10.8.2015

MONDAY, 8/10

Petra, Tamannae

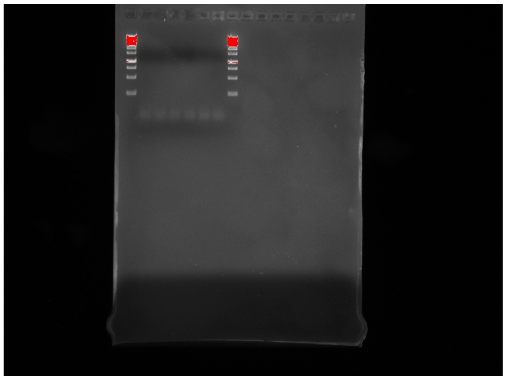
Did a new PCR reaction for amphiphilic protein with linker, because purification of the reaction done last week was unsuccesful (A260/A280: 1,12). Chose Tm according to the amph gradient gel picture from 6.8.15: 71 °C gave the best results.

Did 7x KAPA reaction mix to run 6 different PCR reactions
7 x reaction mix (175 ul):
117,25 ul sterilized water
35 ul 5x buffer
5,25 ul 10mM dNTP mix
5,25 ul 10µM P044
5,25 ul 10µM P045
3,5 ul KAPA HiFi HotStart DNA Polymerase
3,5 ul fixed CAR part 2 DNA dilution (1 ng/ul)

95° *C - 3 min*
98° C - 20 sec
71° C - 20 sec
72° C - 1 min
72° C - 3 min
4° C - forever
Repeated underlined cycles 25 times

Made 1,2 % agarose gel with ETBR. Ran 5 ul samples of each PCR reaction with 1 ul LD on gel: 20 min, 120V. Used Gene O'Ruler 1 kb ladder.

Geldoc_2015-08-10_15hr_33min_amph_linker_pcr.jpg



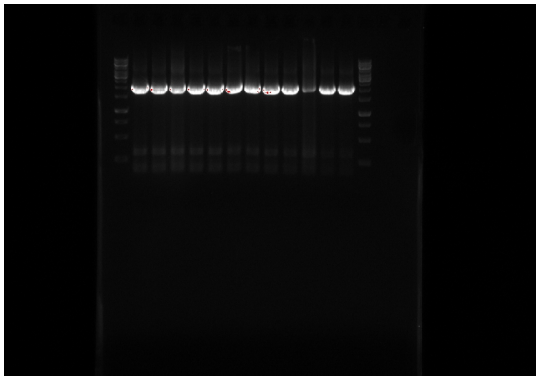
According to gel picture PCR reaction for Amph was unsuccesful. Discarded the reactions.

Restricted pSB6A1 (AH043) backbone Xbal and Spel to do Gibson assebmly and ELIC for celluose system (CenA cex part1 & CenA Cex part 2) and Atob constuct (Atob part 1, fixed part 2, part 3 and part 4). Added 750 ng DNA (55,4 ng/ul = pipet 13,5 ul) to get a suitable final concentration (750 ng / 25 ul = 30 ng/ul).
Followed the protocol. Incubated 90 min (started 13.15) on 37 C before inactivation.
Restriction mix:
2,5µl 10x NEB CutSmart Buffer
13,5 ul plasmid DNA
0,5 ul Spel
0,5 ul Xbal
8 ul water

Stored to freezer.

Made a 1,3 & agarose gel with EtBr. Run yesterday's KAPA PCR product of AtoB part 2 (AtoB2) for 25 min with 120 V. Pipeting order was: 1. ladder 1 ul 2.-13. gradient AtoB2 6 ul 14. ladder 2 ul

Geldoc_2015-08-10_13hr_21min.jpg



Did PCR purification of Amph NOTERM and AtoB2 according to the kit protocol. Added 68,4 ul and 76 ul binding buffer respectively. NanoDrop results:

Table1

Sample	DNA (ng/ul)	A260/A280
Amph NOTER	39,5	1,81
AtoB2	278,5	1,84

Did restrictions of Amph NOTER, GFP and pSB1C3 (linear AH009) following mostly the protocol to create biobricks. However, the incubation time was 1 h.

Then ligated restricted Amph NOTER and GFP with restricted pSB1C3 following the T4 ligase kit protocol. Incubated 1 h.

