6.7.2015

MONDAY, 7/6

Petra, Tamannae

Did PCR reaction for betaglucosidase BglX (AH021)

25µl reaction mix for BglX
12,5µl 2x Mastermix
1,25µl 10µM P019
1,25µl 10µM P020
7,4 ul template DNA (AH021) dilution, 1:100
2,6 ul sterile H2O

Did gradient PCR for VioB to determine the right annealing temperature for th primers.

225 ul PCR reaction mix for VioB gradient:
112,5 µl 2x Mastermix
11,25µl 10µM P024
11,25µl 10µM P025
3,1 ul template DNA (AH020) dilution, 1:10
86,9 ul sterile H2O

7.7.2015

TUESDAY, 7/7

Petra, Tamannae

We weren't sure if our lab's BL21(DE3) strain really was the DE3 strain or just BL21. As our bluechromoprotein is not being produced, we will try to transform them into BL21(DE3) which we received from Pauli Kallio. These are surely BL21(DE3)

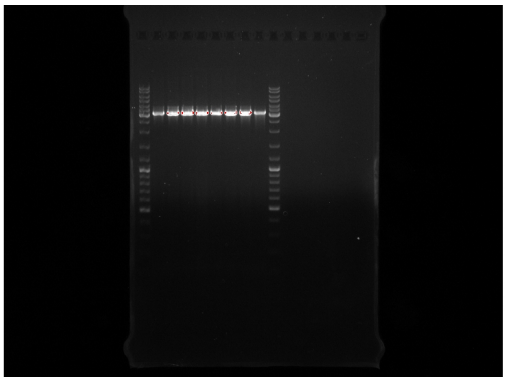
Making of competent cells (PK BL21(DE3)
Followed the protocol. Refreshed o/n, added 50 ul kanamycin. Left to incubator at 11.15.
First OD600 measurement was made at 12.52: OD600 0,259 -> continued the protocol. Poured the 49 ml culture to one 50 ml falcon tube and centrifuged. After the 10 minute centrifufation most of the supernatant was discarded and the cell pellet stood on ice for 10 extra minutes due to occupied laminar. Continued the protocol with full amounts: added 40 ml ice-cold 0.1 M CaCl2 and 5 ml ice-cold 0.1 M CaCl2 solution containing 15% glycerol.

Made two 1,3 % agarose gel with EtBromide

Ran gradient PCR reactions of VioB for 1h with 100 V.

1. ladder 2 µl 2. VioB 2,4 µl (2 µl PCR mix, 0,4 µl 6x LD) 65,0 °C 3. 65,6 °C 4. 66,4 °C 5. 68,1 °C 6. 68,9 °C 7. 70,6 °C 8. 71,3 °C 9. 72,0 °C 10. ladder

Geldoc_2015-07-07_12hr_48min.jpg

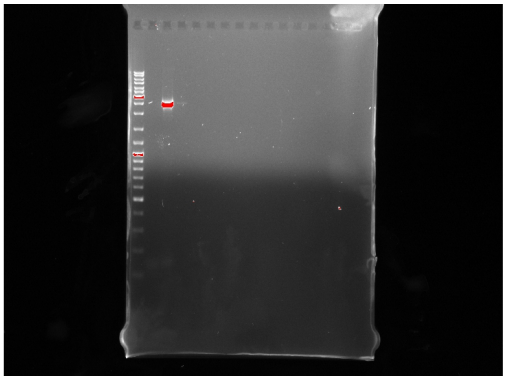


Did PCR reaction for BglX again today because yesterday's PCR program might not have started.

Ran 6.7. & 7.7. BglX in the gel 1h with 100 V.

1. ladder 2 µl 2. 6.7. BglX 2,4 µl 3. 7.7. BglX 2,4 µl

Geldoc_2015-07-07_15hr_18min.jpg



Did PCR purification of 5. 68,1 °C 6. 68,9 °C 7. 70,6 °C (VioB, AH027) and 7.7. BglX

Table1

Sample	DNA (ng/ul)
5. 68,1 °C	47,6
6. 68,9 °C	46,2
7. 70,6 °C	45,0
7.7. BglX	28,7

8.7.2015

WEDNESDAY, 7/8

Petra, Tamannae

Nanodropped yesterday's PCR purifications.
ADD RESULTS

Tested transformation efficiency of PK BL21(DE3) competent cells = DEL01 competent cells.
Used only #37 control as transformed DNA (not transformation efficiency kit). #37 has chloramphenicol resistance and DEL01 kanamycin recictance so the assumption is that the transformed cells would grow on kan+cam plate.
Made a test kan+cam plate: Took a ready-made common kanamycin plate. Mixed 15 ul chloramphenicol from stock with 50 ul sterilized water in a microfuge tube. Pipetted the mix on a kanamycin plate and spred with plating stick. The plate has ~34 ug/ml chloramphenicol.
Plated 100 ul transformation mix on kan+cam plate, 50 ul on cam plate and 50 on kan plate.AB

9.7.2015

THURSDAY, 7/9

Present: Petra, Tamannae

Our modeling team suggested that they could find more information for another BglX than the one we have taken from AH21. Thus we will take another one from AH029. Diluted BglX from plate 1, hole 8J following the protocol. Renamed AH029.

Transformed AH029 to Top10 and AH016 (4 tubes) and AH018 (4 tubes) to PK BL21(DE3), which is named DEL01 following mostly the protocol. The 30 min ice incubation were 5 minutes longer and the 5 min ice incubation were about 8 minutes longer..

Transformation mixes were plated to CAM plates; did 200 µl plates for AH016 & AH18 (total: 8 plates) and 50 µl & 150 µl plates for AH029.

Made IPTG plates. Assumed that each plate has 20 ml LB-agar. Mixed the amounts of IPTG listed on table 1 with 50ul sterile water and plated the mixes on chloramphenicol plates.

Table1

Final IPTG concentration	Amount of stock (500 g/l) used
0,05 M	0,48 ul
0,5 M	4,8 ul
1 M	9,5 ul

10.7.2015

FRIDAY, 7/10

Yesterday's transformations were successful (plates were full of colonies).

Plated transformed AH016 (total: 16 lines in a plate) & AH018 (total: 16 lines) to the 0,05 mM, 0,5 mM and 1,0 mM IPTG plates, which also have CAM, over the weekend in RT.