LAB Notebook

June

4.6.

- Prepared chemically competent *E. coli* DH5α cells
- Prepared electrocompetent *E. coli* DH5α cells, we tested and optimized the protocol

5.6.

- Prepared LB+ampicillin plates
- Performed the transformation in order to test the competent cells prepared on 4.6.
- Lab inventory
- Prepared the chloramphenicol stock

6.6.

- Lab inventory
- Prepared LB+chloramphenicol plates

7.6.

- Tested Biobrick protocol
- Lab inventory

10.6.

- Optimized Biobrick protocol

11.6.

- Whole team performed BioBrick protocol in order to obtain basic laboratory skills.

12.6.

- Prepared antibiotic stocks. (ampicillin, chloramphenicol, kanamycin and tetracycline stock)
- Performed Mini prep (Plasmid extraction)

13.6.

- Designed DNA for Twist Biosciences order

14.6.

- Performed Mini prep (plasmid extraction)
- Prepared liquid culture from transformants

17.6.

- Designed DNA for Twist Biosciences order
- Prepared LB+chloramphenicol plates

18.6.

- BioBrick tutorial

19.6.

- Analysed of the BioBrick tutorial results
- Prepared of recovery medium for *V. natriegens*

20.6.

- Researched about *V. natriegens*

24.6.

- Prepared growing medium for *V. natriegens* (LB+v2 salts)
- Prepared plates for *V. natriegens* (LB+v2+antibiotic)
- Prepared electroporation buffer for *V. natriegens*

25.6.

- Prepared overnight culture of *V. natriegens*
- Performed Mini prep (plasmid extraction)

26.6.

- Finished lab safety form
- Prepared electrocompetent *V. natriegens* cells

27.6.

- Tested *V. natriegens* electrocompetent cells

28.6.

- Prepared more plates (LB+antibiotic for *E.Coli*)
- Prepared 1% agarose gel
- Ran a gel

July

1.7.

- Tested electrocompetent DH5a cells
- Gram staining of *V. natriegens*

2.7.

- Prepared chemically competent *E. coli* DH5α cells that will be needed in future experiments
- pure cultures from overgrown *V. natriegens* plates were made

3.7.

- Tested chemically competent DH5a cells that were prepared earlier by transforming plasmid (pSBIA3 004450, amp selection, c= 99,3 ng/ul) to DH5a cells
- Made *V. natriegens* starter culture for competent cell preparation

4.7

- 1 L of electroporation buffer was prepared *V. natriegens* recovery medium was prepared by adding sucrose to LB + 1x V2 salts solution, which was prepared the previous day
- Made different antibiotic containing plates to be tested with *E. coli* and *V. natriegens* that have different plasmids.

5.7.

- Prepared electrocompetent *V. natriegens* cells
- Made 5 digestions with 5 plasmids using different enzymes. Afterwards, the agarose gel electrophoresis was run to gain more information about the vectors acquired from the University of Helsinki

8.7.

- pQE-30 plasmid transformation was performed to DH5a CC cells
- Prepared additional LB-amp 100y plates
- Tested how successful the preparation of electrocompetent *V. natriegens* cells was; unfortunately, no colonies appeared

9.7.

- tested how successful the preparation of electrocompetent *V. natriegens* cells was with pQE30 plasmid. We obtained nice results with a number of colonies.
- Made plates for *E.coli* electroporation and performing the electroporation on *E.coli* DH5alpha cells to test the plasmids obtained from the University of Helsinki on different antibiotics

10.7.

- Transformed plasmids from the University of Helsinki into *V. natriegens* with electroporation. The best growth was seen on the following plates:
    - LB+V2+amp & pUC18
    - LB+V2+ery & pMG60
    - LB+V2+cm & pSU18
    - LB+V2+kan & pET28a
    - LB+V2+amp & pQE30
    - where amp=ampicillin, kan=kanamycin, cm=chloramphenicol, ery=erythromycin.
- Extracted pQE-30 plasmid for future use from *E. coli* DH5a o/n culture

11.7.

