Labjournal Conductive Curli

14.07.2014
Transformed in commercial competent TOP10 cells:

- From Lu lab for curli production
  - PAYC002 pzA-CmR-rr12-pL(tetO)-CsgA
  - rr12y(rii)g pzE-KanR-rr12y-pLuxR-gfp
  - PAYC003 pzA-CmR-rr12-pL(tetO)-CsgA His
  - rrjt12(11)g pzE-AmpR-rr12-pL(tetO)-gfp
  - PAYC005 pzA-CmR-rr12y-pLuxR-8x CsgA His
  - PAYC008 pzA-CmR-rr12y-pLuxR-gfp
  - PAYC006 pzA-CmR-rr12y-pLuxR-CsgA
  - PAYC007 pzA-CmR-rr12y-pLuxR-CsgA His

Cultivate AYCE189 strain from Lu lab on LB agar plates (KanR)

15.07.2014

- Cultivate the transformed constructs from 14.07.2014 in LB medium for miniprep
  - Also cultivate mKate (amp), eGFP (amp) and Rhamnose promoter - Bba_K914003 (cm)
  - And the strain AYCE189 from Lu lab is also cultivated (for a glycerolstock)

16.07.2014

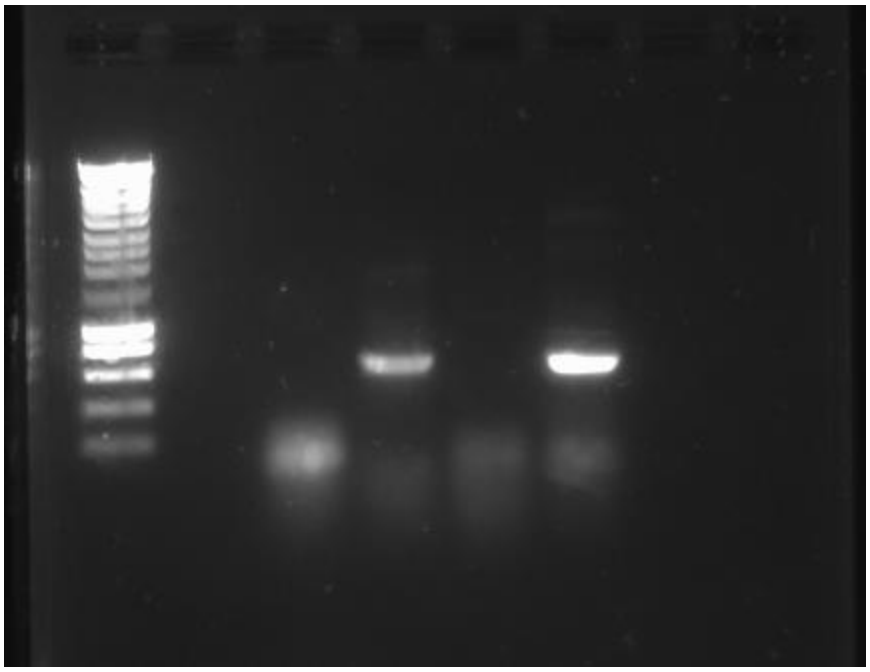
- Miniprepped the samples cultivated on 15.07.2014, except for the strain AYCE189.
- Measured the concentration of the isolated DNA with nanodrop:
  - PAYC002 122.8 ng/ul
  - rr12y(rii)g 52.6 ng/ul
  - PAYC003 187.0 ng/ul
  - rrjt12(11)g 32.3 ng/ul
  - PAYC005 27.8 ng/ul
  - PAYC008 22.3 ng/ul
  - PAYC006 37.0 ng/ul
  - PAYC007 22.3 ng/ul
  - mKate 39.2 ng/ul
  - eGFP 165.1 ng/ul
  - Rhamnose 47.7 ng/ul
- Cultivated the samples again in shakeflasks.
- Made glycerolstocks of all the cultivated samples from 15.07.2014.

17.07.2014
Cultivated AYE189 (KanR) in LB medium and in shaker (37 degrees)

18.07.2014
PCR eGFP + RBS using Taq polymerase. The PCR reaction included:

Mastermix (2x)	12,5ul
eGFP FW [5pmol/ul]	2,5ul
eGFP RV [5pmol/ul]	2,5ul

eGFP (165.1 ng/ul)	0,2ul
MilliQ	7,3ul
Total	25,0ul



eGFP is the 4th lane. The rest are the ccm promoter from Ajo-Franklin lab (belong to the ET module). The ladder is smart ladder. Therefore, the eGFP band corresponds to a fragment of around 800 bp (745 bp was expected).

23.07.2014
Purification of the eGFP (+RBS) PCR product using QIAquick kit. 15 ul of PCR product were used for the purification. DNA concentration of the purified eGFP (+RBS): 23.2 ng/ul.

30.07.2014
Anne

PCR of csgA, csgA-his, csgB, csgBA, pRham
PCR mix (for 2x25 ul) :

	ul
template	1
FW	3
RV	3
mgso4 50 mM	1
dNTP 10 mM	1,5

pfx buffer 10x	5
enhancer	5
pfx polymerase	0,4
MQ	30,1
total	50

Primers (5µM):
Fw pRha
Rev pRha
Fw csgB
Rev csgB
Fw csgA
Rev csgA
Rev csgAHis
Fw csgBA
Rev csgBA

Templates:

K540000(105 ng/ ul) (for all csgX PCR's)

Prhamnose (87 ng/ul) (for rhamnose PCR)

PCR cycling conditions:
Cycle conditions

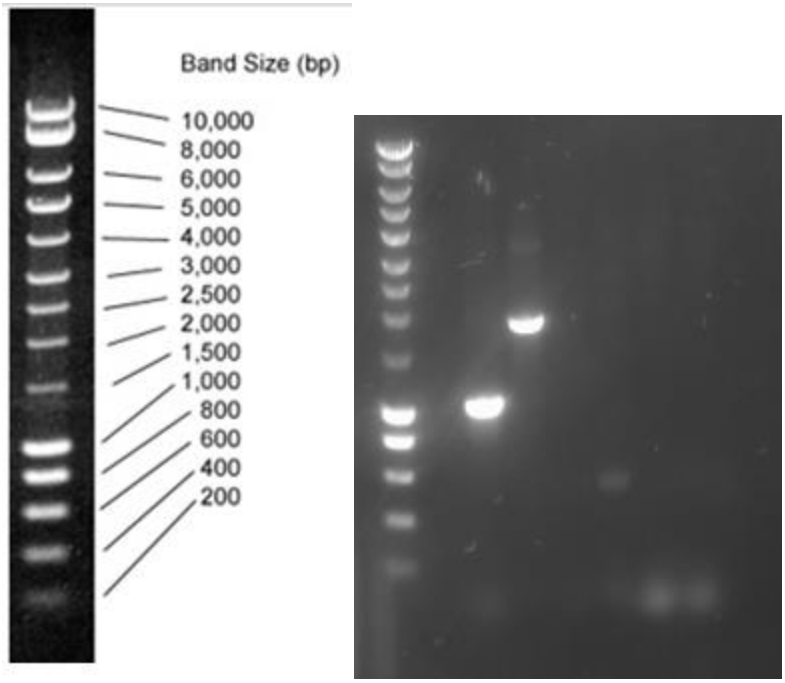
cycling conditions	minutes	°C	
1	3	94	
2	1	94	
3	1	55	
4	1	68	go to step 2, 29x
5	5	68	

→ 4 degrees
Bring 3 ul + 1 ul loading of the PCR mixture on 1.5 % agarose gel (for Prhamnose) and 1% agarose gel (CsgX products).

From left to right :
-EZ load marker
-Prhamnose 175 bp
-ccm promoter (promoter, not discussed here)

Prhamnose was obtained by PCR, although nonspecific products were seen as well. . The rest (20 ul) of the the PCR sample was loaded on gel an purified (see red box) using Qiagen gel purification kit.

From left to right:
-marker DNA SmartLadder
-csgBA (1047bp)
-csgA (531bp)
-csgA-His (531 bp + aprox 20 bp)
-csgB (531 bp)



csgBA and csgB were obtained by PCR. csgA (-HIS) not, and PCR conditions will be optimized. The rest (20 ul) of the the PCR samples (csgBA and csgB) were loaded on gel and purified using Qiagen gel purification kit.

31.07.2014

Anne

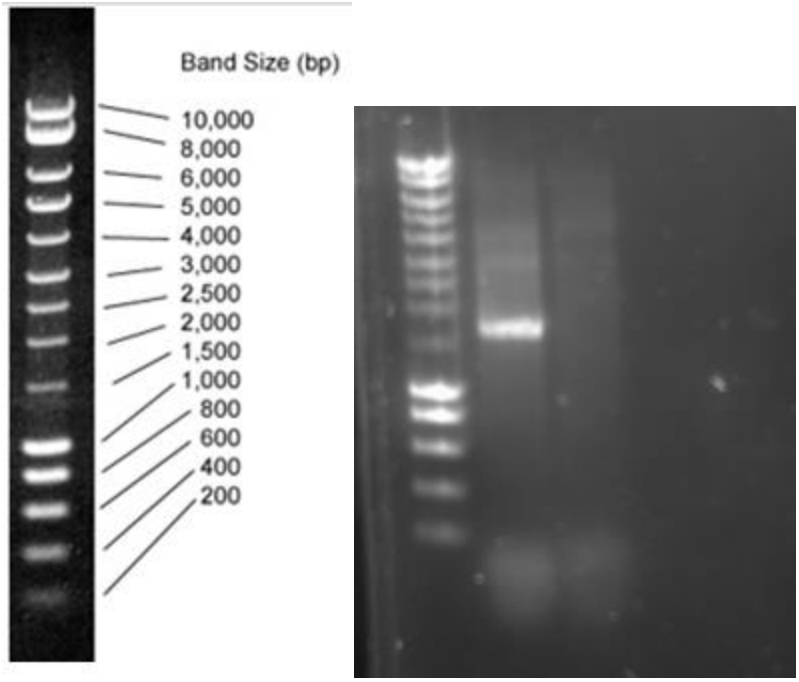
PCR of csgA and csgA-HIS.