Did transformation of ligated Amph NOTER and GFP with pSB1C3 to Top10 CHEM competent cells following the protocol. Also checked yesterday's CHEM competent cells by pipeting 1 ul #37. Plated 50 ul and 100 ul Amph NOTER and GFP to CAM plates for o/n.

11.8.2015

TUESDAY, 8/11

Petra, Tamannae

Made both Gibson assembly and ELIC for AtoB and cellulase construst (CenA).

Table1

Construct	Length (bp)	Concentration (ng/ul)	DNA (ng) needed for 0.05 pmol	DNA (ul) needed for 0,05 pmol
AtoB part 1	1311	106,1	40,51	0,38
fixed AtoB part 2	2000	278,5	61,8	0,22
AtoB part 3	1772	284,3	54,75	0,2
AtoB part 4	1414	52,1	43,69	0,84
pSB1C3	2070	25	63,96	2,6
Total:				4,24
Water:				**5,76**

Gibson for AtoB with pSB1C3:
Pipetted DNA (AH009, AtoB part 1, Fixed AtoB part 2, AtoB part 3, AtoB part 4) to 1,5 ul tube according to Table 1. Added 5,76 ul water. Added 10 ul NEBuilder HiFi DNA Assembly MasterMix. Incubated the mix in 50 C for 60 minutes. Used 2 ul for transformation.

Table2

Construct	Lenght	Concentration (ng/ul)	DNA (ng) needed for 0.03 pmol vector & 0,06 pmol insert	DNA (ul) needed for 0.03 pmol vector & 0,06 pmol insert
CenA part 1	1891	122	70,11	0,57
CenA part 2	1302	114,3	48,28	0,42
pSB1C3	2070	25	38,38	1,54
Total:				2,53
Water:				**7,47**

Gibson for CenA with pSB1C3:
Pipetted DNA (pSB1C3, CenA cex part 1, CenA cex part 2) to 1,5 ul tube according to Table 2. Added 7,47 ul water. Added 10 ul NEBuilder HiFi DNA Assembly MasterMix. Incubated the mix in 50 C for 15 minutes. Used 2 ul for transformation.

ELIC for AtoB with pSB1C3

Table3

Construct	Length (bp)	Concentration (ng/ul)	fmol DNA needed for ELIC	DNA (ng)	DNA (ul)
AtoB part 1	1311	106,1	95,16	77,09	0,73
fixed AtoB part 2	2000	278,5	95,16	117,6	0,42
AtoB part 3	1772	284,3	95,16	104,2	0,37
AtoB part 4	1414	52,1	95,16	83,15	1,6
pSB1C3	2070	25	31,72	40	1,6
Total:					4,72
Water:					**5,28**

ELIC for CenA with pSB1C3

Table4

Construct	Lenght	Concentration (ng/ul)	fmol DNA needed for ELIC	DNA (ng)	DNA (ul)
CenA part 1	1891	122	95,16	111,2	0,91
CenA part 2	1302	114,3	95,16	76,57	0,66
pSB1C3	2070	25	31,72	40	1,6
Total:					3,17
Water:					**6,83**

ELIC for AtoB and CenA with pSB1C3:

Pipetted DNA (pSB1C3, AtoB part 1, Fixed AtoB part 2, AtoB part 3, AtoB part 4, CenA cex part 1, CenA cex part 2) to two 1,5 ul tubes according to Tables 3 and 4. Added water according to the tables. Total reaction volume for ELIC was 10 ul in both tubes. Incubated reaction mixes in room temperature for 60 minutes. When doing transformation, added competent cells straight to the same tube where reaction mix was.

Transformation of Gibson and ELIC reactions intoTOP10,
Followed the protocol. Added 200 ul SOC after heat shock. Transfered ELIC reactions to 2 ml tubes after adding SOC media. Plated 50 ul and 200 ul of each reaction on chloramphenicol plates (8 plates in total) and left to 37 C incubator overnight.

Made a new 10 ng/ul aliquot of Amph

- 1 ul Amph stock (100 ng/ul) to 9 ul sterilized water

Gradient PCR for amph (WITH linker):
Did a 8-sample-gradient KAPA PCR for amphiphilic protein with linker (amph + linker) because yesterday's PCR reaction with Tm=71 failed.