- Purified genomic DNA from *V. natriegens*
- prepared o/n DH5a cultures for future plasmid extractions
- Tested if the ordered primers for TatABCDE and copperoxidase signal sequence work and what would be the optimal temperature. 15 PCR solutions were prepared and the PCR was done with the Dream Taq recommended settings and temperature gradient 61-63 C and left run overnight

12.7.

- The PCR results from previous day were run on a 1% agarose gel for 42 mins @120 V. The temperature gradient might have been too high, so the experiment will be repeated at lower temperature.

15.7.

- Running PCR again to get quality gel of Tat complex and signal peptides to later extract them from the 2% agarose gel. The following bands were isolated, weighted and stored in the freezer over night:
    - Cop50 (Copperoxidase in 50°C): 0,200g
    - Cop51 (Copperoxidase in 51°C): 0,162 g
    - Cop52 (Copperoxidase in 52°C): 0,174 g
    - Hyp50 (Hypoth_BA890 in 50 °C): 0,178 g
    - Hyp51 (Hypoth_BA890 in 51 °C): 0,122 g
    - Hyp52 (Hypoth_BA890 in 52 °C): 0,083 g
- Preparing fresh pure cultures for LB (E. coli) and LB + v2 salts (*V. natriegens*) plates. O/n culture of *V. natriegens* + pET-28a(+) for plasmid extraction tomorrow.

16.7.

- Purified and measured plasmid DNA concentration of pET-28a(+) that had been growing since the previous day. Yields were uniformly 2-8 ng/ul, so no high yields were achieved.
- Extarcted DNA from six 2% agarose gel fragments (cop 50, cop 51, cop 52, hyp 50, hyp 51, hyp 52). The DNA concentrations were as follows:
    - Cop 50: 15,9 ng/ul
    - Cop 51: 22,3 ng/ul
    - Cop 52: 20,6 ng/ul
    - Hyp 50: 20,7 ng/ul
    - Hyp 51: 27,5 ng/ul
    - Hyp 52: 19,2 ng/ul

17.7.

- PCRed the two signal sequences that didn't look so fresh on the previously run gel, Lipoprotein signal sequence and Tetrathianate signal sequence, and extracted them from the gel after PCR and gel electrophoresis. Based on the image taken of the gel: Lipo 50, Lipo 51 and Tetra 51 were chosen for gel extraction.
- After the extraction the DNA concentrations were as follows:
    - Lipo 50: 10,2 ng/ul
    - Lipo 51: 22,1 ng/ul
    - Tetra 51: 29,9 ng/ul

18.7.

- plasmid purifications from plasmids pSB1C3 R0010, pSB1C3 K1033931 and pSB1C3 10500. All plasmids were from DH5alpha cells. The concentrations were as follows:
    - pSB1C3 R0010 62,3 ng/uL,
    - pSB1C3 K1033931 56,8 ng/uL,
    - pSB1C3 10500 71,4 ng/uL
- Making digestions for GFP, *V. natriegens* and its signal sequences (Lipoprotein, Hypothetical protein BA890, Copperoxidase, and Tetrathianate signal sequences)
- Making chloramphenicol (CAP) containing plates for *V. natriegens*

19.7.

- The signal sequence digestion reaction samples were by mistake, not stored properly overnight. Deciding to still continue with the protocol and ligated the digested GFP and the digested signal sequences together as a step for making the constructs. The ligation was performed following the biobrick tutorial protocol.