PCR conditions and mix are the same as decribed in **PCR of csgA, csgA-his, csgB, csgBA, pRham 30.07.2014,** with as adjustment increasing the annealing temperature of the primers to 60 degrees.

Bring samples on 1% agerose gel, run @ 100V 40 minutes
4 ul PCR mixture was mixed with 1 ul loading

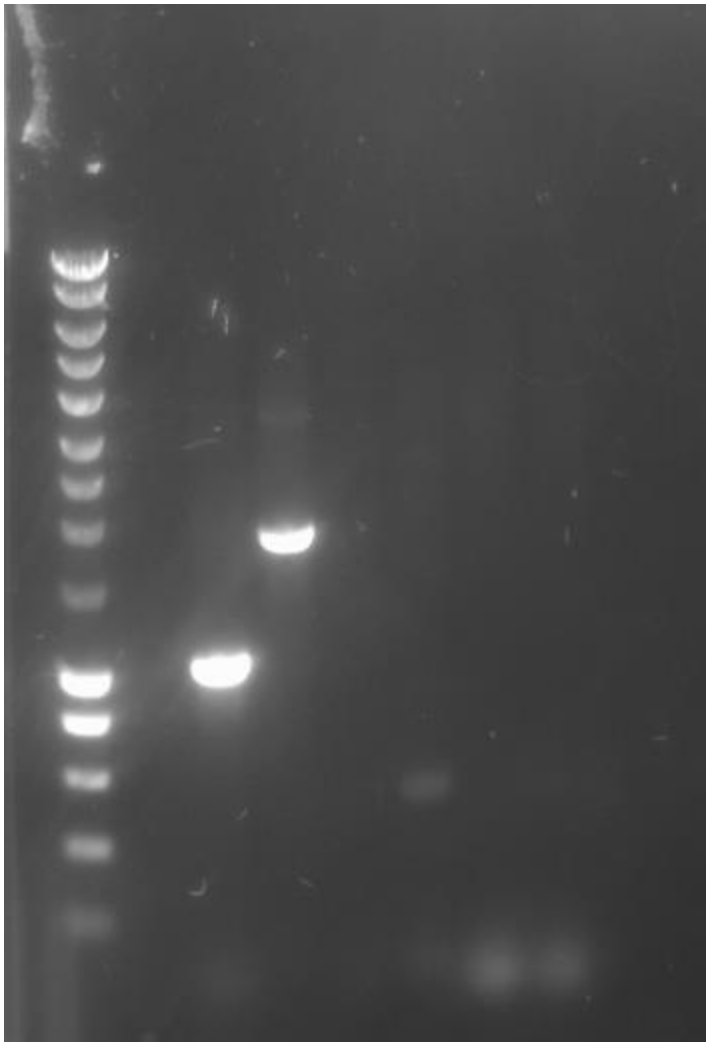
from left to right:

marker
csgA (531 bp)
csgA-HIS (531 + approx. 20 bp)



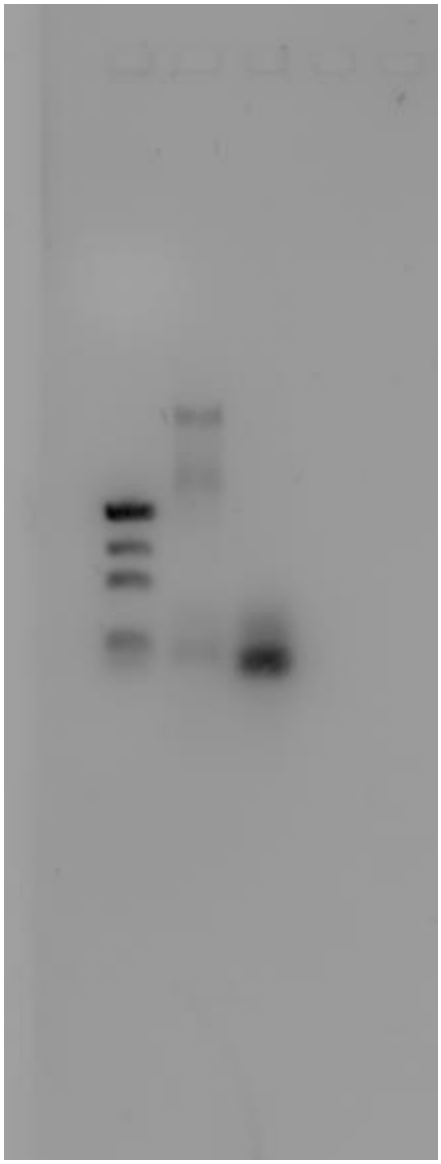
no bands at expected sizes were observed

Joan
Gels of Golden Gate fragments and CsgBA
1%Agarose gel. From left to right: Smart Ladder; Empty; CsgBA amplified from K540000; CsgA for Golden Gate amplified from K540000; CsgA-His for Golden Gate amplified from K540000; CsgB for Golden Gate amplified from K540000. The last two lanes are from the ET module (Ajo-Franklin promoter + mtrCAB PCRed at 55 and 58^{o}C of extention temperature respectively).



CsgBA and CsgB for Golden Gate seem to have the desired size (about 1kb and 500bp).

0.7%Agarose gel. From left to right: EZ Ladder; Rhamnose promoter for the Golden Gate amplified from K914003; pFab promoter for the ccm cluster (for the ET module).



The Rhamnose promoter shows several bands. The pFab promoter seems to have the right size.

01.08.2014
Anne

restriction of csgBA and plasmid + rhamnose promoter

	ul
DNA csgBA (30ng/ul)	20
PstI-HF	0.5
XbaI	0.5
cutsmart	4

MQ	15

incubate 3h @ 37 degrees

	ul
DNA rhamnose promoter (78 ng/ul)	20
xbaI	0.5
SpeI-HF	0.5
cutsmart	4
MQ	15

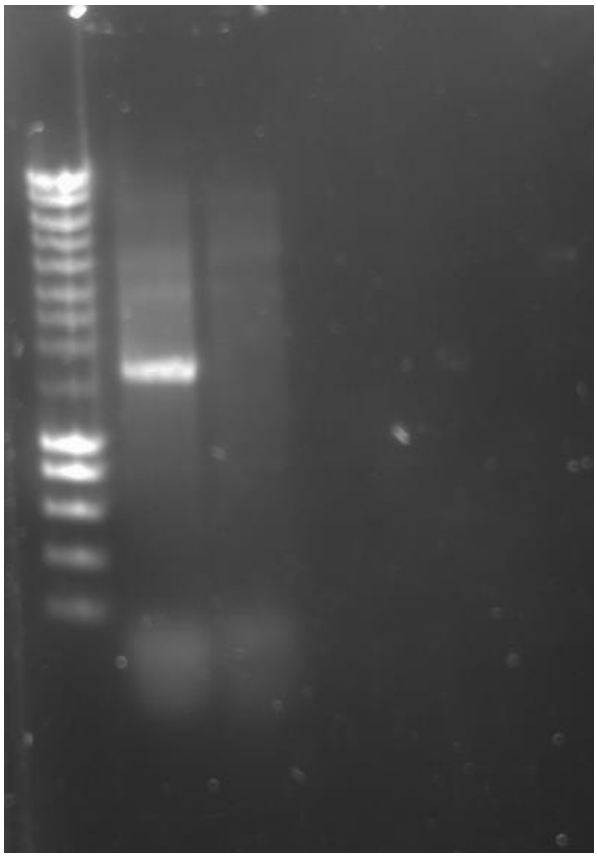
incubate 1.5h @ 37 degrees

samples were purified using nucleospin gel and PCR clean-up kit.

Joan

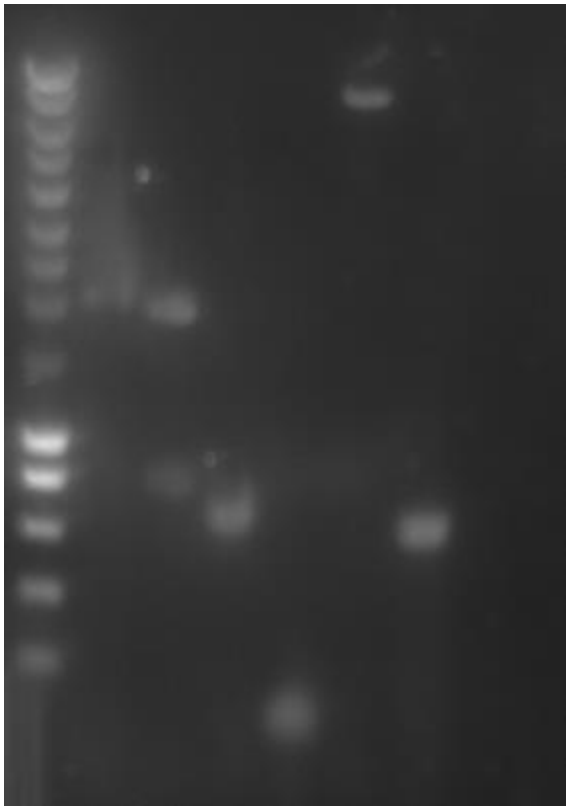
Gels

1% Agarose gel. From left to right: Smart Ladder; csgA and csgA-His



There is a clear band on the lane of csgA, but it is around 1600bp, which is bigger than the expected size of csgA (500 bp).

For the construction of the [constitutive promoter + eGFP], the following 1% Agarose gel was run:



Lanes 1, 3 and 7 correspond to: Smart Ladder; p[Anderson constitutive J23110] cut with SpeI and PstI; and eGFP cut with XbaI and PstI respectively.
The p[Anderson constitutive] shows two bands because it contains RFP downstream the promoter, and this gene is surrounded by 2 SpeI restriction sites (being the latest the one corresponding to the suffix)..

