29.6.2015

MONDAY, 6/29

Tamannae, Petra

Started making chem competent TOP10 cells.
Used the old TOP10 stock for o/n cultures. Open wetware recommends that streptomycin concentation should be between 10 and 50 ug/ml, so did 3 different o/n cultures to test how much antibiotic our TOP10 cells can tolerate (we assume 50 ug/ml is good concentation since it is commonly used in our lab). Our streptomycin stock concentration is 50 mg/ml:

- 10 ug/ml -> add 0,4 ul stock to 2 ml LB
- 25 ug/ml -> add 1 ul stock to 2 ml LB
- 50 ug/ml -> add 2 ul stock to 2 ml LB

Transformed our linear backbones (AH007-1, AH007-2, AH008-1, AH008-2, AH009-1, AH009-2) following the protocol. For AH009 plated 200 µl tranformation mix and for other plated about 50 µl to right plates.

Did o/n cultures of BL21(DE3) AH016 and AH018 in 2 ml LB with CHL (total 16).

Tomorrow:
Refreshing the culture at 9.00:

- use the culture that has 50 ug/ml streptomycin concentration if there is growth
- 0,5 ml o/n culture in 50 om LB
- Add 50 ul streptomycin from stock

The first OD check at 10.30

30.6.2015

TUESDAY, 6/30

Petra, Tamannae

Refreshed TOP10 o/n culture to make competent cells

- erlenmayer flask
- 50 ml LB
- 0,5 ml o/n culture
- 50 ul streptomycin

Stored to incubator with shaking at
9.00
First OD measurement done 10.54
OD600 was 0,3340.

Made IPTG dilution for blue color measurement:

- 1:100, 300 ul in total
- 3 ul IPTG
- 297ul LB

Refreshed AH016 and AH018 o/n cultures of BL21 and put to incubator at 11.45

Pipetted 200 ul cell culture to well plate A1 (control). Pipetted 200 ul refreshed cultures to well plate (triplicates). Added IPTG dilution:

- 0,48 ul for 0,05 mM concentration
- 4,8 ul for 0,5 mM concentration
- 9,5 ul for 1 mM concentration

Put the plastic cover on well plate and left to 37 C, 230 rpm at 16.15
Strarted measuring blue chromoprotein at 16.50. Protocol measures 588, 850 and 600 nm (MM suggestion) every 30 min for 16 hrs, incubation: 37 C and shaking.

Measurement data:

Measurement_data_30.615.xlsx

According to the data sheet tbe bacteria didn't produce any blue color.

Checked yesterday's plasmid backbone transformations:
Realized that the DNA transformed yesterday was linear, so there shouldn't be growth in any plate. Linearized plasmid backbones need to be always ligated with an insert.
Results: There was growth in almost every plate.
AH007-1: Red colonies, one white colony
AH007-2: Red colonies
AH008-1: Red and white coloies
AH008-2: White colonies
AH009-1: No growth
AH009-2: Red colonies, one white colony
CTRL1 (AH007-1 on chlor plate): Red and white colonies, <10
CTRL2 (AH008-2 on chlor plate): 3 red colonies and 1 white colony
We assume that our linearized plasmid backbone DNA contains original, non-linearized plasmids with red-color producing insert.

1.7.2015

WEDNESDAY, 7/1

Present: Petra, Tamannae

Followed transformation efficiency protocol to check yesterdays chemically competent cells. Used 1:50 #37 as DNA (so no different concentrationt) and checked two chem competent tubes, so in total six plates were made.

Made multi-antibiotic plates for Kallio's propane producing strain. Followed the protocol for chloramphenicol plates but added antibiotics according to Table 1.

Table1

Antibiotic	Short name	Stock concentration (mg/ml)	Amount added to 500 ml LB (ul)	Final concentration (ug/ul)
Ampicillin	amp	100	500	100
Chloramphenicol	cam	34	500	34
Spectinomycin	spec	100	250	50
Streptomycin	str	50	200	20

PCR reaction for VioA
Added sterilized water to primer tubes according to Technical Datasheet. Spinned the DNA to the bottom before. Let Tthe tubes sit 10 min on rt and placed to freezer to a box named "iGEM 2015 primers".

Made a AH020 dilution 1:100, 100 ul -> 2,876 ng/ul

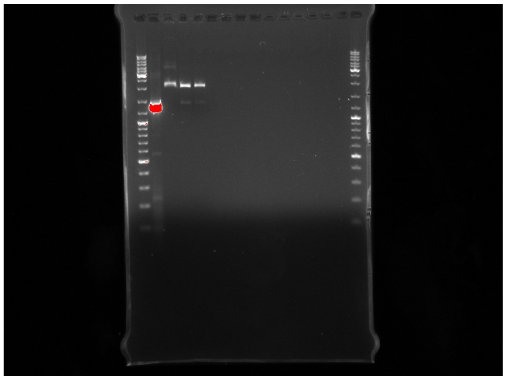
VioA PCR reaction mix
12,5µl 2x Mastermix
1,25µl 10µM P012
1,25µl 10µM P013
0,35 ul diluted AH020 (2,876 ng/ul) -> 1 ng template DNA
9,66 ul sterilizd water

Marked the tube: 1. PCR

2.7.2015

THURSDAY, 7/2

Geldoc_2015-07-02_11hr_56min_VioA_PCR_results__interlab.jpg



Petra, Tamannae

Gel pic: 1. ladder 2. PCR VioA reaction 3.-5 Interlab stuff

Running PCR reactions on gel
Made 1.3 % agarose gel: 0,65 g agarose to 50 ml buffer.
Made a gel mix: 5 ul PCR reaction and 1 ul 6xLD
Pipetted PCR reaction gel mix to a well. Ran some interlab samples at the same time. Started gel run at 10.34: 100V, 1h.