- Eight ligation samples were prepared and loaded to a 1% agarose gel. After the gel run, each band was cut and the DNA extracted with the following concentrations:
    - 1: Lipo 1 + GFP: 0,9 ng/ul
    - 2: Lipo 2 + GFP: 1,4 ng/ul
    - 3: Cop 1 + GFP: 0,9 ng/ul
    - 4: Cop 2 + GFP: 1,0 ng/ul
    - 5: Tetra 1 + GFP: 0,8 ng/ul
    - 6: Tetra 2 + GFP: 2,0 ng/ul
    - 7: Hypo 1 + GFP: 1,8 ng/ul
    - 8: Hypo 2 + GFP: 1,1 ng/ul
- Cleaned the signal sequences that were made the previous day. The concentrations were as following:
    - Cop 51: 73,6 ng/uL
    - Tetra 50 61,4: ng/uL
    - Lipo 50 73,8: ng/uL
    - Hypo 51 71,6: ng/uL
- Later, digesting the signal sequences that were cleaned up.

22.7.

- Performing GFP+SS digestion, ligation and digestion again with the following reagents:
    - *Signal sequences*:
    - Hypothetical protein BA880 = H51
    - Lipoprotein = L50
    - Copperoxidase = C51
    - Tetrathionate = T50
    - *GFP with following concentrations:*
    - No.3, 18,9 ng/uL, for H51
    - No.5, 18,7 ng/uL, for L50
    - No.2, 17,2 ng/uL, for C51
    - No.5, 16,8 ng/uL, for T50

23.7.

- Continued the SS + GFP construct experiment by doing vector (pQE-30) digestion and vector + SS + GFP ligation

30.7.

- Prepared *V. natriegens* electrocompetent cells

31.7.

- tested the *V. natriegens* EC cells made the previous day; decided to test the cells with PQE30 on LB+V2+cm plates. *V. natriegens* electroporation was done with PQE30.
- Amplified *E. coli's dxs* gene using PCR. After, the PCR products were run on an agarose gel. However, PCR did not succeed.

August

1.8.

- Amplifying *E. colis dxs* gene using PCR after (second attempt). This time the annealing temperature was lowered from 55°C to 53°C. PCR did succeed, as bands were clearly seen. The concentrations were measured using NANODROP and were as follows:
    - 1 ul dxs: 333,7 ul/ng
    - 2 ul dxs: 708,8 ul/ng

5.8.

- Electroporation of *V. natriegens* cells for pQE-30 transformation to test different antibiotic concentrations. After the electroporation and overnight incubation, it seemed that the ampicillin 25 ug/ml plates were marked wrong. The plate marked “negative control” had colonies and the marked “pQE-30” had no colonies.
- In the chloramphenicol 10 ug/ml plate there were a handful of very small colonies. In the chloramphenicol 20 ug/ml plate there were no colonies. We suspect that the antibiotic stock is somehow ruined and/or concentration is not the one that's written on the side.

7.8.

- Prepared *V. natriegens* electrocompetent cells

8.8.

- Tested the electrocompetent *V. natriegens* cells made the previous day with three plasmids and two types of plates. Plates & plasmids pairs:
    - *V. natriegens* + pQE-30 on amp (25 ug/mL)
    - *V. natriegens* + pET-28(+) on amp (25 ug/mL)
    - *V. natriegens* + pET-28(+) on kan (100 ug/mL)
    - *V. natriegens* + pUC-18 on amp (25 ug/mL)
    - *V. natriegens* + H2O on amp (25 ug/mL) and kan (100 ug/mL)
    - We were able to see a number of colonies the following day and were therefore able to conclude that the EC *V. natriegens* cells were prepared succesfully.

9.8.

- Prepared *V. natriegens* glycerol stocks

12.8.

- Ran a PCR protocol to amplify dxs of *V. natriegens* and running a gel for later purification. The PCR program was run with a gradient 52-53-54 C which was chosen based on the eurofins info sheet. After this a 75ml 1% agarose gel was prepared. Since it looked like +53C was too high a temperature, the +54C PCR product was not run on a gel. The amplified fragment of size around 100 kb was cut from lane 1 and weighed: the gel weighed 0.161 grams.

13.8.