Made a template DNA dilution

- 1 ul 10 ng/ul amph aliquot (10 ng/ul) to 9 ul sterilized water

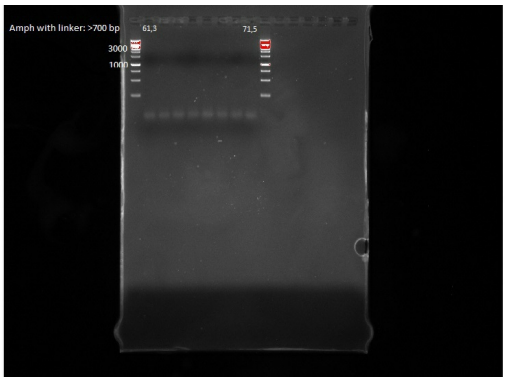
9x reaction mix (225 ul):

150,75 ul sterilized water
45 ul 5x buffer
6,75 ul 10mM dNTP mix
6,75 ul 10µM P044
6,75 ul 10µM P045
4,5 ul KAPA HiFi HotStart DNA Polymerase
4,5 ul fixed Amph DNA dilution (1 ng/ul)

95° *C - 3 min*
98° C - 20 sec
56,5-71,5° *C - 20 sec*
72° C - 1 min
72° *C - 3 min*
4° *C - forever*
Repeated underlined cycles 25 times

Checking PCR results:
Made a 1,3 % agarose gel with ETBR. Ran 5 ul samples of each PCR reaction with 1 ul LD. Used Gene O'Ruler 1 kb ladder. Ran the gel for 20 min, 120V.

EDITED_GPCR_amph_with_linker_Geldoc_2015-08-11_14hr_30min.jpg



Accorging to gel picture above PCR reaction for amph with linker was unsuccesful. Discarded all the PCR samples.

Yesterday's transformation of Amph NOTER and GFp was successful as there were some colonies in the plates.

Did colony PCR reactions of Amph NOTER (7 reactions) and GFP (6 reactions) according to the protocol.

PCR reaction mix for an Amph NOTER colony (25 ul):
15,25 µl H2O
5µl 5 x Buffer
0,75 µl 10mM dNTP mix
0,75 µl 10µM p046
0,75 µl 10µM p051
0,5 µl KAPA HiFi HotStart DNA Polymerase
2 ul template DNA from the chosen colonies

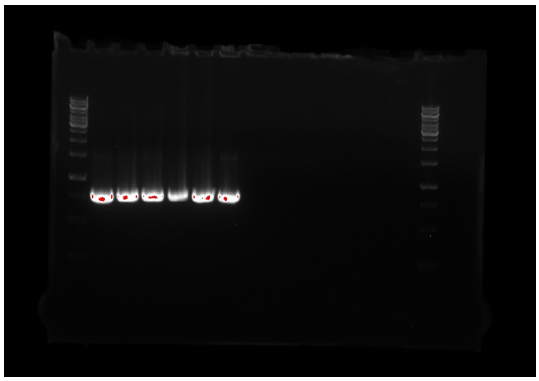
PCR reaction mix for a GFP colony (25 ul):
15,25 µl H2O
5µl 5 x Buffer
0,75 µl 10mM dNTP mix
0,75 µl 10µM p043
0,75 µl 10µM p050
0,5 µl KAPA HiFi HotStart DNA Polymerase
2 ul template DNA from the chosen colonies

The PCR program for the both reactions (Amph NOTER & GFP).
95° *C - 3 min*
98° C - 30 sec

66,5° *C - 15 sec*
72° C - 50 sec
72° C - 1,5 min
4° *C - forever*
Repeated underlined cycles 25 times

Made a 1,3 % agarose gel with EtBr. Run colony PCR products in the gel for 25 min with 120 V. Pipeting order was: 1. ladder 2 ul 2.-7. GFP colonies 1-6 6 ul 8.-14. Amph NOTER colonies 1-7 6 ul 15. ladder 2 ul

Geldoc_2015-08-11_16hr_08min_GFP_Amphnoter.jpg



Did o/n cultures of Amph NOTER and GFP colonies in 2 ml LB adding 2 ul CAM.

12.8.2015

WEDNESDAY, 8/12

Petra, Tamannae

Checked yesterday's Gibson and ELIC plates:
Colonies (both colorless and red) found from the plates

- AtoB Gibson 50 ul and 200ul
- CenA Gibson 50 ul
- CenA ELIC 20 ul and 200 ul

No colonies on plates

- AtoB ELIC 50 ul and 200 ul
- CenA Gibson 200 ul

Chose 4 colonies from AtoB plates and 12 colonies from CenA plates for screening (colony PCR). Chose all the colorsess colonies from the plates and included some reddish colonies.

