14.9.2015

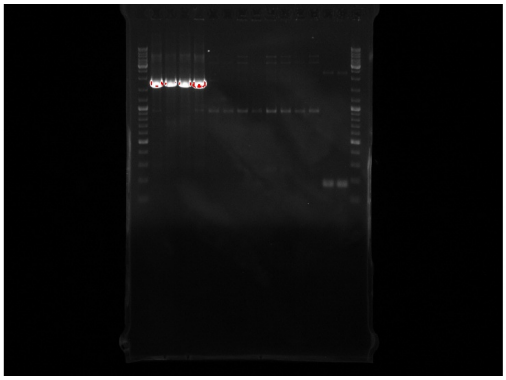
MONDAY, 9/14

Petra

Checking last Friday's colony PCR reactions on gel:
Made a 1,2 % agarose gel with ETBR. Ran 5 µl samples of each PCR reaction on gel. Used 1 µl GeneRuler ladder to each sample. Ran the gel for 1 h, 90 V.
Pipetting order:

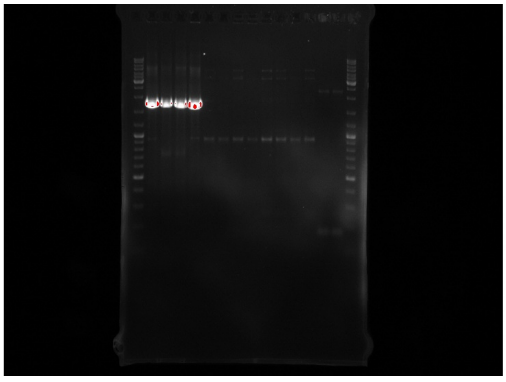
1. Ladder
2. CAR gibson 2 (in AH043)
3. CAR gibson 3 (in AH043)
4. CAR gibson 4 (in AH043)
5. CAR gibson 7 (in AH043
6. CAR gibson 2#1 (in pSB1C3, the BioBrick backbone)
7. CAR gibson 2#2(in pSB1C3)
8. CAR gibson 3#1 (in pSB1C3)
9. CAR gibson 3#2 (in pSB1C3)
10. CAR gibson 4#1 (in pSB1C3)
11. CAR gibson 4#2 (in pSB1C3)
12. CAR gibson 7#1 (in pSB1C3)
13. CAR gibson 7#2 (in pSB1C3)
14. AtoB gibson 6 (in pSB1C3)
15. AtoB gibson 6 (in pSB1C3), 2nd sample
16. Ladder

Geldoc_2015-09-14_10hr_51minFINAL_CAR_ATOB.png



Ran the gel for additional 15 min to separate the ladder a bit more. According to this gel picture CAR in AH043 and CAR in pSB1C3 samples are right-sized. AtoB colony PCR was not successful.

Geldoc_2015-09-14_11hr_41min_FINAL_2_CAR_ATOB.png



This picture shows the same gel after running it for 1h (90 V). Chose the sample from well 10: CAR Gibson 4 #1 in pSB1C3 to be sent to iGEM registry.

Sending biobricks to iGEM registry:

Table 1 shows the names of the biobricks to be sent to the registry. Pipetted DNA to the well plate provided by iGEM according to the Table 1:

Table1

Biobrick	Sample name	Concentration (ng/µl)	DNA pipetted to well plate (ng)	DNA pipetted to well plate (µl)
Propane producing plasmid 1: CAR	CAR Gibson 4 #1 in pSB1C3	28,5	250	8,8
Amphiphilic protein	Amph noter C1	61,1	500	8,2
Fusable GFP	GFP C3	438,4	500	1,2

Pipetting order:
A1: CAR Gibson 4 #1 in pSB1C3
B1: Amph noter C1 (=Amph without terminator)
C1: GFP C3

Covered the plate with a lid. Dried the well plate in a laminar hood (room temperature) for 5 hours. Stored the plate in fume hood o/n.

Tamannae

Did confocal microscopy samples of o/w cultures of GFP+Amph 1 & 2 and AH045 + Amph 4, 5 & 6. First, pelleted cells with a centrifuge for 2 min for 3000 rcf. Then resuspended the pellet with 200 ul fresh growth media, which was LB in our case. And then did 1:1000 and 1:100 dilutions with H2O. CM-results will be done by tomorrow. The samples and the bacterial cultures were given to Ritva Heinonen at VTT, who will send us images of the bacteria during the following days.

15.9.2015

TUESDAY, 9/15

Anna assembling AH045+GFP+AH011 in pSB1A3 -> AH046
Tuukka packing and sending BioBricks

Attached a foil cover on the dried BioBrick plate and closed the lid. Covered the plate with bubble wrap and sent it with UPS-letter.

Restricted AH045 + GFP 11 & AH045 + GFP 15 from 26.8. with EcoRI & SpeI.
Restricted AH011 (12.6, AH011 2)with XbaI & PstI.
Restricted linearized pSB1A3 with EcoRI & PstI.

