13.7.2015

MONDAY, 7/13

Petra

Made 2 o/n cultures of AH029 (bglX). Added 1,5 ul chloramphenicol to 2 ml LB. Chose 2 colonies for o/n cultures, one small (#1) and one big (#2). Left to 37C incubator with shaking at 14.40.

14.7.2015

TUESDAY, 7/14

Miniprepped yesterday's o/n cultures (AH029 #1 and AH029 #2). Used Macherey-Nagel NucleoSpin Plasmid EasyPure kit. Followed the kit protocol. Nanodrop results of the miniprep are on Table 1.

Table1

Plasmid	Concentration (ng/ul)	A260/A280
AH029 #1	138,2	1,59
Ah029 #2	126,7	1,87

Samples of both minipreps will be run on gel later to check whether both small and big colonies of the original plate contain AH029 plasmid.

Made 2 o/n cultures of psB3T5 backbone that we got from M&M today. Added 2 ul tetracyclin to 2 ml LB.

Made 1:10 dilutions of Ycia-sfp-car part1 and Ycia-sfp-car part 2 for PCR.

- Original concentration 10 ng/ul
- 1 ul DNA + 9 ul water -> 1 ng/ul
- used 1 ul both dilutions for PCR reaction mix

25 ul reaction mix for Ycia-spf-car part1
As in protocol
+ 1 ul 1:10 Ycia-spf-car part1 dilution
+ 9 ul water

25 ul reaction mix for Ycia-spf-car part2
As in protocol
+ 1 ul 1:10 Ycia-spf-car part2 dilution
+ 9 ul water

Made 1:10 dilutions Ycia-spf-car part3, AtoB Part1 and AtoB Part 2 for PCR.

- Original concentration 10 ng/ul
- 1 ul DNA + 9 ul water -> 1 ng/ul
- used 1 ul both dilutions for PCR reaction mix

25 ul reaction mix for Ycia-spf-car part3
As in protocol
+ 1 ul 1:10 Ycia-spf-car part3 dilution
+ 9 ul water
BUT we ran this PCR mix with the same program as for AtoB part2

25 ul reaction mix for AtoB Part1
As in protocol
+ 1 ul 1:10 AtoB Part1 dilution
+ 9 ul water

25 ul reaction mix for AtoB Part2
As in protocol
+ 1 ul 1:10 AtoB Part2 dilution
+ 9 ul water

Ran all the PCR reactions according to the protocols.

Made 1,3% agarose gel: 0.65g agarose & 50 ml TAE buffer.
Ran PCR reactions and AH029 #1 & #2 on gel. Didn't cut AH029 samples before running them on gel so the results won't be correct. Mixed the gel made by Petra to another gel, so the gel that we used was 1,5% instead of 1,3%.
Pipeting order in the gel was:

1. ladder 2 µl 2. AH029 #1 6 µl 3. AH029 #2 6 µl 4. AH035 2,4 µl 5. AH036 2,4 µl 6. AH037 2,4 µl 7. AH038 2,4 µl 8. AH039 2,4 µl 9. ladder 2 µl

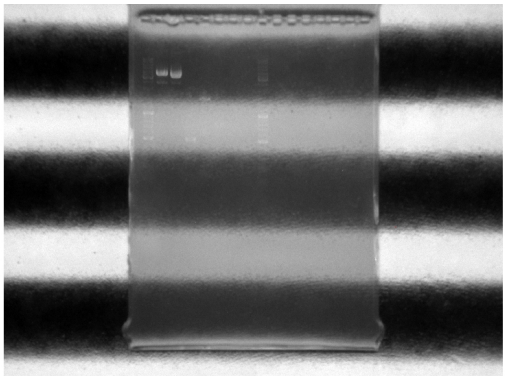
Purified PCR reactions with GeneJt PCR purification kit. Followed the protocol.

