22.6.2015

MONDAY, 6/22

Petra, Tamannae

Nanodropped plasmid bacbones AH007 and AH009 purified from gel. DNA concentrations (table 1) were so small that we decided to do new o/n cultures of all the backbones (AH007, AH008, AH009).

Table1

Backbone	Concentration (ng/ul)	A260/A280
AH007-1	8,4	1,56
Ah007-2	7,3	1,33
AH007-3	11,0	2,25
AH009-1	13,0	1,42
AH009-2	11,5	1,42
AH009-3	11,5	1,46

Did two 5 ml o/n cultures of each backbone, antibiotic concentration 25 ug/ul

- AH007: kanamycin stock 2,0 ul
- AH008: ampicillin stock 2,5 ul
- AH009 chloramphenicol stock 3,7ul

Stored to 37C incubator with shaking
Marking:
Backbone number, antibiotic used.

Did four identical o/n cultures of AH018 2-1, the only known colony containing right sized plasmid

- 2 ml LB
- 1,5 ml chloramphenicol stock (antibiotic concentration 25 ug/ul)

Stored to 37C incubator with shaking
Marking:
AH018 2-1, BL21, o/n #1
....
AH018 2-1, BL21, o/n #4

23.6.2015

TUESDAY, 6/23

Refreshed o/n cultures of AH018 in 2ml LB with CAM (4 tubes).

Made 50ml an agarose gel (1,3 %) and added 5,0 µl SYBR Safe reagent to it. Used two combs placed together to create double sized wells.

Did minipreps of o/n cultures of AH007, AH008 and AH009 with GIAprep Spin Miniprep Kit

- 2 tubes of each: 7-1, 7-2, 8-1, 8-2, 9-1, 9-2.
- Only two tubes were red at the beginning: AH008 (8-1) and AH007 (7-2)
- step 1: pelleted 5 ml cultures to 2 ml centrifuge tubes. 3 centricugations at 13000 rpm (1min)
- Did the optional step 7 from the protocol

NanoDrop results of minipreps are in Table 1.

Table1

Sample	DNA (ng/µl)	A260/AH280
AH007-1	251,3	1,86
AH007-2	382,3	1,86
AH008-1	540,4	1,86
AH008-2	208,0	1,86
AH009-1	128,3	1,86
AH009-2	257,9	1,86

Restricted minipreps following the protocol.
Restriction mix:
DNA (miniprep) 23,5 µl
NEB Buffer 3.1 5,0 µl
EcoRI and PstI: total 2,0 µl (1,0 µl/enzyme)
H2O 19,5 µl
Total volume 50,0 µl

Ran the gel (100V, 30 min).
Pipeting order was

1. 5,0 µl ladder
2. 60,0 µl AH007-1
3. 60,0 µl AH007-2
4. 60,0 µl AH008-1
5. 60,0 µl AH008-2
6. 60,0 µl AH009-1
7. 60,0 µl AH009-2
8. 5,0 µl ladder

Blue chromoprotein measurement for AH018

Added IPTG to the tubes culture of AH018 containing 2 ml culture at 13.30

- 0,05 mM IPTG -> 48 ul
  calculaion: (0,05 mmol/l*M(IPTG)*2ml)/500mg/ml
- 0,5 mM IPTG -> 480 ul

- 1 mM IPTG -> 954 ul

and incubated at 37 C with shaking for 30 min.
After incubation pipetted one blank sample and triplicates of each sample with different IPTG concentration to 96 well plate, 200 ul each.
Measured the plate every 30 min on 588nm and 850 nm with Gen5 program. Between measurements the well plate was incubated at 37C with shaking.
NOTE: there's a machine that can do all this by itself: measure the samples, heat and shake itself between measurements. We couldn't use it today because it was booked for somebody else, so we had to do the measurements manually with the plate reader in our lab.

14.00 First measurement - not done
14.00-14.20 samples at rt without shaking due to preparations
14.30 2nd measurement - proceeded on time
15.00 3rd measurement - on time
15.30 4th measurement - on time
16.00 5th measurement - the last made, no bue & no change in measurement data from the beginning

Measurement data:

Blue_chromoprotein_measurement_data_23.6.15.xlsx

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

-> gel purification kit

- Binding buffer added:
- 7-1: 22,8
- 7-2: 19,6
- 8-1: 31,6
- 8-2: 28,0
- 9-1: 43,2
- 9-2: 48,6 ul

-> final elution in 50ul
-> stored in -20C
-> concentration not measured

24.6.2015

WEDNESDAY, 6/24

Petra & Tamannae
All the linearized, gel purified backbones are marked with date, plasmid name and the word "linearized". There are 2 tubes of each backbone.
Example: 23.6 AH007 linear

Nanodropped linearized backbones purified from gel yesterday. Nanodrop results are in table 1.