- Due to unknown bands in dxs PCR performed the previous day, the dxs PCR was rerun with different annealing temperatures. The gradient was tested with +54C, +55C and +56C temperatures. PCR program according to Thermo Fisher PCR Master Mix manual. Results were somewhat successful. The bands were cut, weighed and stored in -20 C freezer.

14.8.

- PCR:ed the small amount of SS + GFP/hGH synthetic DNA from Twist Biosciences, and running a 1,25% agarose gel.
- DNA was purified using Macherey-Nagel's Nucleospin PCR cleanup kit. Eluted DNA solution was measured for concentration with Nanodrop. The DNA concentrations (ng/ul) were as follows:

(1) YGFP + TorA 52C+53C	421,7
(2) YGFP + aminotransferase 52C+53C	388,2
(3) YGFP + lipoprotein 52C+53C	373,3
(4) YGFP + copperoxidase 52C+53C	422,3
(5) YGFP + tetrathionate 52C+53C	286,5
(6) YGFP + PN96_04110 52C+53C	295,2
(7) hGH + TorA 52C+53C	419,5
(8) hGH + aminotransferase 52C+53C	288,4
(1) YGFP + TorA 54C	296,9
(2) YGFP + aminotransferase 54C	269,7
(3) YGFP + lipoprotein 54C	228,3
(4) YGFP + copperoxidase 54C	255,9
(5) YGFP + tetrathionate 54C	221,2
(6) YGFP + PN96_04110 54C	260,8
(7) hGH + TorA 54C	26,7
(8) hGH + aminotransferase 54C	124,0

15.8.

- Extracted the dxs DNA from gel according to gel extraction protocol. As a result, we
    - got four tubes with dxs with following concentrations:
- New dxs: 9 ng/ul
    - dxs 1: 42 ng/ul
    - dxs 2: 57,8 ng/ul
    - dxs 3: 38,6 ng/uL
- Digested ss + GFP PCR fragments and pQE-30 vector with PstI and HindIII restriction enzymes. Reaction conditions were set up according to Thermo Fisher recommendations.
- Reaction conditions for ss+GFP and pQE-30 ligation were measured: for ligation reactions 5,4 ul of ss + GFP ligation mixtures, and 1 ul of pQE-30 ligation mixture was used.

16.8.

- Analysed of the digestion run (of a gel).

20.8.

- Ran PCR reactions

21.8.

- Ran products of PCR reactions in a gel

22.8.

- Prepared o/n culture
- Analysed the gel

23.8.

- Performed the induction
- Checked the cells under the UV light
- Performed PCR screening of the fragments
- Performed transformation with arabinose/ rhamnose plasmids

26.8.

- Planned colony PCR
- Ran PCR products from 23.8. on the gel

27.8.

- Performed colony PCR of ss+GFP colony
- Ran a gel of a colony PCR

28.8.

- Transformation of PC203 and PC201 plasmids into *V. natriegens*

29.8.

- Started starter culture
- Performed GFP+ss extraction
- Performed transformation

30.8.

- Started LB+v2 culture with transformants

September

2.9.

- Amplified tfox. The prepared reactions were put on PCR for 1h10mins:
    - tfox 67 C
    - tfox 67 C neg ctrl
    - tfox 68 C
    - tfox 68 C neg ctrl
    - tfox 69 C
    - tfox 69 C neg ctrl

3.9.

- Transformed plasmids from Aboa & LiU into *V. natriegens*

4.9.

- Preformed gel purification
- Performed PCR of the constructs
- Started a culture with Aboa and LiU plasmids

5.9.

- Run and analyse gels

7.9.

- Performed dns and TatO PCR

8.9.

- Performed tfoX PCR
- Ran gel on dns and TatO PCR products
- Performed PCR for PC201 with primers PC20x_dPluR/dProm fwd and PC201_dPluR rev (anneal 62,7) & PC203 with primers PC20x_dPluR/dProm fwd & PC203_dPluR rev (anneal 62,3)
- Continued work with LiU plasmids
- Performed PC201 digestion w/ EcoRI & XbaI

9.9.

