25.5.2015

MONDAY, 5/25

9:15 Present: Juuso, Anna, Tamannae

9:20 Petra, Arto arrived.

13:40 Tuukka, Tamannae and Petra arrived

14:17 Added 1*ul* DNA for AH007, AH008 and AH009
14:26 Added 1*ul* DNA for RFP-control

Transformations for propagations:

Table1

Plasmid name	Plate	Well
AH001	Plate 1	19E
AH002	Plate 3	13A
AH003	Plate 4	4G
AH004	Plate 2	2J
AH005	Plate 4	1H
AH006	Plate 3	3F
AH007	Plate 4	2H
AH008	Plate 4	6B
AH009	Plate 4	4B

Proceeded with the transformation protocol (TOP10 cells).

-> followed the protocol
14:17 Added 1*ul* DNA for AH007, AH008 and AH009
14:26 Added 1*ul* DNA for RFP-control
- 14:36 included a H2O control to the original RFP-control (H37) (!!!)

14:48 Moved AH007, AH008 and AH009 to heat shock (after 31 min inc) for 60s
14:50 AH007, AH008 and AH009 moved to ice for 5 mins
14:56 Moved RFP-control to heat shock
14:57 RFP-control to ice 5 mins
15:03 added 200 *ul* SOC to each tube
15:08 Incubatin samples 37C 60 mins
Created new RFP-control with same protocol as earlier

((-> made double plates of each sample, one plate with 50µl, one with 200µl, and older and newer RFP-controls (#37) with 200 µl
-> +37°C o/n 17 h))

Making of o/n cultures of successful transformants (AH001-AH004 and AH006) and BL21 transformation efficiency transformants.

17.00 Added 2 ml LB to six tubes (one for each transformant and one for control)

Added 1,47 ul chloramphenicol stock to each tube (25ug/ul)
Followed the protocol
17.15 Incubated with shaking overnight in 37 C

26.5.2015

TUESDAY, 5/26

MiniPreps for o/n cultures
-followed QIAprepSpin Miniprep Kit (Kit protocol can always be found with the kit)
-2ml o/n cultures - repeated step 1 twice
-1 min centrifugation in step 6

Table1

Sample	Concentration	A260/280
AH001	57,3ng/µl	1,87
AH002	38,6ng/µl	2,13
AH003	11,0ng/µl	1,91
AH004	35,0ng/µl	1,87
AH006	42,0ng/µl	
BL21 #37 control	83,0ng/µl	1,88

15.30 Anna, Petra and Juuso arrived

Propagating AH005 & AH019

- AH019 was diluted from the kit
- followed Diluting kit plasmids protocol & Transformation protocol
- AH019 was incubated on ice for 50min before heatshock of which 20 minutes without DNA

PCR to test BL21 transformants (the plasmid in them)

- Plasmid extracted with MiniPrep this morning, concentration 83,0ng/µl
  - -> use 0,5µl for as PCR template
- Use the same program and Master mix for colony PCR
  - unlike in the protocol, dilute the colony in 25µl of water (not 50µl) and use 0,5µl of treated dilution as PCR template
  - denaturation 5min, 95C
- Control sample of water (use 0,5µl)

Prepared 10µM dilutions of primers (10µl 100µM primer + 90 µl H20)

- VF2 10µM
- VR 10 µM

Master Mix (x4)
67µl H2O (Calculate the amount of water based on how much DNA you use)
20µl 5 x Buffer
3µl 10mM dNTP mix
3µl 10µM forward primer
3µl 10µM reverse primer
2µl KAPA HiFi HotStart SNA polymerase
(**0,5µl** Template DNA) DO NOT INCLUDE IN THE MASTER MIX

Pipette 24,5µl of master mix into each PCR tube. Add the desired template into each tube.

1. Plasmid DNA
2. Colony DNA
3. Water control

PCR program:
95° *C* 3min
98 °*C* 20s
57° *C* 30s
72° *C* 1min 30s
72° *C* 5min
4° *C* forever

Inoculated AH007, AH008 and AH009 in 2ml LB
AH007: 2ml LB + 1µl Ampicillin
AH008: 2ml LB + 0,8µl Kanamycin
AH009: 2ml Lb + 1,5µl Chloramphenicol

Calculations for antibiotics:
Amp
100000µg/ml x X = 50µg/ml x 2ml

(50µg/ml x 2ml) / 100000µg/ml
=1µl

Kan
50000µg/ml x X = 20µg/ml x 2ml

(20µg/ml x 2ml) / 50000µg/ml
=0,8µl

Chlor
34000µg/ml x X = 25µg/ml x 2ml

(25µg/ml x 2ml) / 34000µg/ml
=0,0014705ml
=1,5µl

27.5.2015

WEDNESDAY, 5/27

MiniPreps for o/n cultures
-followed QIAprepSpin Miniprep Kit (Kit protocol can always be found with the kit)
-2ml o/n cultures - repeated step 1 twice
-1 min centrifugation in step 6
-AH007 and AH009 were mixed and contaminated with each other
->proceeded with 1ml cultures of each

Table1

Sample	Concentration	A260/280
AH007	7,9ng/µl	1,94
AH008	12,1ng/µl	1,98
AH009	8,1ng/µl	1,92

Proceeded with Restrictiong AH002, AH006, AH007 & AH008 according to Restriction digestion protocol

- Doubled the reaction volume because the DNA yields were so small
  - Pipet 5µl 10x NEB CutSmart Buffer into the tube
  - Add about 250ng DNA
  - Add 1µl of each enzyme you want to restrict with
  - Adjust total volume to 50µl with sterile water
- 31,6µl AH007 (=249,64ng)
  - 11,4µl H2O
- 6,5µl AH002 (=252,85ng)
  - 36,6µl H2O
- 20µl AH008 (242ng)
  - 23µl H2O
- 5,9µl AH006 (=247,8ng)
  - 37,1µl H2O

NOTE! There was something inside the restricted tubes, this was noted after the inactivation. Consistently in all tubes, maybe contamination?