Table1

Backbone	Concentration (ug/ul)	A280/A260
AH007-1	20,6	1,67
AH007-2	15,3	1,72
AH008-1	27,4	1,81
AH008-2	7,3	1,70
AH009-1	16,7	1,77
AH009-2	20,8	1,69

Decided to use AH009-2 as a backbone for AH016 and AH018. Since AH011, AH013 and AH014 have all AH008 as backbone, decided to try to form AH016 and AH018 with AH007-1 backbone too ->AH023 and AH015

Table3

Plasmid name	Construct	Antibiotic resistance
AH016	AH011 +AH013 +AH009	CHL
AH018	AH011 +AH015 +AH009	CHL
AH023	AH011 +AH013 +AH007	AMP
AH025	AH011 +AH015 +AH007	AMP

Restriction digestion

Table2

Reaction (plasmid)	Restriction enzymes	Buffer	Amount of DNA needed	Water needed
AH007	EcoRI & PstI	3.1	12,2	9,4
AH009	EcoRI & PstI	3.1	12,0	9,4
AH011	EcoRI & SpeI	1	3,0	18,4
AH013	XbaI & PstI	CutSmart	5,1	16,4
AH015	XbaI & PstI	CutSmart	5,1	16,4

Restricted all the plasmids needed according to the table. Followed the protocol, but +37 °C incubation wes 45 min.

Ligations were done following the Thermo Scientific T4 DNA Ligase: Sticky-end ligation protocol.
Reaction mixture was:
Restriction mix 5,0 µl for each plasmids (50 ng/plasmid)
10x T4 DNA Ligase Buffer 2,0 µl
T4 DNA Ligase 0,2 µl
H_2O 2,8 µl
Incubation was done at +22 °C for 1 h and inactivation at +70 °C for 5 min.

Transformation was done in BL21(DE3) following mostly the protocol, but we added 5,0 µl ligation mix and ice incubation was 1h 50min.

25.6.2015

THURSDAY, 6/25

Petra, Tamannae, Milla

Made chloramphenicol plates. Followed the protocol.
Got 43 plates when using 500 ml of agar, so each plate contain ~12 ml agar.

Chloramphenicol test:
Tested whether our CAM stock is still working properly or not after it spent one night outside freezer.
Labelled one newly-made CAM agar plate and one older agar plate for control. Plated four different AH008 colonies on both of the plates. AH008 has kanamycin resistance so it shouldn't grow on chloramphenicol plates. Left the plates to 37C incubator overnight.

IPTG
Calculated the amount of IPTG needed to a plate to make concentrations of 0,05 mM, 0,5 mM and 1 mM.
Estimated that chloramphenicol plates have 12 ml agar

- 0,05 mM -> 0,2 ul IPTG
- 0,5 mM -> 2,8 ul IPTG
- 1 mM -> 5,7 ul IPTG

Mixed all the IPTG amounts with 50 ul water and plated the IPTG-water mixes to chloramphenicol plates. Stored the plates in cold room before use.

There was no growth in any plates transformed yesterday, so decided to induce 12 colonies of AH018 from a plate made earlier (the one we used tho measure blue light for the first time and failed). Plated each colony to the 3 plates having different IPTG concentration.The amount of IPTG used before was too big for the cultures, so we ´decided to use same plate again.

Did o/n cultures of TOP10 transformants (AH016, AH018; total 8 tubes) in 2 ml LB with 1,5 µl CHL. We want to transfer TOP10 plasmids to the strain BL21(DE3).

26.6.2015

FRIDAY, 6/26

Petra, Tamannae, Linda

Checked yesterday's IPTG plates. There was growth on every concentration, but none of the colonies were blue.

Did minipreps of AH016 and AH018 o/n cultures made yesterday using the new Macherey-Nagel, NucleoSpin Plasmid EasyPure kit. Nanodrop results are on Table 1.

Table1

Sample	Concentration (ng/ul)	A280/A260
AH016 2-1	40,3	1,84
AH016 2-2	62,0	1,85
AH016 3-1	35,3	1,89
AH016 3-2	54,7	1,84
AH018 2-1	28,3	1,93
AH018 2-2	53,6	1,84
AH018 3-1	57,5	1,82
AH018 3-2	56,8	1,84

Transformed AH016 and AH018 to BL21 and plated 200 ul in CHL plates (total 8).

IPTG calculatios for tuesday:
Well plate well: 200 ul
Wanted IPTG concentrations: 0,05 mM, 0,5 mM, 1 mM
IPTG stock: 500 ug/ul
-> 0,0048 ul, 0,048 ul and 0,095 ul IPTG to make the wanted concentrations. Can't pipet such small amounts, 1:99 IPTG dilution needed.

IPTG dilution 1:99, 5 mg/ml, volume 20 ul:
Pipet 0,2 IPTG