NanoDrop results of PCR purifications are in Table 2:

Table2

Sample	ng/µl	A260/280
CAR part 1 (AH035)	31,4	1,69
CAR part 2 (AH036)	38,1	1,75
CAR part 3 (AH037)	28,5	1,67
AtoB part 1 (AH038)	11,4	1,55
AtoB part 2 (AH039)	10,8	1,50

Geldoc_2015-07-14_17hr_21minCAR_ATOB_PCR.jpg



According to the gel picture the only succesful PCR reaction was CAR part 2. However the concentration we got from PCR purification might not be enough for Gibson assembly. Decided to try PCR for CAR part 2 again later.

o/n liquid culture of Interlab Measurement study's GFP plasmid in TOP10. We need the GFP protein from this to test our amphiphils later on. This is called AH044 in the propane project.

15.7.2015

WEDNESDAY, 7/15

Only AH036 is storaged from yesterday's PCR purification (result from analysing the gel picture).

Made new 1:10 dilutions of P006, P007, P011 and P018.

- 2 ul primer to 18 ul water

Made new 1 ng/µl template dilutions of YciA-sfp-CAR part 3 and AtoB part 2.

- 2 µl DNA stock and 18 µl H2O

Gradient PCR for CAR part 3 and Atob part 2

- 12 tubes per gradient

Reaction mixes (13x25 ul =325 ul)
AtoB part 2

162,5 ul 2x Mastermix
16,25 ul 10µM P006
16,25 ul 10µM P007
13 ul 1:10 AtoB part 2 template dilution
117 ul water

CAR part 3

162,5 ul 2x Mastermix
16,25 ul 10µM P011
16,25 ul 10µM P018
13 ul 1:10 YciA-sfp-CAR part 3 template dilution
117 ul water

Did PCR reactions o/n of AtoB part 3 and AtoB part 4 following the protocol.

Made 1,3 % agarose gel with EtBr. Run the gradient PCR reactions of Car part 3 (I) and AtoB part 2 (II).

(PLACEHOLDER)

Double-click to attach file.

MiniPrep for AH044 B, 167 ng/ul

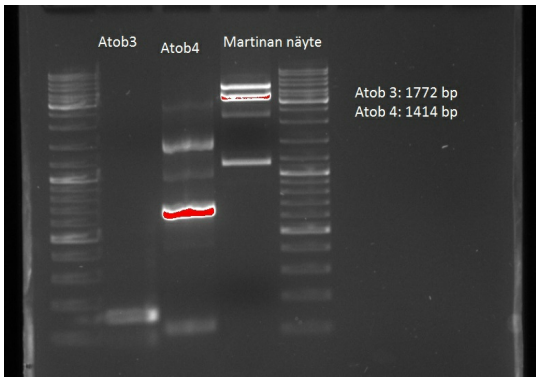
16.7.2015

THURSDAY, 7/16

Petra, Tamannae

Checking yesterday's PCR reactions:
Made 1,3% agarose gel with ETBR.
Ran yesterday's PCR reactions (AtoB part 3 and AtoB part 4) on gel (100V, 40min). Didn't run Atob part 2 and CAR part 3 from gradient PCR as planned because the samples were ruined when spinning the tubes (the spinner didn't work well with 8-tube strips and threw the samples out. Nothing was left to analyze them).

Edited_PCR_Atob3_Atob4_16.7.15.jpg



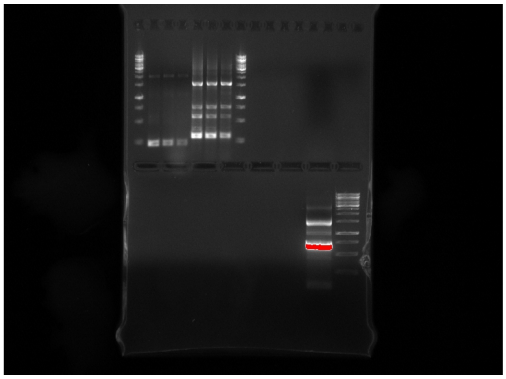
Did PCR reactions with DMSO to Atob part 2 and CAR part 3. Made 3 reaction mixes of each template DNA and added DMSO in 3 different concentrations according to the list below.