04.08.2014
Anne and Mathijs
colony PCR of potential transformants with prhamnose + csgBA construct.

6 colonies and one colony that appeared on the plate without insert were checked.
PCR mix:

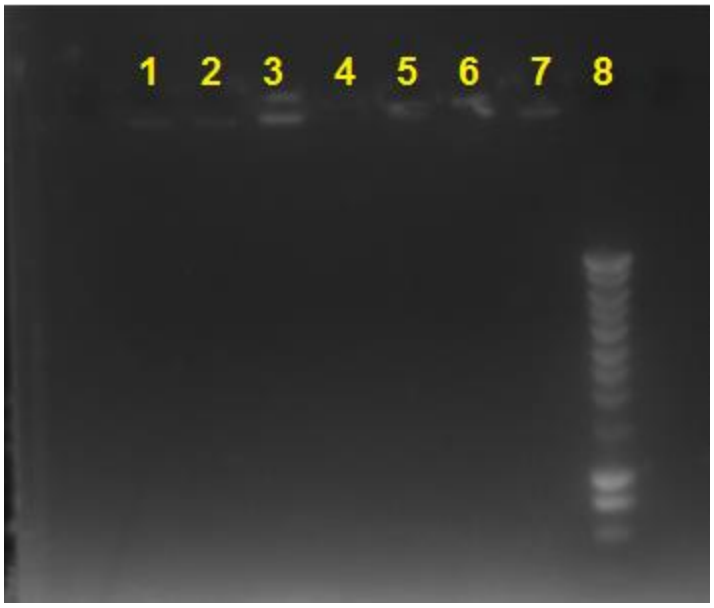
	ul
FW csgBA (5uM)	2.5
RV csgBA (5uM)	2.5
template = single colony	/
Taq master mix 2x concentrated	12.5
Taq polymerase	0.2
MQ	7.3

Cycle conditions

cycling conditions	minutes	°C	
1	5	94	
2	1	94	
3	1	55	
4	1	72	go to step 2, 29x
5	5	72	

→ 4 degrees

PCR result
Loaded the PCR products on 1 % agarose gel for 45 minutes at 100 V.



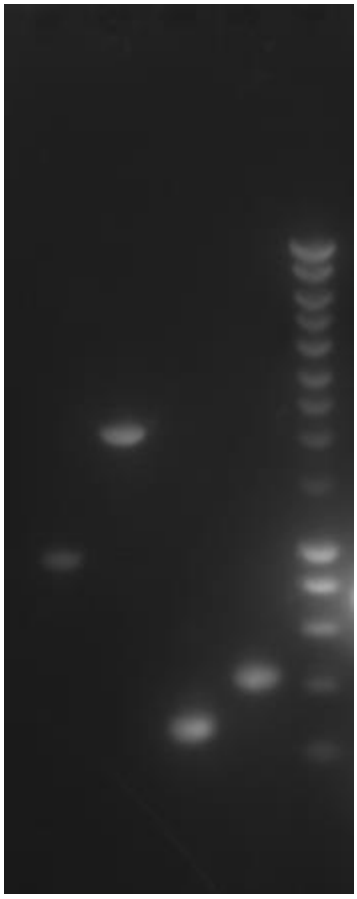
PCR of pRhamnose-CsgB-CsgA (expected size 3283 bp) (52)
15 ul of sample with 5 ul of loading dye
1. CsgBA first colony
2. CsgBA second colony
3. CsgBA third colony
4. CsgBA fourth colony
5. CsgBA fifth colony
6. CsgBA sixth colony
7. CsgBA negative control
8. Smartladder

The PCR did not work out as expected, there were no bands to see on the gel.

04.08.14
Anne
check both cut and purified rhamnose promoter plasmid and csgBA on gel
load samples (4ul product, 1 ul loading) on 1% agerose gel, 100V 40 mins

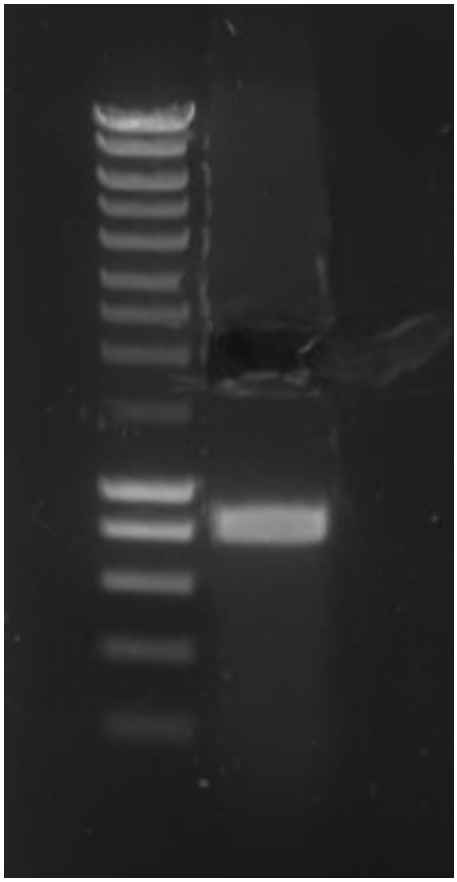
from left to right:

csgBA(aprox. 1kb)
rhamnose plasmid (aprox. 2 kb)
not this experiment
not this experiment
smartladder



Both cut products were successfully purified

Joan
As the plasmid containing p[Anderson constitutive J23110] + RFP showed two bands, it was necessary to run it again on a gel to be able to purify the desired band (containing the promoter and the backbone). Therefore, the plasmid was cut again using SpeI and PstI. An agarose gel was run with the restricted product. The gel showed the two expected bands and the upper one containing the constitutive promoter and the backbone was cut and purified with the Macherey-Nagel kit. The concentration of digested plasmid after purification was 57.1ng/ul.



05.08.2014
Anne and Janna:
Innoculated colonies from the plate with pRham-CsgBA (52) in 3 ml liquid LB medium with chloramphenicol. We used numbers 1 and 2 from the plate with transformants from 04.08.2014. Put in the stove at 37 C for 5 hours. Made glycerolstock of one culture, made minipreps of both cultures.

Mariëlle and Anne
Ligation of csgBA in K914003 (plasmid with pRhamnose)

For restriction see: restriction of csgBA and plasmid + rhamnose promoter on 01.08.14

ratio's 1:3 and 1:5 plasmid:insert were chosen

	ul	ul	ul
	1;3	1;5	MQ
K914003 restricted 46 ng/ul	2.2	2.2	2.2
csgBA restricted 40 ng/ul	3.5	6.0	/
T4 polymerase	0.5	0.5	0.5
buffer	1	1	1

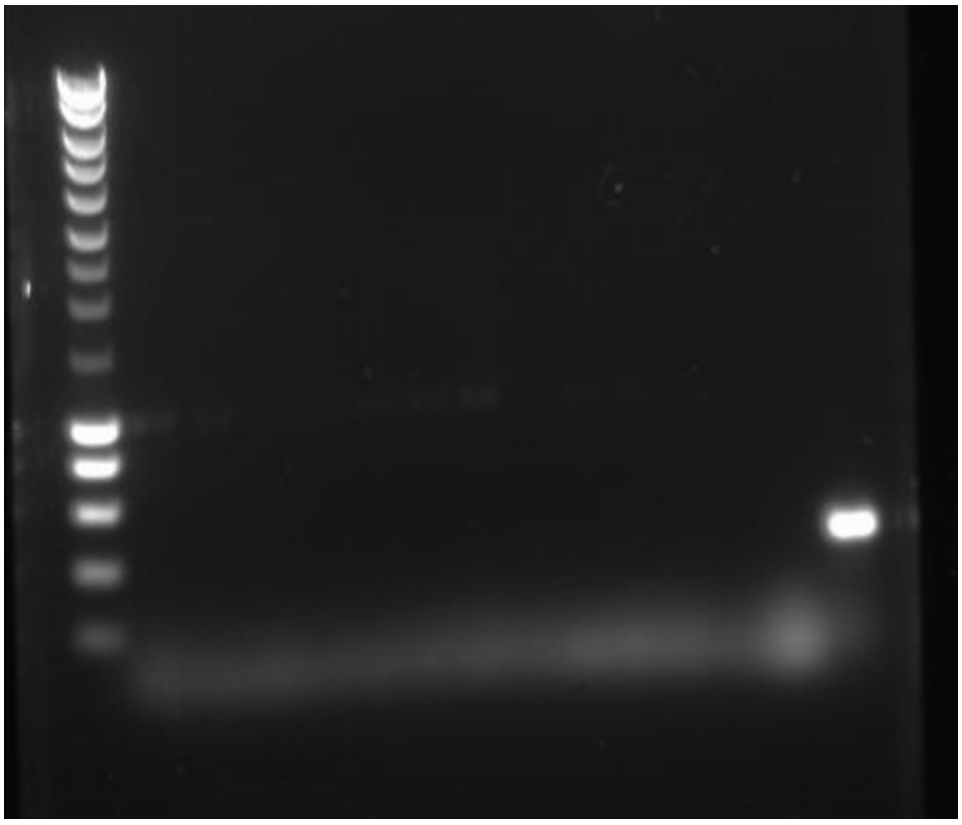
MQ	1.8	0.3	6.3

ligate 1hr @ rt.