Chosen AtoB colonies:
Colony number, plate, color:
1. Gibson, colorless
2. Gibson, reddish
3. Gibson, reddish
4. Gibson, reddish

Chosen CenA colonies:
1. Gibson, red
2-4. ELIC, colorless
5. ELIC, reddish
6-12. ELIC, colorless

Made DNA samples of colonies for colony PCR following the protocol. In the meantime plated all the colonies used on a fresh chloramphenicol plate (1 plate for AtoB and 1 for CenA colonies). Incubated the plates on 37 C.

At this point realized that cellulose construct is missing betaglucosidase (BglX), so Gibson and ELIC for that needs to be done again. All the pieces needed for Gibson for cellulose construct are CenA cex part 1, CenA cex part 2 and BglX. Discarded CenA colony PCR DNA samples and continued with AtoB samples.

5 x Reaction mix for AtoB (115 ul):
76,25 µl H2O
25µl 5 x Buffer
3,75 µl 10mM dNTP mix
3,75 µl 10µM P001
3,75 µl 10µM P011
2,5 µl KAPA HiFi HotStart DNA Polymerase

Added **2 ul** AtoB template DNA from each colony to PCR tubes (1 tube per colony, 5 tubes in total). Added 25 ul reaction mix for every tube. Total reaction volume on every tube: 25 ul.

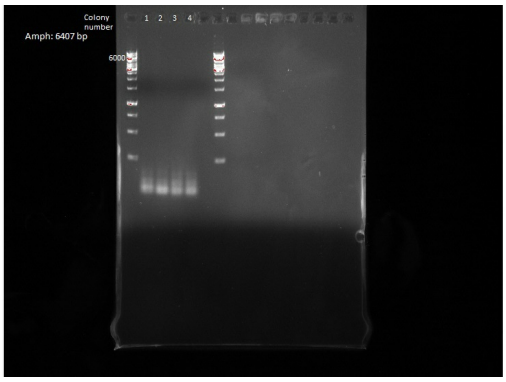
KAPA colony PCR for AtoB from Gibson assembly: PCR program
95° *C - 3 min*
98° *C - 30 sec*
64° *C - 20 sec*
72° *C - 6 min*
72° *C - 7 min*
4° *C - forever*
Repeated underlined cycles 25 times

AtoB construct length: 6407 bp

Checking AtoB colony PCR results:

Made a 1,3 % agarose gel with ETBR. Mixed 5 ul sample of each PCR reaction with 1 ul LD and pipetted to the wells. Ran the gel for 30 min, 120 V.

EDITED_CPCR_AtoB_whole_Geldoc_2015-08-12_17hr_58min.jpg



According to gel picture above none of the colonies contain the right construct.

Made a new Gibson assembly for CenA and Atob constructs.

Table1

Construct	Length (bp)	Concentration (ng/ul)	DNA (ng) needed for 0.05 pmol	DNA (ul) needed for 0,05 pmol
AtoB part 1	1311	106,1	40,51	0,38
fixed AtoB part 2	2000	278,5	61,8	0,22
AtoB part 3	1772	284,3	54,75	0,2
AtoB part 4	1414	52,1	43,69	0,84
pSB1C3	2070	25	63,96	2,6
Total:				4,24

Gibson for AtoB with pSB1C3:
Pipetted DNA (pSB1C3, AtoB part 1, Fixed AtoB part 2, AtoB part 3, AtoB part 4) to 1,5 ul tube according to Table 1. Didn't add water. Added 4,24 ul NEBuilder HiFi DNA Assembly MasterMix. Incubated the mix in 50 C for 60 minutes. Used 2 ul for transformation.

Table2

Construct	Lenght	Concentration (ng/ul)	DNA (ng) needed for 0.03 pmol vector & 0,06 pmol insert	DNA (ul) needed for 0.03 pmol vector & 0,06 pmol insert
CenA part 1	1891	122	58,43	0,48
CenA part 2	1302	114,3	40,23	0,35
BglX	2440	345,9	75,39	0,22
pSB1C3	2070	25	63,96	2,56
Total:				3,61
Water:				6,39

Gibson for CenA with pSB1C3:
Pipetted DNA (pSB1C3, CenA cex part 1, CenA cex part 2) to 1,5 ul tube according to Table 2. Added 6,39 ul water. Added 10 ul NEBuilder HiFi DNA Assembly MasterMix. Incubated the mix in 50 C for 60 minutes. Used 2 ul for transformation.

