 and 2500 bp. Original MP6 and mutated MP6 were restriction digested and run on the same gel. It seemed like the mutation worked as expected, as the original MP6 showed two bands (4200 and 2500 bp), while the mutated MP6 showed one band on (7000 bp).



Day 71

Tuesday, 10 October 2017 11:21 am

PCR
Result of PCR of backbone and mutated MP6:



Gibson assembly (BioLabs kit)
See protocol for details.

Amounts used:

Reagent	**Volume (uL)**
Vector = pSB1C3* (22 ng/uL)	5
Insert = MP6mut (155 ng/uL)	1.3
dH_2O	3.7
Master mix (buffer)	10

*Standard iGEM shipping backbone

Incubated in thermocycler at 50 degrees for 1 hour.

Transformation
Assembly product (pSB1C3 + MP6mut) transformed into chemically compotent DH5alpha. 10 uL 2M Glc added together with 900 uL LB.

Plated 100 uL on LB + CM + Glc 15:50.

Day 72

Thursday, 12 October 2017 12:55 pm

Plasmid extraction
DH5alpha with mutated MP6 and backbone pSB1C3 (from transformation on Tuesday) miniprepped and measured on Nanodrop: 300-500 ng/uL. The high number is due to 16.5 hours between inoculation and miniprep, and that a high-copy plasmid (pSB1C3) has been used.

Mutated MP6 was restriction digested and run on agarose gel electrophoresis. Something went wrong and caused smearing on the gels. Tried again with undigested plasmid, but the same happened.

Four new cultures started 17.10.

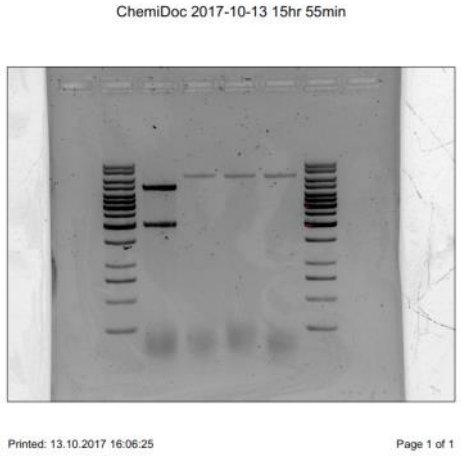
3D printing
LED boxes and new chemostat lids designed and 3D-printed.

Day 73

Friday, 13 October 2017 12:10 pm

Plasmid extraction

New MP6mut cultures miniprepped and restriction digested. Agarose gel electrophoresis:



Now the biobrick (MP6 mutator) is ready for being washed, dried, tested and sent!

Chemostats

Two new chemostat lids 3D printed.

Day 74

Tuesday, 17 October 2017 3:40 pm

Transformation
DH5alpha was transformed with pLA230 and MP6, and arabinose or glucose was added.

DNA submission
Our biobrick was prepared for submission to the iGEM Headquarters:

- 10 uL of a 25 ng/uL miniprep of the biobrick was placed into a well in a 96 well plate covered with a lid
- The DNA was then dried down at 50 degrees in a thermocycler
- The wells were then covered with adhesive foil:

- The sealed plate was covered with a plastic plate and labeled.

Day 75

Wednesday, 18 October 2017 9:52 am

Plating
Plated out DH5alpha with pLA230 + MP6mut + Ara/Glc:

Plates with LA + Amp + Glc
- pLA230 + MP6mut + Ara (10^{-4}-10^{-8})
- pLA230 + MP6mut + Glc (10^{-5}-10^{-8})

Plates with LA + Glc (control)
- pLA230 + MP6mut + Ara (10^{-1}-10^{-5})
- pLA230 + MP6mut + Glc (10^{-1}-10^{-4})

Incubated in 37 C.

Transformation
pLA230 and MP6mut were transformed into supercompetent DH5alpha 16:30.

The old MP6 with the new protocol was started to compare with the new MP6mut.

Day 76

Thursday, October 19, 2017 9:44 AM

Counted colonies from yesterday of MP6(biobrick)+pla230.