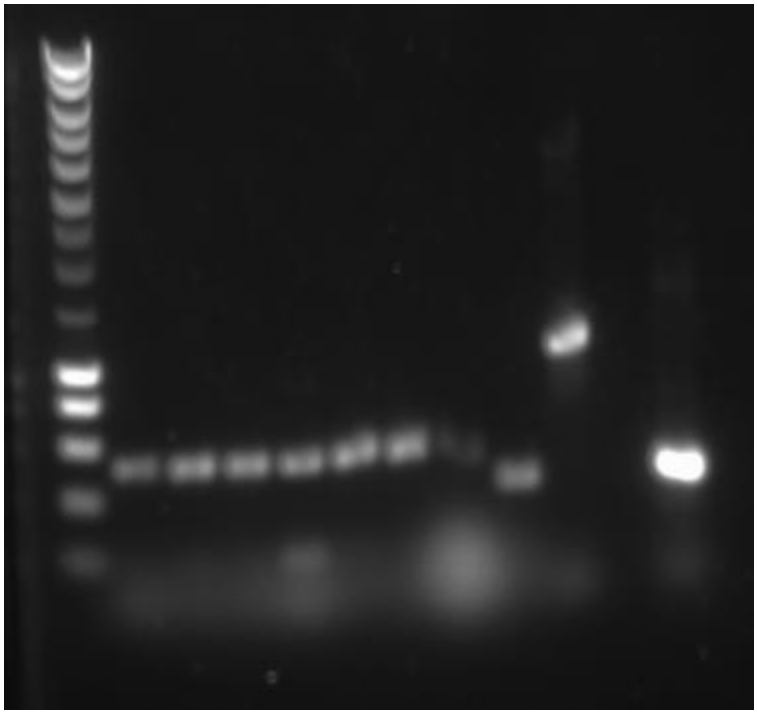
transform competent *E. coli* dh5α bacteria according to the protocol (only 1:5 and negative control was transformed).

06.08.14
Joan
Electrophoresis gel. From left to right:

- Smart Ladder (lane 1)
- Colony PCR of 13 colonies supposedly carrying the construct: pAnderson+csgBA using the csgBA FW and RV primers (lanes 2-13); and using the standard VF2 and VR primers (lane 14). In both cases the expected size is slightly bigger than 1kb
- Negative control: PCR of an empty plasmid using the standard VF2 and VR primers (lane 15)



csgBA was also amplified from K54000, to compare with the size of the band in this last gel. The resulting band (from K540000) is in lane 10 of the following gel. The size matches (slightly above 1kb)



07.08.14
Anne

PCR of csgA, csgAHIS, csgB Golden Gate

content master mix (used for both curli and mtrCAB GG)

	1x 25 ul	MM 10X
FW	1.5	15
RV	1.5	15
template	0.5	5
dNTP	0.75	7.5
enhancer	2.5	25
mgSO4	0.5	5
pfx	0.2	2
10x buffer	2.5	25
MQ	15.05	150,5
total	50	250

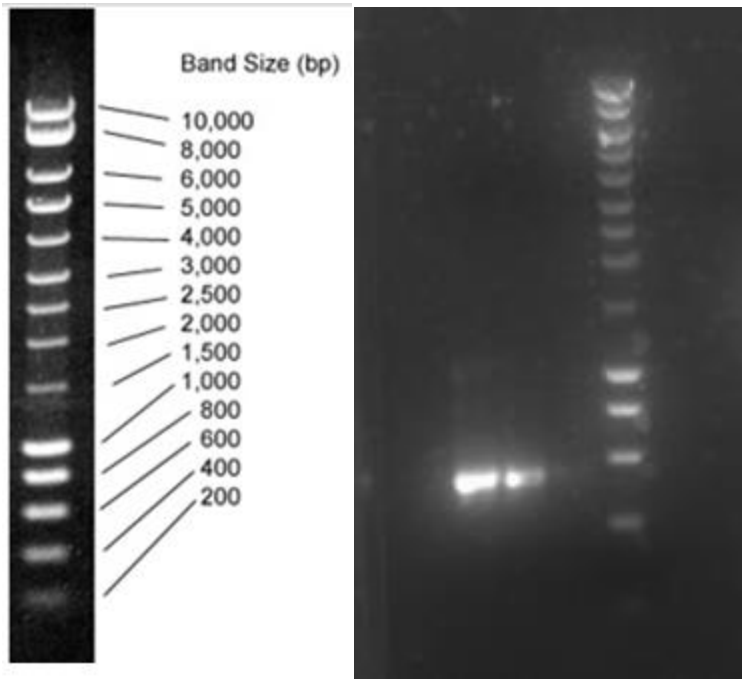
FW and RV primers: [5uM]
template: K540000 (nr 23) 50 ng/ ul

PCR cycling conditions:

min	temp C
3	94
1	94
1	56
45s	68
5	68
pause	4

load samples (4ul PCR product, 1 ul loading) on 1% agerose gel, 100V 40 mins

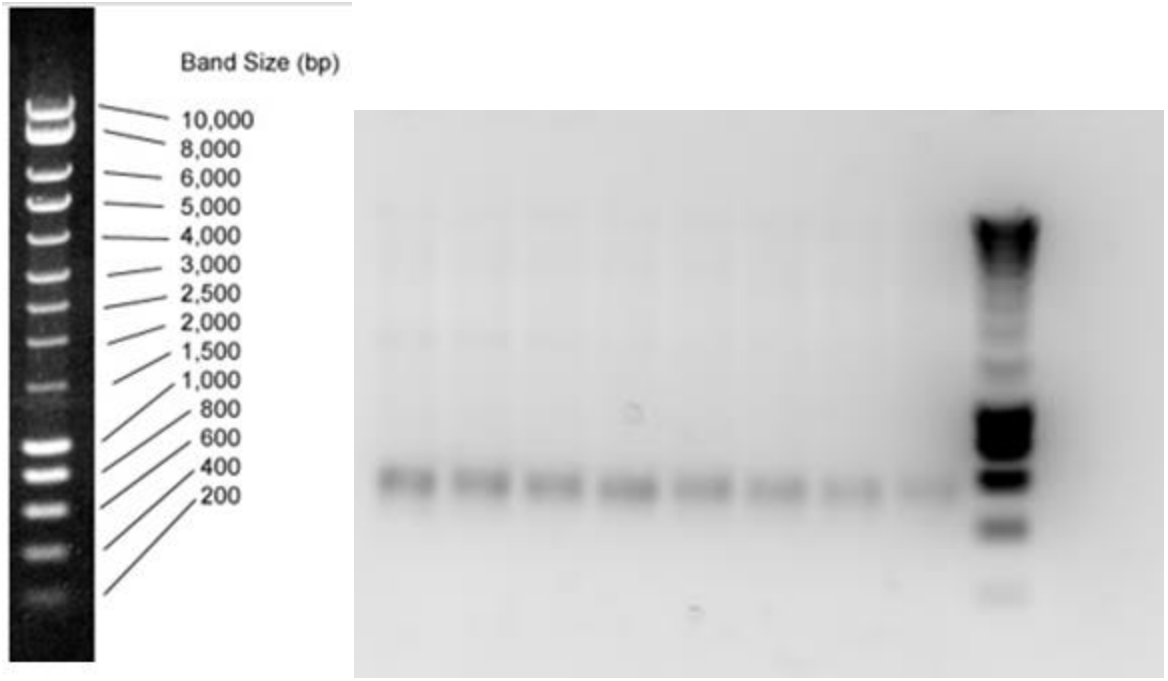
from left to right:
csgA (531 bp)
csgAHIS (531 + aprox 20)
csgB (549bp)
smart ladder



CsgA and CsgAHIS products were successfully PCR-ed. A weak signal is seen for csgB, therefore these PCR will be repeated, applying a gradient (only annealing temperature will be adjusted).

load samples (4ul PCR product, 1 ul loading) on 1.5% agerose gel, 100V 40 mins
From left to right:

csgB: (549bp) (50, 50.9, 52, 53,2 54.4, 56.8, 58, 59,8.)
smart ladder



a clear product of aprox 550 bp was formed, with higher concentrations for lower annealing temperatures.

Janna

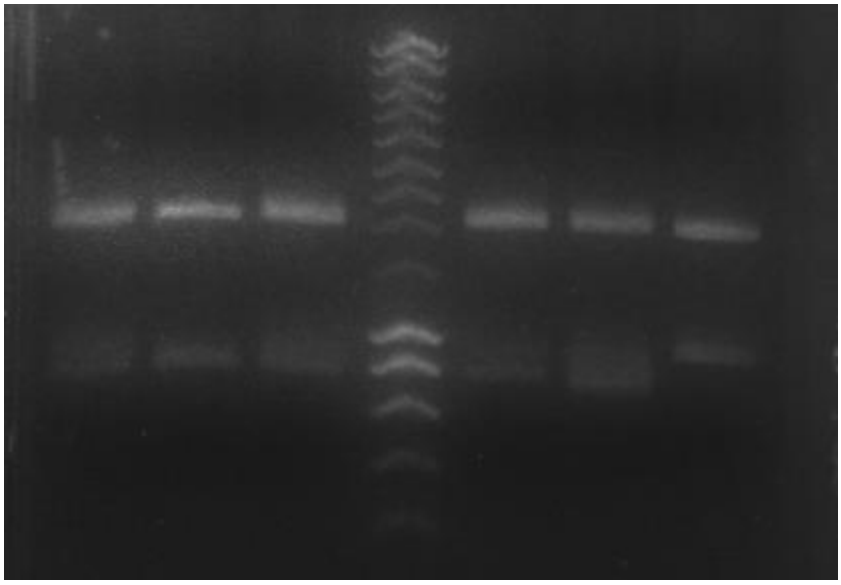
Restriction of CC 53 eGFP and CC 52 CsgBA

Restricted CC 53 eGFP culture 1 to 6 and CC 52 CsgBA culture 1 and 7 with EcoR1 and Pst1. The used buffer is CutSmart, the restriction enzymes were the HF version. The restriction was conducted for 1 hour at 37 degrees Celsius. Restriction scheme:

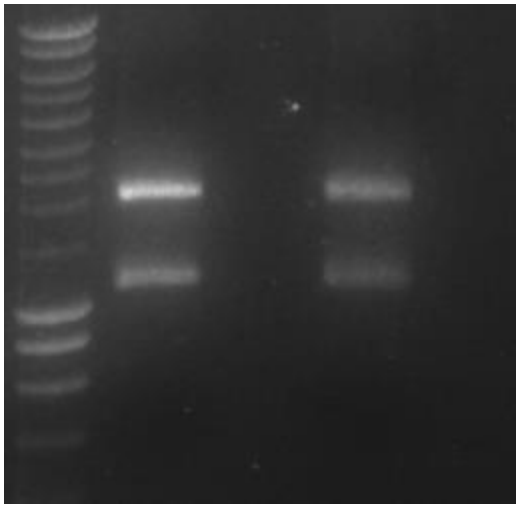
	ng/ul	DNA (ul)	EcoR1 (ul)	Pst1 (ul)	Buffer (ul)	MilliQ (ul)
53 cul 1	73.2	6	0.5	0.5	3	20
53 cul 2	81.9	5	0.5	0.5	3	21
53 cul 3	86.2	5	0.5	0.5	3	21
53 cul 4	100.0	4	0.5	0.5	3	22
53 cul 5	137.7	3	0.5	0.5	3	23
53 cul 6	91.6	4	0.5	0.5	3	22
52 cul 1	76.8	6	0.5	0.5	3	20
52 cul 7	133.3	3	0.5	0.5	3	23

Restriction result

Loaded the samples on 1 % agarose gel for 40 minutes at 100 V. Added 5 ul loading dye to each 30 ul sample.