ELIC for CenA with pSB1C3

Table4

Construct	Lenght	Concentration (ng/ul)	fmol DNA needed for ELIC	DNA (ng)	DNA (ul)
CenA part 1	1891	122	95,16	111,2	0,91
CenA part 2	1302	114,3	95,16	76,57	0,66
BglX	2440	345,9	95,16	143,5	0,41
pSB1C3	2070	25	31,72	40	1,6
Total:					3,58
Water:					6,42

ELIC for CenA with pSB1C3:

Pipetted DNA (pSB1C3, CenA cex part 1, CenA cex part 2, BglX) to two 1,5 ul tubes according to Table 4. Added 6,42 ul water to the reaction. Total reaction volume for ELIC was 10 ul . Incubated reaction mix in room temperature for 45 minutes. When doing transformation, added competent cells straight to the same tube where reaction mix was.

Transformation of Gibson and ELIC reactions into TOP10.
Followed the protocol. Added 200 ul SOC after heat shock. Transfered ELIC reaction to 2 ml tube after adding SOC media. Plated 50 ul and 200 ul of each reaction on chloramphenicol plates (6 plates in total) and left to 37 C incubator overnight.

Miniprepped yesterday's o/n cultures of Amph NOTER and GFP following the kit protocol. However, Amph NOTER colony 4 LB was red, so it wasn't miniprepped.

NanoDrop results of the minipreps:

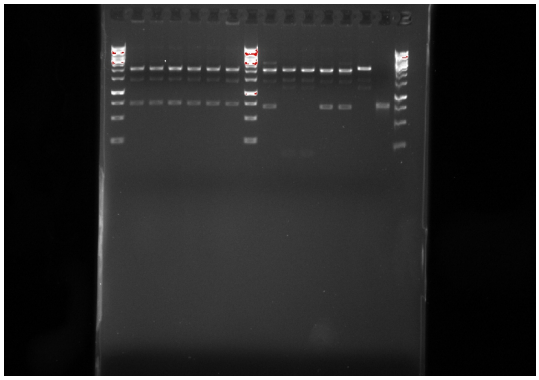
Table5

Sample	DNA (ng/ul)	A260/A280
GFP c1	48,6	1,86
GFP c2	51,0	1,86
GFP c3	438,4	1,83
GFP c4	60,0	1,85
GFP c5	53,2	1,85
GFP c6	49,7	1,86
Amph NOTER c1	61,1	1,85
Amph NOTER c2	27,2	1,89
Amph NOTER c3	33,0	1,84
Amph NOTER c5	56,4	1,84
Amph NOTER c6	54,5	1,86
Amph NOTER c7	40,7	1,86

Restricted with XbaI & PstI Amph NOTER, GFP and Amph with linker following mostly the protocol. However, the incubation time was 1 h.

Made a 1,3 % agarose gel with EtBr. Run the restrictions of Amph NOTER, GFP and Amph with linker in the gel for 25 min with 120 V. Pipeting order was: 1. ladder 2 ul 2.-7. rest. GFP colonies 1-6 6 ul 8. ladder 2 ul 9.-14. rest. Amph NOTER colonies 1-3,5-7 6 ul 15. Amph with linker 6 ul 16. ladder 2 ul

Geldoc_2015-08-12_18hr_58min_GFP_Amphnoter_Amphwithlink.jpg



Stored the GFP and Amph with linker restrictions in - 20 °C and discarded amph NOTER restrictions.

13.8.2015

THURSDAY, 8/13

Petra, Tamannae, Tuukka

Made chloramphenicol plates. Followed the protocol.

Checking yesterday's Gibson and ELIC transformations:

- AtoB Gibson 50 ul: no colonies
- AtoB Gibson 200 ul: 5 colonies -> to screening
- CenA Gibson 50 ul: 1 colony -> to screening
- CenA Gibson 200 ul: 5 colonies -> to screening
- CenA ELIC 50 ul: 2 colonies - > to screening
- CenA ELIC 200 ul: the only plate with some red colonies, chose 4 colorless colonies for screening

Total colonies for screening:

- AtoB: 5
- CenA: 12

Made DNA samples of the chosen colonies for colony PCR according to colony PCR protocol. In the meantime plated all the used colonies on a fresh chloramphenicol plates. Stored the plates on 37 C incubator.