La + Glucose + Amp:

Plasmid	Concentration	Number of colonies
PLA+MP6+Ara	10^-1	48
''	10^-2	1
''	10^-3	1
''	10^-4	0
''	10^-5	0
PLA+MP6+Glc	10^-1	3
''	10^-2	1
''	10^-3	0
''	10^-4	0

Controls (LA + Glucose):

Plasmid	Concentration	Number of colonies
PLA+MP6+Ara	10^-4	>300
''	10^-5	287
''	10^-6	28
''	10^-7	4
''	10^-8	0
PLA+MP6+Glc	10^-5	>300
''	10^-6	67
''	10^-7	3
''	10^-8	1

Split and induced culture from yesterday with Arabinose and Glucose (MP6 (original) + pla230) (10:00).

Transformed dh5alpha with MP6(biobrick) and pla230 again.

Day 77

Saturday, October 21, 2017 9:11 AM

Mutation rate results

Results from plating MP6(biobrick) + pLA230 transformed bacteria plated on LA+Glc or LA+Glc+Amp plates with Ara or Glc in medium (see protocol)

(AB added) + Arabinose conc.	Number of colonies
Amp+ Ara 10^0	1 gazillion
Amp+ Ara 10^-1	Estimate >600
Amp+ Ara 10^-2	46
Ara 10^-5	Ca 286
Ara 10^-6	21
Ata 10^-7	5

(AB if added) + Glucose concentration	Number of colonies
Amp+ Glc 10^0	1 gazillion
Amp+ Glc 10^-1	22
Amp + Glc 10^-2	1
Glc 10^-5	Ca 286
Glc 10^-6	41
Glc 10^-7	2

Transformation of plasmids

PLA230 and MP6BB 13 #1 (old) was transformed into DH5a according to the protocol, but in stead of plating the bacteria right away (last step), they were put into a 25 mL culture (total volume) with 25 uL of CM and 25 uL of Kan after 1 hour incubation in 37C. The culture was further incubated in 37Covernight (16 hours). The transformants was put to first incubation at 18.15 pm.

Day 78

Sunday, October 22, 2017 9:50 AM

Split and induced culture from yesterday with Arabinose and Glucose (MP6BB 13 #1 (old)) + pla230) (9:30).

Put on incubation on the Tecan machine with control dh5alpha, same+ T2 and same+our phage.

Day 79

Tuesday, October 24, 2017 11:10 AM

Results from plating of E.coli DH5a transformed with PLA230 and MP6BB 13 #1 (old) , added Arabinose or Glucose and plated with Ampicillin and without (control).

Control: LA + Glc medium

Sugar (dilution)	#colonies
Ara 10^-5	260
Ara 10^-6	41
Ara 10^-7	2
Glu 10^-5	>400
Glu 10^-6	66
Glu 10^-7	5

Medium: LA + Glc + Amp

Sugar (dilution)	#colonies
Ara 10^0	Too many
Ara 10^-1	3
Ara 10^-2	0
Glu 10^-0	Too many
Glu 10^-1	9
Glu 10^-2	0

Made cultures of 6 dilutions of DH5alpha + CFP: 1/10, 1/20, 1/40, 1/80, 1/160 and 1/320.
Incubated in 37 degrees, 225 rpm.

Characterisation of biobrick
Transformed DH5a with pLA230 and MP6(biobrick), incubated at 17.10 for 1 h in 37C before addition of CM and Kan (25 uL of each in 25 mL LA medium) and further incubated in 15 hours (16 hours in total).

Day 80

Friday, October 27, 2017 11:24 AM

Results from plating of E.coli DH5a transformed with PLA230 and MP6BB 13 #1 (old) , added Arabinose or Glucose and plated with Ampicillin and without (control).

Control: LA + Glc medium

Sugar (dilution)	#colonies
Ara 10^-5	245
Ara 10^-6	28
Ara 10^-7	5
Glu 10^-5	342
Glu 10^-6	44
Glu 10^-7	4

Made cultures of 6 dilutions of DH5alpha + CFP: 1/10, 1/20, 1/40, 1/80, 1/160 and 1/320. Incubated in 37 degrees, 225 rpm.

Characterisation of biobrick
Transformed DH5a with pLA230 and MP6(biobrick), incubated at 17.10 for 1 h in 37C before addition of CM and Kan (25 uL of each in 25 mL LA medium) and further incubated in 15 hours (16 hours in total).

Transferred 2.5 mL of DH5alpha + CFP into new LB + CM medium at 22:00.

Chemostats
Added 1 uL of phage stock into the reactor at 21:45.