Gel 1
1. CC 53 cul 1 (expected sizes around 2 kb and around 800 bp)
2. CC 53 cul 2
3. CC 53 cul 3
4. Smartladder
5. CC 53 cul 4
6. CC 53 cul 5
7. CC 53 cul 6



Gel 2
1. Smartladder
2. CC 52 cul 1 (expected sizes around 1200 bp and 2 kb)
3. Empty lane
4. CC 52 cul 7

We can see that all restrictions worked out, as both the backbone and the inserts are visible. This means that all tested colonies have the expected construct.

08.08.14
Mariëlle

Purify GG samples

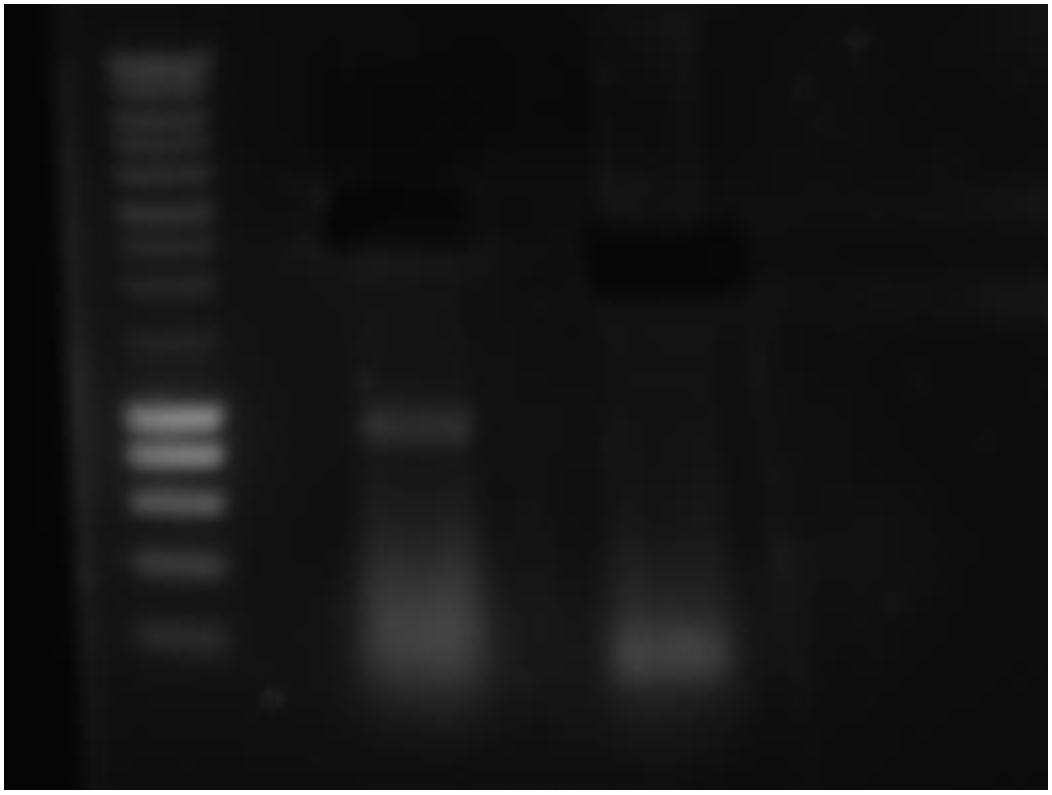
Qiagen (QiaQuick) PCR purification kit was used to purify samples.

08.08.14
Joan
Prepare the sequencing mixture for the construct pAnderson+eGFP+DT (CC53)

Glycerol stock and miniprep:

- The synthesised pFab promoter for ccmAH
- The intermediate construct carrying NapC and CymA together
- NapC alone

Load on gel the digested parts I20260 and B0017 both cut with EcoRI and SpeI, which are the backbones that we want to use for the Golden Gate Assembly. The upper band (empty backbone opened were purified)



08.08.14 & 11.08.14
Mariëlle and Anne

Golden Gate assembly Curli

Digestion of pSB1C3, cut E,S

	ul
pSB1C3 (1ug)	10

SpeI-hf	0.5
EcoRI-hf	0.5
cutsmart	3
MQ	16

incubate 1.5h 37degrees
Purify from gel, using qiagen gel purification kit.

golden gate mix

	ng/ul	ul
pSB1C3 cut E,S	15.5	4.4
prham	5.2	8
csgB	40	2
csgA-HIS	48	1.6
T4 ligase buffer		2
ligase	400 u	1
BsaI	10 u	1
total		20

and

	ng/ul	ul
pSB1C3 cut E,S	15.5	4.4
prham	5.2	8
csgB	40	2
csgA	52	1.6
T4 ligase buffer		2
ligase	400 u	1
BsaI	10 u	1
total		20

PCR reaction conditions

step	min	degrees	
1	3	37	
2	4	16	go back to step 1 (49x)
3	5	50	
4	5	80	

→ 4 degrees

12.08.14
Anne
Transformation of DH5 alpha cells with GG ligation mixture

25 ul competent DH5 alpha cells (C2987I, NEB) were transformed with 7.5 ul GG50 and GG51 ligation mixture (with and without HIS) according to associated protocol.
Bacteria were plated on LB+CAM

12.08.14
Anne

Golden Gate assembly Curli

Same procedure as described in Golden Gate assembly Curli 08.08.14 & 11.0.814 was used, but then different prhamnose PCR and digested backbone was used in the final ligation mix.

13.08.14
Anne
colony PCR of GG50 and GG51 transformants

PCR MM 23x

FW GG rham	3 ul [100uM]
RV GG csgA 2nd (for GG50)/ RV GG csgA 2nd HIS (for GG510)	3 ul [100uM]
2 x MasterMix TAQ	287.5 ul
MilliQ	281.5 ul
Total	575 ul

Colonies were ented with a pipet tip, inoculated in 25 ul MM, and streaked on LB+ CAM.

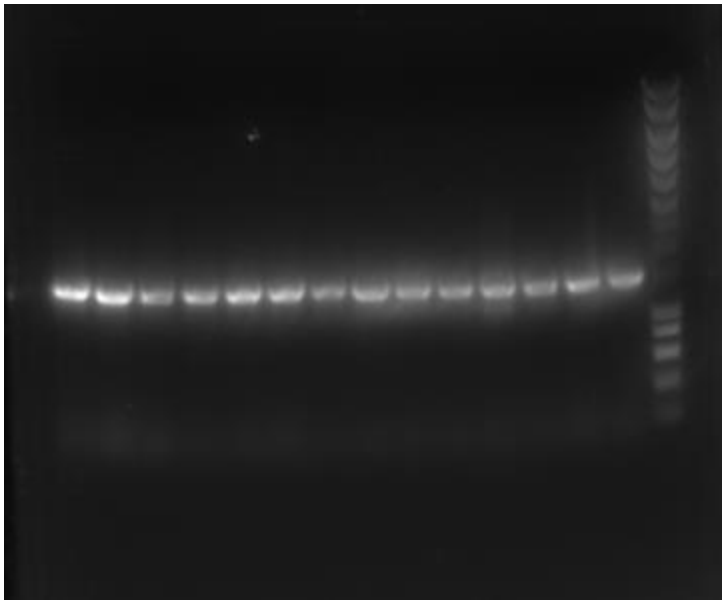
Cycle conditions

cycling conditions	minutes	°C	
1	5	94	
2	1	94	
3	1	56	
4	1.30	72	go to step 2, 29x
5	5	72	