6x KAPA reaction mix for AtoB (138 ul)
91,5 µl H2O
30µl 5 x Buffer
4,5 µl 10mM dNTP mix
4,5 µl 10µM P001
4,5 µl 10µM P011
3 µl KAPA HiFi HotStart DNA Polymerase

Added **2 ul** AtoB template DNA from each colony to PCR tubes (1 tube per colony, 5 tubes in total). Added 23 ul reaction mix for every tube. Total reaction volume on every tube: 25 ul.

AtoB construct length: 6407 bp

13x Kapa reaction mix for CenA (299 ul)
198,25 µl H2O
65µl 5 x Buffer
9,75 µl 10mM dNTP mix
9,75 µl 10µM P001
9,75 µl 10µM P011
6,5 µl KAPA HiFi HotStart DNA Polymerase

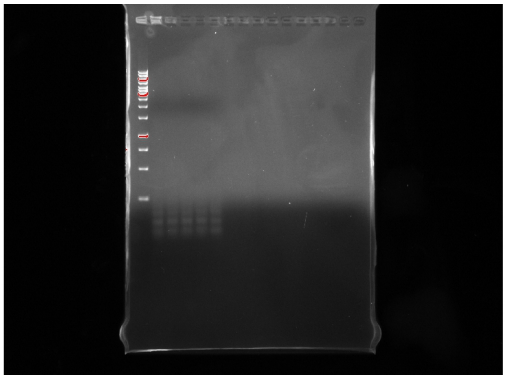
Added **2 ul** AtoB template DNA from each colony to PCR tubes (1 tube per colony, 12 tubes in total). Added 23 ul reaction mix for every tube. Total reaction volume on every tube: 25 ul.

KAPA colony PCR for AtoB from Gibson assembly: PCR program

95° *C - 3 min*
98° *C - 30 sec*
64° *C - 15 sec*
72° C - 5 min
72° *C - 7 min*
4° *C - forever*
Repeated underlined cycles 25 times

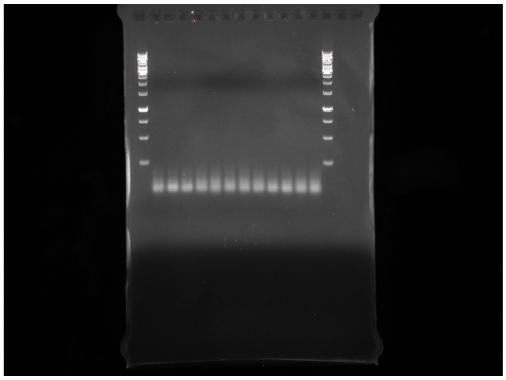
Made 2 1,3 % agarose gels with ETBR. Ran 5 ul samples of every colony PCR reaction with 1 ul ladder. Ran the gels for 40 min, 120 V. Used Gene O'Ruler 1 kb ladder.

Geldoc_2015-08-13_17hr_34min_Cpcr_atob_from_gibson.jpg



According to the gel picture colony PCR for AtoB colonies was unsuccesful -> colonies might not contain AtoB construct.

Geldoc_2015-08-13_17hr_37min_Cpcr_cenA_with_bglx_from_gibson_and_e



According to the gel picture colony PCR for CenA colonies was also unsuccesful -> colonies might not contain Cellulose construct.

Checked the colonies plated on fresh plates earlier today before making colony PCR. Only one colony from AtoB plate (colony 4) and 5 colonies from CenA plate (colonies 5, 8, 9, 10 and 11) were not red. Chose those six colonies and made o/n cultures:

- 2 ul chloramphenicol stock (34 mg/ml) to 2 ml LB
- Tubes left to 37 C incubator with shaking overnight

Did sample tubes for sequencing GFP and Amph NOTER with pSB1C3. In one tube there should be 1,6 ul 5 uM primer and 5 ul DNA template (150-300 ng). However, if DNA amount is less than 5 ul, sterile water should be added so the volume of the tube is 6,6 ul. Used 2 primers: BBa_G00100 and BBa_G00101, so there were 2 tubes for one DNA template. Below is the table which shows how much of each component was pipetted.