Yesterdays' plates did grow fine and they were put to the cold.

Did CAM plates adding 368 µl CAM in 500 ml LB agar. One CTRL plate was done.

Line test: Also plated 29.6. BL21 AH016 and AH018 colonies to 0,05 mM, 0,5 mM and 1 mM IPTG plates, so in one plate there are 32 lines (4 lines for each plates' colonies)

Made long term glycerol stocs of Pauli Kallio's strains: BL21(DE3), pACYC backbone and promane producing BL21(DE3). Used the o/n cultures made yesterday. Followed the protocol exept used dry ice instead of liquid nitrogen for cooling the tubes. The cryogenic tubs are marked:

- BL21(DE3), 2.7.2015, kan, iGEM2015
- pACYC, 2.7.2015, cam, iGEM2015
- propane BL21(DE3), 2.7.2015, amp100, cam34, str20, spec50, iGEM2015

The tubes were stored to -80 C freezer.

Took a picture of the gel: see above. Found that there id right-sized band and purified the rest of the PCR reaction with GeneJET PCR purification kit. Followed the protocol. Centrifuged always 1 min in 13000 rpm.
Nanodtop results:

Table1

Gene	Concentration (ng/ul	A260/289
VioA (AH026)	74,8	1,59

Named the purified VioA: AH026
Stored to freezer to the same box with primers. The tube is marked with VioA, AH026, 2.7.15 and DNA concentration.

Did PCR reaction for VioB and VioE following mostly the protocol. Because primers were not diluted to 10 mM before pipeting them, primers' concentration were 100 mM.
PCR reaction mix for the both:
1:100 template DNA (AH020) 0,35 µl
100 mM 1,25 µl primer (VioB p024; VioE p034)
100 mM 1,25 µl primer (VioB p025; VioE p035)
2x Master mix 12,5 µl
H20 9,65 µl

3.7.2015

FRIDAY, 7/3

Tamannae, Milla, Victor

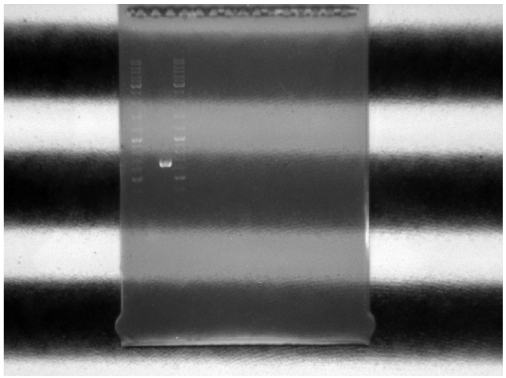
Line test: no blue lines
The reason why our IPTG results aren't positive might be because we are using *E. coli* strain BL21 not BL21(DE3), which has T7 RNA polymerase gene.

Made 1,3 % agarose gel (twice) in 50 ml 1x TAE buffer with EtBromide.

Ran PCR reactions twice in the gel for 1h with 100 V (to make sure the first):
The First (Picture 1)

1. ladder 2 µl 2. VioB 2,4 µl (2 µl PCR reaction mix + 0,4 µl 6x LD) 3. VioE 2,4 µl 4. ladder 2 µl

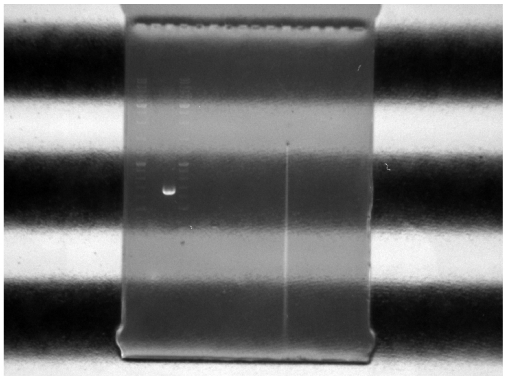
Geldoc_2015-07-03_16hr_26min.jpg



The Second (Picture)

1. ladder 2 µl 2. VioB 6 µl (5 µl PCR reaction mix + 1 µl 6x LD) 3. VioE 6 µl 4. ladder 2 µl

Geldoc_2015-07-03_18hr_35min.jpg



While the second gel was running, PCR purification were done for the both VioB and VioE following ThermoFisher's PCR purification protocol (added BB 18 µl).

NanoDrop results of PCR purifications:

Table1

Sample	DNA (ng/ µl)	A260/A280
VioB	19,4	1,59
VioE	50,9	1,76

So the results say that VioB hasn't amplified probably because the concentrations of primers were too high (100 mM to 10 mM).

Also made CAM plates adding 368 µl CAM to LB agar.