→ 4 degrees
negative control: no template

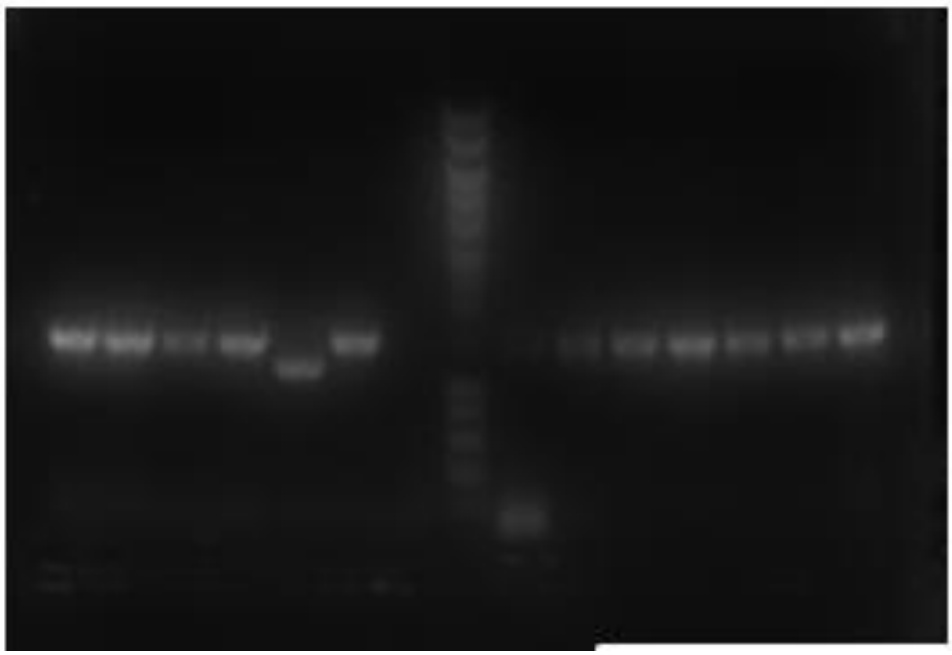
run samples on 1% agerose gel, 100V 40 min

from left to right:
samples 1-14 GG51 (1148bp)
Smartladder



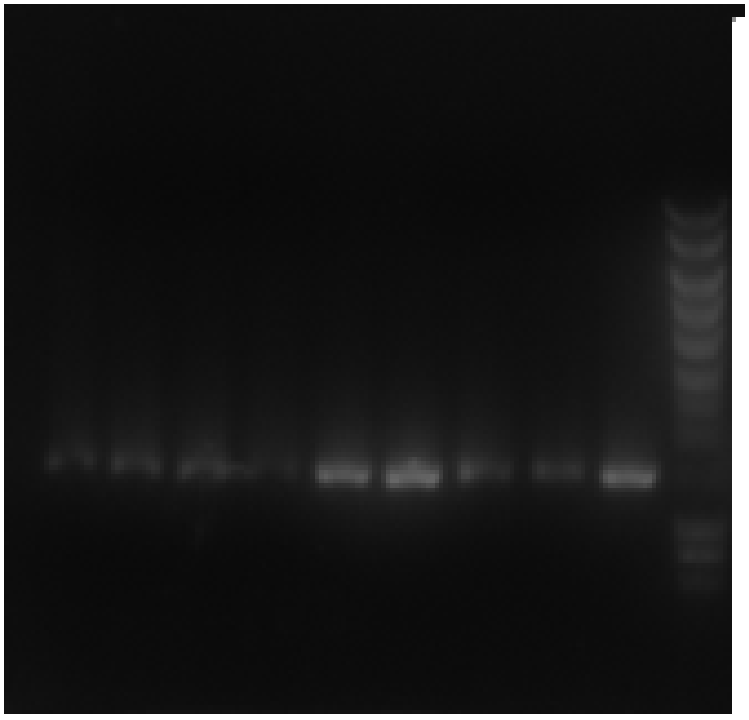
all fragments formed meet the expected size

from left to right:
samples 15-20 GG51 (1148bp)
negative control
Smartladder
samples 1-7 GG50 (1177 bp)



All fragments formed meet the expected size, except for sample 19 GG51 and sample 1 GG50, which do not meet the expectations and are therefore not further analysed.

from left to right:
samples 8-16 GG50 (1177 bp)
smartladder



all fragments formed meet the expected size

13.08.14

Anne

Transformation of DH5 alpha cells with GG ligation mixture

25 ul competent DH5 alpha cells (C2987I, NEB) were transformed with 7.5 ul GG50 and GG51 (from 12.08.14) ligation mixture (with and without HIS) according to associated protocol.
Bacteria were plated on LB+CAM

Janna

PCR of transformants CC 53

Construct check of BL21 transformants with CC 53 (eGFP). The expected size of this construct is around 800 bp.

MasterEp:

2.5 ul	FW[eGFP]	x 4 =	10 ul
2.5 ul	RV[eGFP]	x 4 =	10 ul
12.5 ul	2x MasterMix TAQ	x 4 =	50 ul
7.5 ul	MilliQ	x 4 =	30 ul

Negative control: primers no colony. Positive control: eGFP plasmid from freezer.

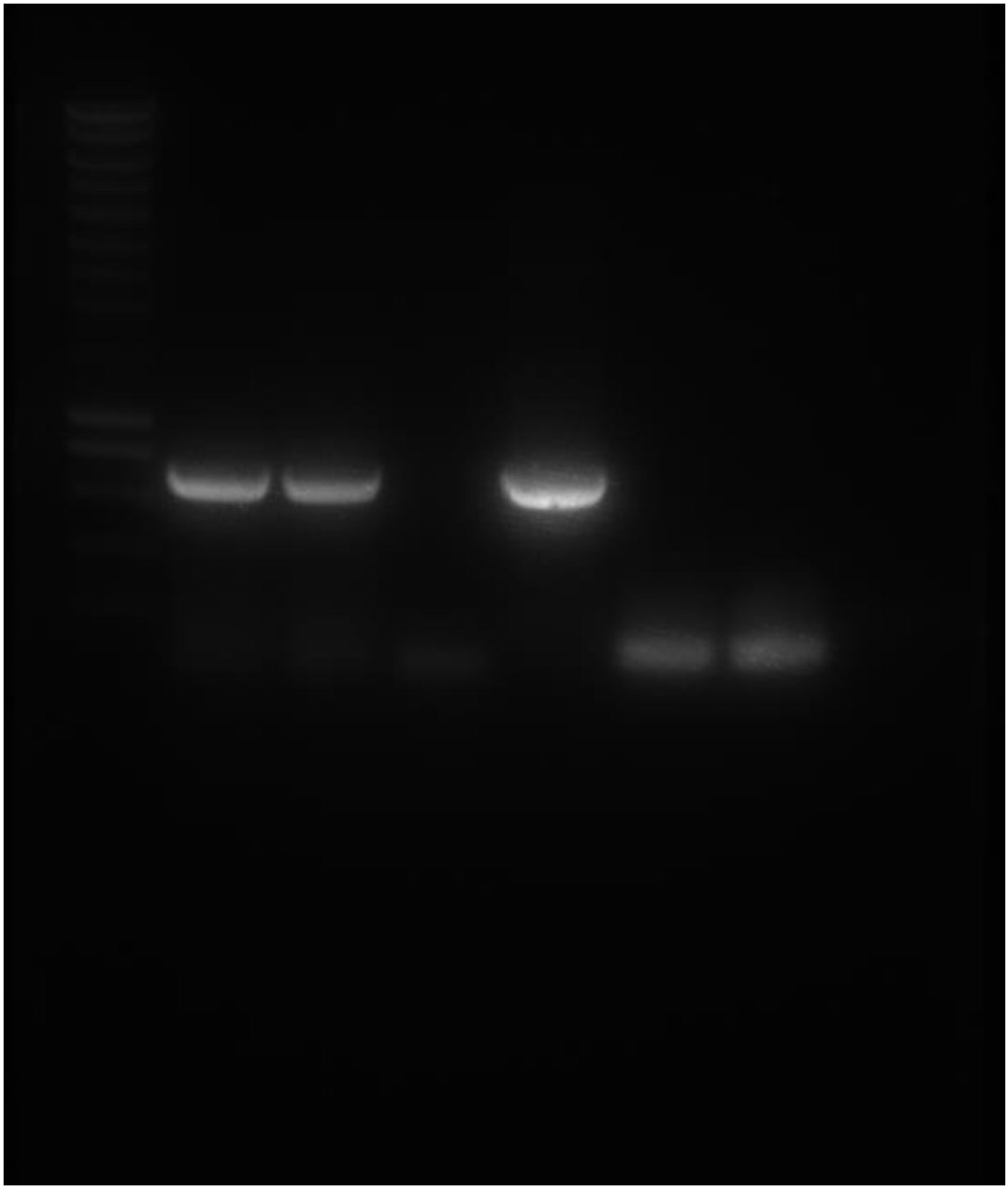
Cycling conditions:

1.	5 minutes	94 C	
2.	1 minute	94 C	
3.	1 minute	56 C	
4.	1 minute	72 C	go back to 2. - 29 x
5.	5 minutes	72 C	
6.	pause	4 C	

Loaded the PCR products on a 1 % agarose gel at 100 V for 40 minutes. Added 5ul loading dye to each sample, generating a total of 30 ul per sample. Loaded 20 ul of each sample and used 5 ul SmartLadder.

1. SmartLadder
2. CC53 c1
3. CC53 c2
4. CC53 negative control
5. CC53 positive control

rest of the samples is from another module.



The product from the PCR is indeed around 800 bp. The negative control is clean, there is no product to see in this lane. The positive control shows that the PCR worked. So the cc53 is indeed inserted into the BL21 strain.

14.08.14
Joan
Culture 4 colonies of GG50 and 4 colonies of GG51
grow the single colonies overnight on shake flasks.

Janna
Colony PCR of GG50 and GG51
Did a colony PCR of pSB1C3-p[Rham]-CsgB-p[And]-CsgA (GG50) and pSB1C3-p[Rham]-CsgB-p[And]-CsgA:HIS (GG51). The goal was to check if the construct was present in the plasmids. The expected size of both constructs is about 1200-1300 bp.