Made two restrictions for each, one with ~250ng DNA and one with ~500ng DNA. For the backbone, the second restriction was 375ng.

Table1

Construct	DNA (ul)	H2O (ul)
AH045+GFP11 1	4	17.5
AH045+GFP11 2	8	13.5
AH045+GFP15 1	5	16.5
AH045+GFP12 2	10	11.5
AH011 1	5	16.5
AH011 2	10	11.5
pSB1A3 1	10	11.5
pSB1A3 2	15	6.5

Incubated in +37°C for 1 hour
20 min inactivation in +80°C

Ligation with Thermo's T4 ligase. Made 2 parallel reactions for each restriction.

Restriction 1, 1:3 insert:vector ratio (20µl reaction)
2µl T4 buffer
0,2µl T4 ligase
6µl GFP11
5µl pSB1A3
5,5µl AH011
1,3µl H2O

Retriction 1, 1:5 insert:vector ratio (30µl reaction)
3µl T4 buffer
0,3µl T4 ligase
9,4µl GFP11
5µl pSB1A3
9,3µl AH011
3µl H2O

Restriction 2, 1:3 (20µl reaction)

2µl T4 buffer
0,2µl T4 ligase
3µl GFP11 in tube 1 / GFP15 in tube 2
5µl pSB1A3
2,8µl AH011
7µl H2O

Restriction 2, 1:5 (20µl reaction)
2µl T4 buffer
0,2µl T4 ligase
4,7µl GFP11 in tube 1 / GFP15 in tube 2
5µl pSB1A3
4,7µl AH011
3,4µl H2O

Incubated ligation mixes in room temperature for 12minutes. 5min inactivation in +70°C.

Made 5 transformation tubes of each ligation mix. 5µl ligation mix for each 50µl of chem competent cells. Incubated on ice for 30min. 1min heatshock in +42°C. 45min recovery in +37°C. Plated each tube on a plate in its entity (=50µl/plate).
-> plates in +37 o/n

16.9.2015

WEDNESDAY, 9/16

Got images from VTT which can be seen in Figure 1.

Aalto-Helsinki_GFP-amph_vs_ctrl.jpg

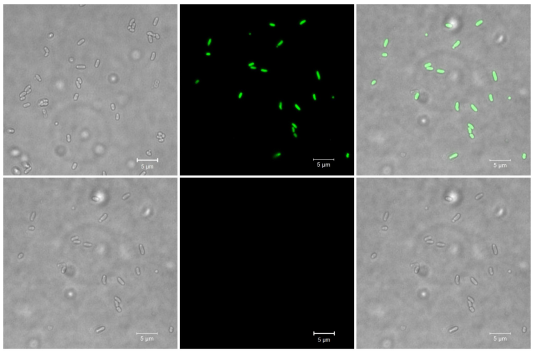


Figure 1. Upper row: *E. coli* expressing GFP fused to the N-terminal end of our amphiphilic protein. Bottom row: Control with amphiphilic protein. On the left, a light microscope picture and in the middle a fluorescent microscope picture of the same cells (excitation at 488 nm, detection 493-598 nm). On the right the two pictures to the left merged, showing that GFP is expressed in transformed cells, but not in control cells. Amphiphilic structures can't be seen in any picture.

Yesterday's AH046 didn't grow on transformation plates... Tamannae suspected that the problem might be in the AH011 construct, as the blue chromoprotein may be inverted and the terminator is for T7. Anna is suspecting that there's something wrong with the transformation.

Anna will try re-assembling AH046 and a new assembly for AH045+GFP+AH001+pSB1A3 -> AH047.
Made two restrictions of each construct, the first with 250ng DNA and second with 500ng.

Table1

Construct	DNA (ul)	H2O (ul)
AH045+GFP11 1	4	17.5
AH045+GFP11 2	8	13.5
AH045+GFP15 1	5	16.5
AH045+GFP15 2	10	11.5
AH011 1	5	16.5
AH011 2	10	11.5
pSB1A3 1	10	11.5
pSB1A3 2	15	6.5
AH001 1	5	16.5
AH001 2	10	11.5

E&P and X&P with fast digest. E&P incubated at +37 for 25 mins because of star activity, 5 min inactivation in +80.
"Regular" digestion for E&S, because we are not sure of the functionality of the FD SpeI.
X&P and E&S incubated in +37 for 60mins, 20 min inactivation at 65C.

Transformed 5ul of each ligation into TOP10 cells. (50ul cells + 5ul ligation mix)
-Incubated 30mins on ice

- 1 min +42C
- 5 min on ice
- 200ul SOC
- 45min in +37
- Plate all tranformants on amp plates

17.9.2015

THURSDAY, 9/17

Checked yesterday's transformations. No blue colonies detected for AH046 and AH047.