AH005 transformants did not produce colonies, because the backbone carried ampicillin resistance instead of the chloramphenicol resistance (that we presumed it to carry) and we used chloramphenicol plates.

Ligation

Ligation of AH006 & AH007 will produce AH010 with Ampicillin resistance. The correct ligation will be 2333bp long.
Ligation of AH002 & AH008 will produce AH012 with Kanamycin resistance. The correct ligation will be 2233bp long.

For both ligations, two ligation mixes were prepared. One mix contained 2µl of the restricted DNAs (as suggested in the iGEM protocol) and the other one contained 4µl of the restricted DNAs. This was done to test the ligation efficiency. Ligation reaction was done as in Ligation protocol.

Restricting the ligations with EcoRI to run them on the gel and extract the right ligation

Restricted according to the Restriction Digestion protocol

- Used the whole ligation mix (=10µl) as our DNA sample
- Incubated in +37C for 45 minutes to ensure complete restriction but avoid start activity

- Total restriction reaction volume 25µl

Added 5µl 6x LD into the restriction reaction after inactivation

Ran whole reactions of 1,2% agarose gel, 100V, 1h

-> we cannot separate these on the gel. Abort process.

Prepared 2ml o/n cultures from AH007, AH008, AH009, AH019

1µl Amp for AH007

0,8µl for Kan AH008

1,5µl Chlor for AH009 & AH019

-> +37C shaking

28.5.2015

THURSDAY, 5/28

Present: Petra, Arto, Tuukka, Tamannae (until 10.15)

Made 1,2% agarose gel to run colony PCR results
Pipetted 5 ul ladder and 10 ul sample to the gel: 1: ladder, 2: PCR sample 1, 3: PCR sample 2, 4: PCR sample 3
Ran the gel in 120V, 20min

Geldoc_2015-05-28_12hr_14min.tif

thumbnail

Miniprep for AH007, AH008, AH009, AH019
-followed QIAprepSpin Miniprep Kit (Kit protocol can always be found with the kit)
-2ml o/n cultures -> 2ml tube
-1 min centrifugation in step 6

Table1

Sample	Concentration (ng/µl)	A268/280
AH007	47,5	1,89
AH008	41,7	1,90
AH009	51,7	1,90
AH019	26,0	1,84

Restrictions:
We used linearized strandard plasmid pSB1K3 as AH008
Proceeded with the normal protocol, DNA yields were accurate for that.

2,5µl 10x NEB CutSmart Buffer to each tube

10 ul AH008 (pSB1K3)
- 37,5 ul water

4,36 ul AH001
- 43,14 ul water

6,43 ul AH002
- 41,07ul water

22,72 ul AH003
- 24,78 ul water

7,14 ul AH004
- 40,36 ul water

ADD ENZYMES LAST

Finished restrictions with protocol, but added 0,25ul extra XbaI enzyme in AH006.

Ligations
Followed the ligation protocol

Ligation 1, Building 011
AH002 2µl
AH004 2µl
AH008 2µl
1µl 10x T4 DNA Ligase Buffer
3µl water
-> add 0,5µl T4 DNA Ligase
AH001 2µl
AH006 2µl
AH008 2µl
1µl 10x T4 DNA Ligase Buffer
3µl water
-> add 0,5µl T4 DNA Ligase

Ligation 2:
AH002 2µl
AH004 2µl
AH008 2µl
1µl 10x T4 DNA Ligase Buffer
3µl water
-> add 0,5µl T4 DNA Ligase

Ligation 3, building 013
AH002 2µl
AH003 2µl
AH008 2µl
1µl 10x T4 DNA Ligase Buffer
3µl water
-> add 0,5µl T4 DNA Ligase

Added T4 DNA Ligase before water
Sentrifuged 1 min 10 000 rpm before incubation
Finished ligations stored to the fridge with the code names 168, 248, 238

Transforming AH005

29.5

FRIDAY, 5/29

Made a gel (1,2%) to run yesterday's ligations

Restricting the ligations with EcoRI to run them on the gel and extract the right ligation

Restricted according to the Restriction Digestion protocol

- Used the whole ligation mix (=10µl) as our DNA sample
- Incubated in +37C for 45 minutes to ensure complete restriction but avoid start activity
- Total restriction reaction volume 25µl

Added 5µl 6x LD into the restriction reaction after inactivation

Ran whole reactions of 1,2% agarose gel, 100V, 1h
Predicted lengths of plasmids:
168: 3002 bp
248: 2246 bp
238: 2248 bp

Followed Thermo-Scientific GENEJet Extraction Kit instructions, added:
168:390 µl
248: 220 µl
238: 440 µl
of binding buffer to gel slices

followed protocol to end, results were:
AH011: 1.0 ng/µl, A260/A280 2,14
AH013: 1,4 ng/µl, A260/A280 2,04
AH014: 1,7 ng/µl, A260/A280 2,80

started ligation according to protocol, ligations incubated 30 minutes over the recommended time.

Transformed AH011, AH013 and AH014 according to the protocol. In 1 hour incubation (9.), they weren't in the shaker.
Transformed AH005 following mostly the protocol, but incubation time for the point 7 was about 2.5 minutes and for the point 9 40 minutes (20 min without shaking).

Plates were left in RT over the weekend.