MasterEps:
GG50

2.5 ul	FW[Rham]	x 8 =	20 ul
2.5 ul	RV[CsgA GG 2nd]	x 8 =	20 ul
12.5 ul	MasterMix TAQ 2x	x 8 =	100 ul
7.5 ul	RNAse free H2O	x 8 =	60 ul

GG51

2.5 ul	FW[Rham]	x 8 =	20 ul
2.5 ul	RV[CsgA:HIS GG 2nd]	x 8 =	20 ul
12.5 ul	MasterMix TAQ 2x	x 8 =	100 ul
7.5 ul	RNAse free H2O	x 8 =	60 ul

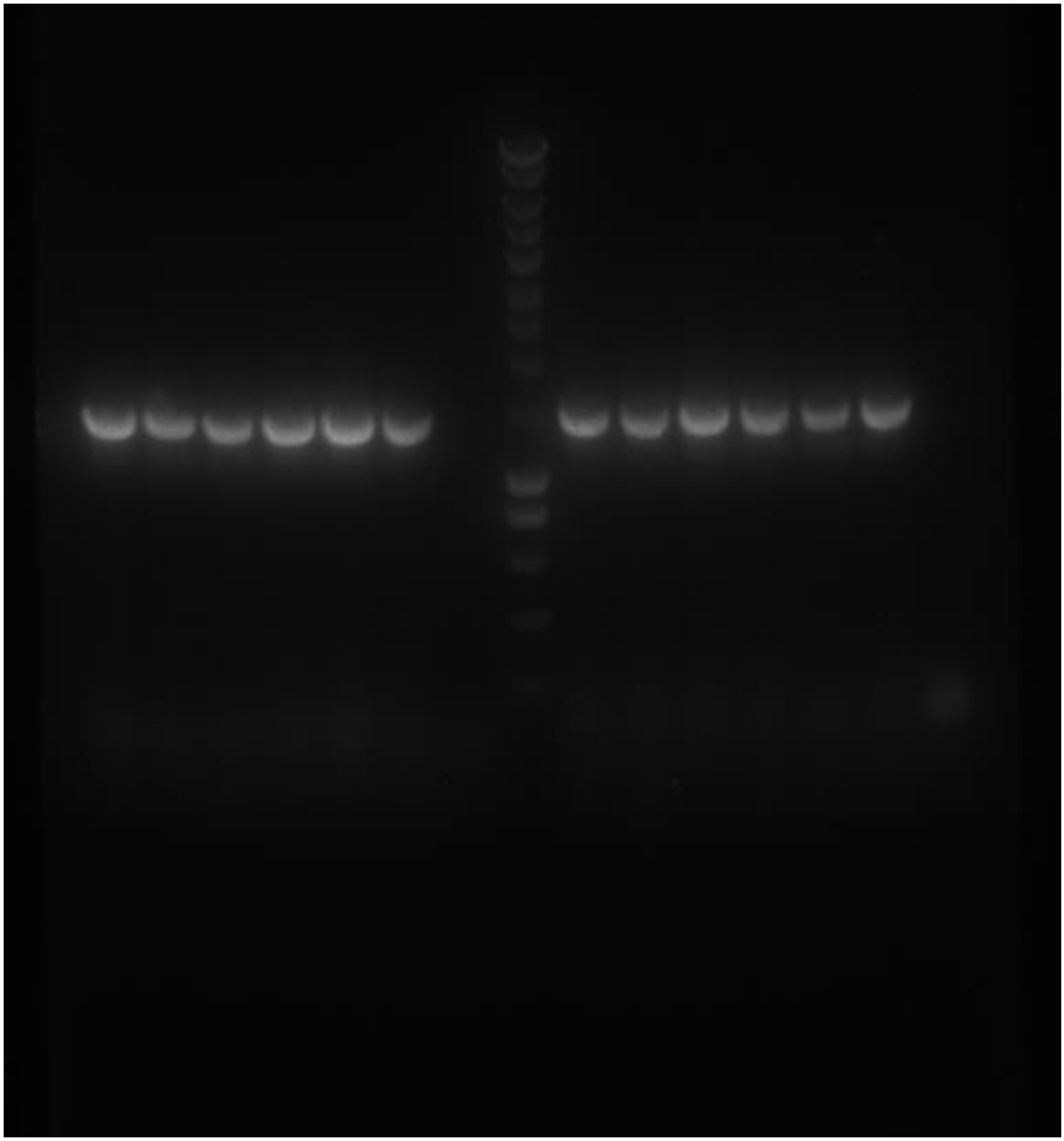
Negative control: primers no colony. Each colony that was put in the PCR, was also streaked on a LB cam plate.

PCR cycling conditions:

1.	5 minutes	94 C	
2.	1 minute	94 C	
3.	1 minute	56 C	
4.	1 min 30 sec	72 C	go back to 2. - 29 x
5.	5 minutes	72 C	
6.	pause	4 C	

PCR result on gel
Loaded samples on 1 % agarose gel for 40 minutes at 100 V. 25 ul sample with 5 ul loading dye. Used 10 ul of this total.
1. GG 50 colony 1
2. GG 50 colony 2
3. GG 50 colony 3
4. GG 50 colony 4
5. GG 50 colony 5
6. GG 50 colony 6
7. GG 50 negative control
8. Smartladder (5 ul)
9. GG 51 colony 1
10. GG 51 colony 2
11. GG 51 colony 3
12. GG 51 colony 4

13. GG 51 colony 5
14. GG 51 colony 6
15. GG 51 negative control



25.08.2014

Transformation:

- CsgB + cc54
  100ul on LB+Kan, 100ul on LB, concentrated on LB+Kan
- CsgB + pUC19
  100ul on LB+Amp, 100ul on LB, concentrated on LB+Amp
- CsgB + milliQ
  100ul on LB+Amp, 100ul on LB+Kan, 100ul on LB

Janna

Minipreps of GG50 and GG51 (2nd)

Used 2 x 1.5 ml and spun down for 4 minutes at 13000 rpm at 20 C. Used 13000 rpm for all steps.
Measured the concentration with nanodrop:
CC GG50 c1: 124.0 ng/µl
CC GG50 c2: 132.9 ng/µl
CC GG51 c1: 109.6 ng/µl
CC GG51 c2: 80.0 ng/µl

Joan
Glycerol stock the cultured streaks from the single cells of the constructs **CC GG50** and **CC GG51** from the 2nd Golden Gate reaction.

26.08.2014
Results of transformation from 25.08.2014: everything is probably infected, because there are a lot of different kind of colonies on all the plates.

Transformation again:

- CsgB + cc54
  100ul on LB+Kan, 100ul on LB, 100ul on LB+Cam
- CsgB + pUC19
  100ul on LB+Amp, 100ul on LB, 100ul on LB+Cam
- CsgB + milliQ
  100ul on LB+Kan, 100ul on LB, 100ul on LB+Cam

27.08.2014
Janna
Send CC GG50 and GG51 for sequencing
Send CC GG50 colony 1 and GG51 colony 1 for sequencing. Used standard forward, standard reverse, CsgB forward and CsgB reverse.
GG50 c1: 4.0 ul DNA, 2.5 ul primer and 3.5 ul water.
GG51 c1: 4.6 ul DNA, 2.5 ul primer and 2.9 ul water.

01.09.2014
Janna
Transformation of CC54 in deltaCsgB (also test)
Made different transformations:
CC54 (2 ul) in dCsgB (30 ul) kanR
pUC19 (0.5 ul) in dCsgB (30 ul) ampR
MilliQ (1 ul) in dCsgB (30 ul)

Used the protocol for home-made competent cells.
Results:
dCsgB CC54

kan	100 ul	growth
amp	100 ul	no growth
kan	200 ul	growth

dCsgB pUC19

kan	100 ul	growth
amp	100 ul	growth
LB	100 ul	growth

dCsgB MQ

LB	100 ul	growth
Kan	100 ul	growth

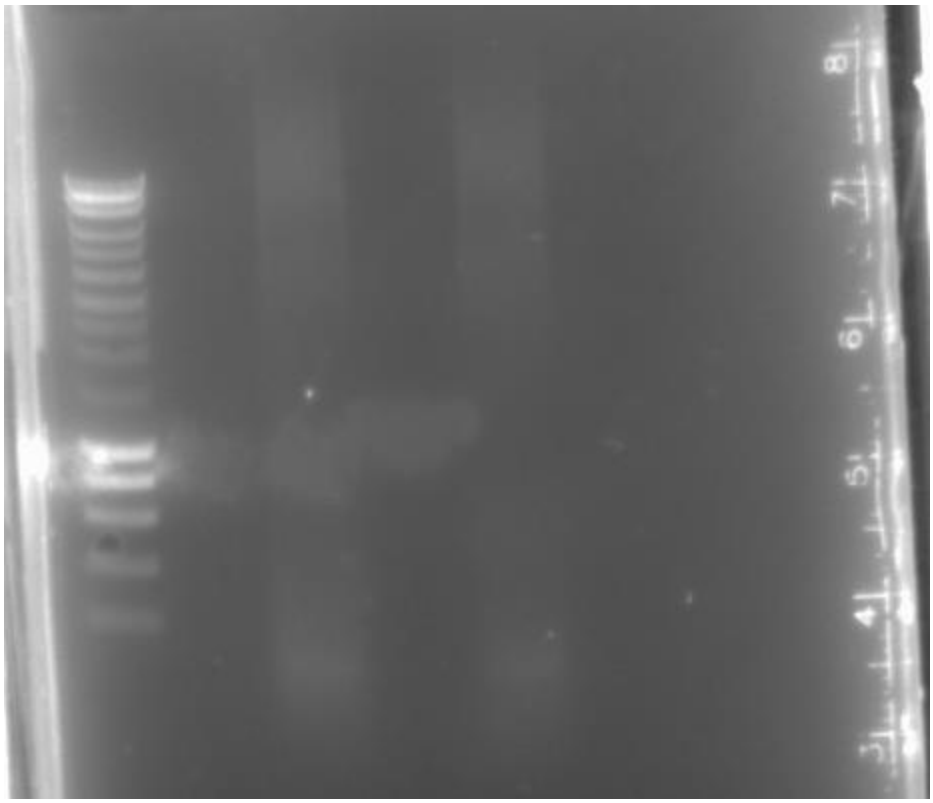
These results were not as expected and from now on we will use electroporation for the dCsgB cells.