Table1

Construct	Colony	DNA (ul)	H20 (ul)
GFP	2	4,0	1
GFP	3	0,6	4,4
GFP	4	3,4	1,6
GFP	5	3,8	1,2
GFP	6	4,2	0,8
Amph NOTER	1	3,4	1,6
Amph NOTER	5	3,6	1,4
Amph NOTER	6	3,8	1,2

The sequencing samples were sent to the centre by bike.

Did restrictions of AH013, AH015 and pSB1A3 following mostly the protocol. However, for AH013 & AH015 the incubation was 1,5 h and for pSB1A3(AH007) 1 h. The restrictrictions were stored in - 20 C.

14.8.2015

FRIDAY, 8/14

Petra, Tamannae

Checked yesterday's o/n cultures

- AtoB colony 4 & CenA colonies 5 and 8 had turned red -> discarded them
- CenA colonies 9, 10 and 11: Colorless, continued with these

Miniprepped CenA colonies 9-11 with NucleoSpin Plasmid EasyPyre kit. Nanodrop results:

Table1

Construct, colony number	Concentration (ng/ul)	A260/A280
CenA 9	53,5	1,88
CenA 10	92,5	1,89
CenA 11	74,9	1,96

Restricted CenA colonies 9-11 with EcoRI and PstI. Digested 250 ul each miniprepped DNA in NEBuffer 3.1 at 37°C.

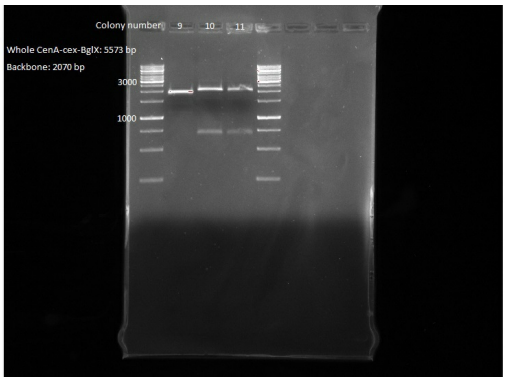
- CenA colony 9: 4,68 ul, water 19,12 ul
- CenA colony 10: 2,7 ul, water 21.1 ul
- CenA colony 11: 3.34 ul, water 20,46 ul

Added 0,5 ul PstI and 0,7 ul EcoRI to each reaction.

Cellulose construct length: 5573 bp

Made a 1,3 % gel with ETBR. Ran 25 ul restriction samples with 5 ul LD. Used Gene O'Ruler 1 kb ladder. Ran the gel for 40 min, 120V.

EDITED_CenA_with_Bglx_whole_Geldoc_2015-08-14_15hr_21min.jpg



According to the gel picture none of the CenA colonies contain the right insert.

Did ligations of yesterday restricted pSB1A3 and GFP with restricted AH013/AH015 following mostly the T4 ligase protocol. However, the incubation in 22 C was 45 min.
Transformed the ligations to TOP10 CHEM competent cells following the protocol. However, the incubation was 115 min. Plated 50 ul and 150 ul to AMP plates and incubated in RT over the weekend.

Did KAPA gradient PCR reaction for CAR amph part 3 & KAPA gradient PCR reaction for CAR amph part 4.
CAR amph part 3 13x reaction mix (325 ul):

217,75 ul sterilized water
65 ul 5x buffer
9,75 ul 10mM dNTP mix
9,75 ul 10µM P018
9,75 ul 10µM P032
6,5 ul KAPA HiFi HotStart DNA Polymerase
6,5 ng Car part 3 template DNA

CAR amph part 4 13x reaction mix (325 ul):
217,75 ul sterilized water
65 ul 5x buffer
9,75 ul 10mM dNTP mix
9,75 ul 10µM P021
9,75 ul 10µM P011
6,5 ul KAPA HiFi HotStart DNA Polymerase
6,5 ng Amph template DNA

The PCR program for CAR amph part 3 & CAR amph part 4 were same.
The PCR program:
95°C - 3 min
98°C - 20 sec
56,5 C - 71,5 C -15 sec
72°C - 90 sec
72°C - 1,5 min
4°C - forever
Repeated underlined cycles 25 times

Stored PCR products in 4 C.