25 ul reaction mix for AtoB Part 2
As in protocol
+ 1 ul 1:10 AtoB Part2 dilution (from yesterday)
+ DMSO & water:
For 1% DMSO concentration: 0,25 ul DMSO + 8,75 ul water
For 3% DMSO concentration: 0,75 ul DMSO + 8,25 ul water
For 5% DMSO concetration: 1.25 ul DMSO + 7,75 ul water

25 ul reaction mix for Ycia-sfp-CAR part 3
As in protocol
+ 1 ul 1:10 Ycia-sfp-CAR part3 dilution
+ 9 ul water
+ DMSO & water:
For 1% DMSO concentration: 0,25 ul DMSO + 8,75 ul water
For 3% DMSO concentration: 0,75 ul DMSO + 8,25 ul water
For 5% DMSO concetration: 1.25 ul DMSO + 7,75 ul water

Ran the reactions following the protocol for AtoB part 2.

Geldoc_2015-07-16_15hr_59min_A135C135Atob4.jpg



Did gradient (56°C-71°C) PCR of AtoB part 3.
Reaction mix (13x25 ul =325 ul)
AtoB part 3

- 162,5 ul 2x Mastermix
- 16,25 ul 10µM P009
- 16,25 ul 10µM P008
- 13 ul 1:10 AtoB part 2 template dilution
- 117 ul water

Ran the PCR-gradients of AtoB part 3 with the gel electrophoresis (1,5% Agarose) which results can be seen in Figure 2. It seems that samples 3-12 were succesful with the size of 1772 bp.

Geldoc_2015-07-16_17hr_32min_Atob3.jpg

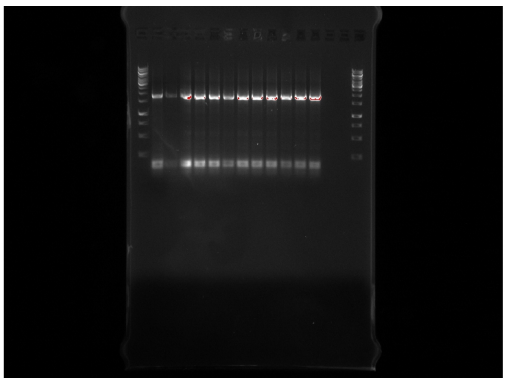


Fig 2. The gel electrophoresis results from the PCR gradient of Atob3.

Extracted the DNA from samples 3-12 and divided them into two tubes to wait the gel purification. The masses of tubes were:

A Atob3 266 mg
B Atob 3 190 mg

17.7.2015

FRIDAY, 7/17

Construct lengths:
CAR 1: 1945 bp, AtoB 1: 1311 bp

Did gel purification of AtoB part 3 which was divided to two parts A and B, and AtoB part 4.
NanoDropped AtoB part 3 and part 4 (Table 1).

Table1

Sample	DNA (ng/µl)	A260/A280
AtoB part 3 A	10,1	1,97
AtoB part 3 B	16,8	1,72
AtoB part 4	4,9	1,85

Did PCR reaction with 5 % DMSO for CAR1 and AtoB1. Added DMSO to check whether it helps to produce better PCR reaction.

25 ul reaction mix for Ycia-sfp-CAR part 1
As in protocol (Primers p001 + p015)
+ 1 ul 1:10 Ycia-sfp-CAR part 1 dilution
+ added 1.25 ul DMSO (5 % concetration in the reaction mix)
+ added 7,75 ul water

25 ul reaction mix for AtoB part 1
As in protocol (Primers p001 + p005)
+ 1 ul 1:10 AtoB part 1 dilution
+ added 1.25 ul DMSO (5 % concetration in the reaction mix)
+ added 7,75 ul water

Ran PCR reactions according to the protocol.

Run PCR products (5 µl DNA + 1 µl 6x LD) in 1,3 % agarose gel with EtBr for 30 min with 120 V. Pipeting order was 1. ladder 2 µl 2. AtoB1 6 µl 3. CAR1 6 µl (Picture 1)

Geldoc_2015-07-17_17hr_21min_AtoB1CAR1.jpg