- Growed PC plasmid constructs with TorA-YGFP
- Performed PCR of dxs plasmids.

10.9.

- Reran PCR for PC201 & PC203 with longer cycles in the 72C region
- Performed screening of inserts

11.9.

- Ran a gel of the PCR of dxs plasmids

- Performed induction with arabinose

12.9.
- Lab inventory

17.9.
- Induced the pc203-ss-ygfp
- Preformed a PCR

18.9.
- Purification of PC201 and 203 w/ PluR removal
- Attempted tat deletion PCR

22.9.
- Performed a torA+hGH and aminotrs+hGH PCR
- Ran a gel
- Extracted of torA+hGH from the gel

23.9.
- Performed gel purification

25.9.
- Conducted an induction experiment of TorA-yGFP in PC201 and PC203

27.9.
- cPCR screened transformed PC201-TorA-YGFP/PC203-TorA-YGFP constructs

28.9.
- Started PC201 and PC203 plasmid constructs building with *TorA* signal sequence including *hGH* gene by digesting the vectors and inserts.

29.9.
- The digestion reactions from the previous day included the following approximate amounts of DNA:
    - PC203: 3,1333... ng/ul
    - PC201: 3,2533... ng/ul
    - TorA-hGH fragment: 6,4 ng/ul
- Continued the hGH construct building by ligation of insert to the backbone
- Analyzed the previous day's cPCR results in a 1% agarose gel
- Transformed the ready hGH constructs to DH5a strain. Transformation was done according to NEB High Efficiency Transformation Protocol (C2987H/C2987I)

30.9.

- Additional testing to obtain broader range evaluation for inducer concentrations.
- pC201-TorA-yGFP colonies 9, 21, and 23 were selected based on cPCR analysis, and pC203-TorA-yGFP colonies 5 and 6 were selected accordingly
- Rebuilding the pC203-TorA-hGH construct.

October

1.10.

- Performed GFP fluorescence measurement in Vibrio Natriegens
- Performed PCR for PC201, PC203, TorA-hGH and Aminotrsf-hGH

2.10.

- Prepared 1% agarose gel
- Ran PCR results in a gel
- Performed tfoX PCR

3.10.

- Tested the cell fixation protocol
- Induced the cells to express TorA-YGFP
- Prepared an o/n culture of PC203 and PC201 plasmids with the still attached PluR insert

4.10.

- Performed imaging of TorA-YGFP in order to test the translocation
- Performed a Mini prep (plasmid extraction) and measured a concentration
- Performed a double digestion for the PCR amplified TorA-hGH fragments
- Analysed digestion reaction in a gel

5.10.

- Analysed ligation reaction in a gel
- Purification of ligation reaction
- Transformation of purified DNA into Vibrio natriegens
- Performed tfoX PCR

6.10.

- Performed colony PCR
- Started plasmid cultures
- Run tfoX PCR in a gel
- Purified tfoX from the gel
- Transformation of tfoX into Vibrio natriegens and *E. Coli*
- Prepared LB+ampicillin plates

7.10.

- Grown cells for DNA extraction

9.10.

- Prepared samples for sequencing

10.10.

- Sent sample to sequencing
- Performed qPCR assay running with pQE30/pUC18/PC203 plasmids
- Performed PCR of hGH with restriction sites
- Performed fractioning

11.10.

- Ran gel with PCR of hGH with restriction sites and performed a gel extraction.
- Ran an SDS-page with the fractioning products

12.10.

- Analysed gels
- Prepared LB+v2+ampicillin plates

13.10.

- Transformation of Tfox and PC203 TorA hGH plasmids into Vibrio Natriegens

14.10.

- Attempted dns deletion

15.10.

- Transformation of BioBricks into *E.Coli*

16.10.

- Performed a qPCR

17.10.

- Worked with BioBricks
- Performed induction

18.10.

- Ran SDS-page and performed Westrn blot

19.10.

- Ran SDS page of LiU plasmids