03.09.2014
Janna
Miniprep of dCsgB cc54
Spun down 2 x 1.5 ml for 3 minutes at 13000 rpm. Used culture 2 and 5. Used the miniprep protocol. Performed all steps at 13000 rpm 20 C.
Measured concentrations with nanodrop:
Culture 2: 18.1 ng/ul
Culture 5: 25.4 ng/ul

04.09.2014
Janna
Restriction of CC54
Cut 600 ng of CC54 (possibly) of 03.09 with EcoRI-HF and PstI-HF.

	ng/ul	Use	EcoRI	PstI	CutSmart	MQ
Culture 2	18.1	33	1	1	4	1
Culture 5	25.4	24	1	1	4	10

40 ul total volume and added 8 ul of Loading Dye and loaded on 1 % agarose gel for 40 minutes.



Left to right: SmartLadder, culture 2, culture 5
No bands are to be seen, so CC54 is not present in the cells.

09.09.2014
Janna

Restriction of K540000 for the backbone

Used K540000 (105 ng/ul) to get the PSB1C3 backbone. Cut it with EcoRI-HF and SpeI-HF.

EcoRI-HF	0.5 ul
SpeI-HF	0.5 ul
CutSmart	2.5 ul
DNA plasmid	5 ul
MilliQ	16.5 ul
Total	25 ul

Used protocol for restriction of plasmid DNA.

Gelextraction of GG parts

Ran all purified PCR products for the Golden Gate reaction of the CC module on gel to get the templates out of the mix. Used the following purified products:

- Rhamnose promoter
- CsgA
- CsgA HIS
- CsgB
- K540000

Used 1 % agarose gel at 95 V for 40 min. Cut the gel with a razorblade and followed the MinElute DNA extraction protocol. Samples were needed for the Golden Gate of 10.09.

10.09.2014
Joan
Golden Gate reaction of GG50 and GG51

Part	ng/uL	uL added
pSB1C3	27.1	3
csgB	25.7	3.15
pRhamnose	8.3	7
csgA	29.7	2.75
T4 ligation buffer		2
T4 ligase		1
BsaI		1
Total		**19.90**

Part	ng/uL	uL added

pSB1C3	27.1	2.95
csgB	25.7	3.11
pRhamnose	8.3	7
csgA-His	11.6	6.9
T4 ligation buffer		2.45
T4 ligase		1.2
BsaI		1.2
Total		**24.81**

PCR cycle for the Golden Gate assembly

Step	min	Temp (oC)	
1	3	37	
2	4	16	go back to 1. - 49 x
3	5	50	
4	5	80	
5	pause	5	

11.09.2014
Joan
Transform via electroporation into the “delta csgB” strain the constructs:

- CC52
- CC54 culture2
- Negative control (no plasmid)

Run the GG CC50 and CC51 from 10.09 on a gel → no band was observed, no picture taken.

12.09.2014
Joan
Transform via electroporation into the “delta csgB” strain the constructs:

- CC50
- CC51

Grow on Shake Flasks and Colony PCR the CC52 and CC54 transformed into delta csgB on the previous day (3 colonies of each).

MasterEps:
For both CC52 and CC54

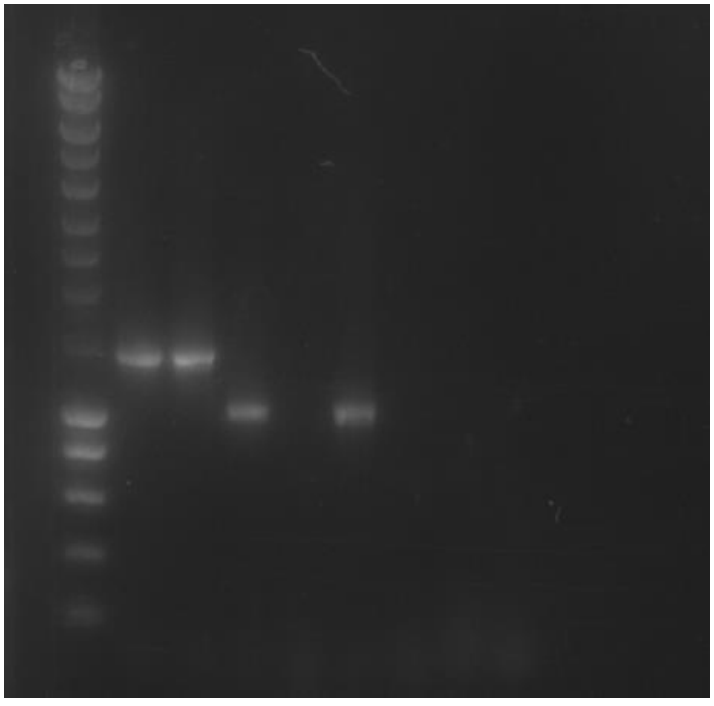
1 ul	VF2 (Standard FW) [20uM]	x 10 =	10 ul
1 ul	VR (Standard RV) [20uM]	x 10 =	10 ul
12.5 ul	MasterMix TAQ 2x	x 10 =	125 ul
10.5 ul	MiliQ H2O	x 10 =	105 ul

Each colony that was put in the PCR, was also streaked on a LB cam plate.

PCR cycling conditions:

1.	5 minutes	94 C	
2.	1 minute	94 C	
3.	1 minute	55 C	
4.	1 min 10 sec	72 C	go back to 2. - 29 x
5.	5 minutes	72 C	
6.	pause	4 C	

Result:



Lanes from left to right:

SmartLadder, CC52 colony 1, CC52 colony 3, CC54 colony 1, CC54 colony 2, CC54 colony 3, negative controls.

Janna
Transformed the GG50 and GG51 into commercial DH5alpha.
Made the following combinations:

- 25 ul DH5alpha with 5 ul GG50 camR
- 25 ul DH5alpha with 5 ul GG51 camR
- 25 ul DH5alpha with 5 ul MilliQ

Used the protocol for transformation of homemade competent cells.

17.09.2014
Janna
Sequencing of GG50 and GG51
Sent GG50 colonies 1, 2, 3 and 4 and GG51 colonies 1, 2, 3, 4, 5 and 6 for sequencing. GG50 colony 4 and GG51 colony 1 appear to have the right construct.

Electroporation of dCsgB with CC54 and CC52
Used dCsgB + CC52 (already made by Joan) and the plasmid CC54 colony 2 (38 ng/ul). Followed the electroporation protocol and made three eps:

- 40 ul cells with 1.5 ul CC54 - 1
- Again 40 ul cells with 1.5 ul CC54 - 2
- 40 ul cells with 1.5 ul MilliQ

The results were as follows:

- cells with MilliQ
  - cam+kan 100 ul growth
- cells with cc54
  - cam+kan 100 ul - 1 no growth
  - cam+kan 300 ul – 1 no growth
  - cam+kan 100 ul – 2 growth
  - cam+kan 300 ul – 2 growth
  - cam 100 ul – 2 much growth
  - kan 100 ul – 1 some growth

The results are not that positive, but do a colony PCR anyway, maybe the construct is in there.

19.09.2014
Janna
Colony PCR of dCsgB CC52 CC54
Did a colony PCR on 5 colonies of the 17.09 plate cam+kan dCsgB CC54 CC52 – 2 – 300 ul. Tested all colonies both for eGFP and CsgBA, using two rows of PCR eps.
Master Eps:
CC 52 used the FW CsgBA and RV CsgBA primers.

2.5 ul	FW[CsgBA]	x 8 =	20 ul
2.5 ul	RV[CsgBA]	x 8 =	20 ul
12.5 ul	MM TAQ 2x	x 8 =	100 ul
7.5 ul	RNase free H2O	x 8 =	60 ul

CC54 used the FW eGFP and RV eGFP primers.

2.5 ul	FW[eGFP]	x 8 =	20 ul

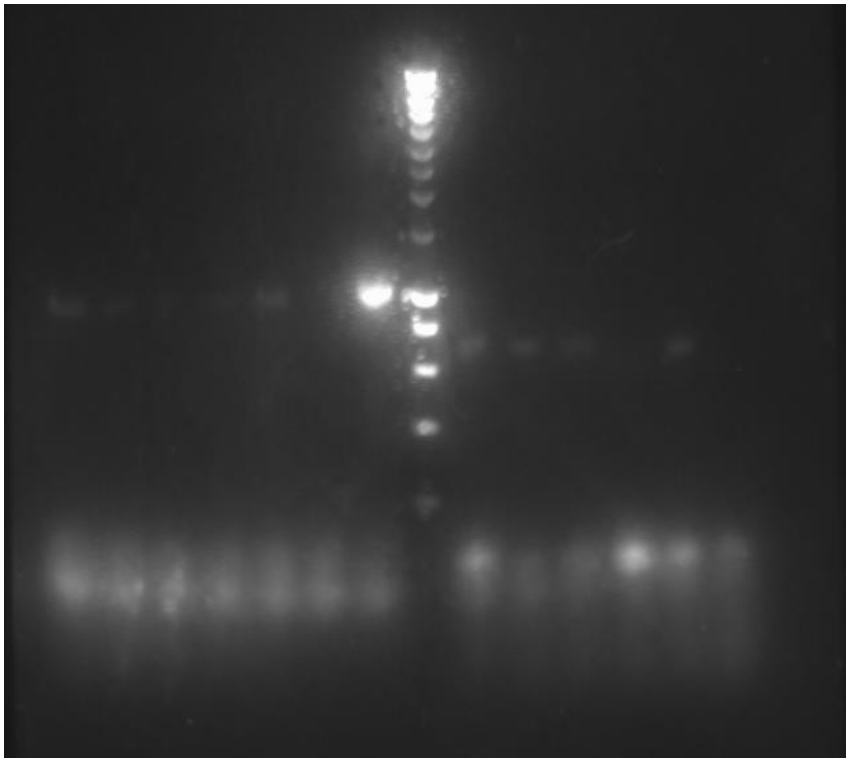
2.5 ul	RV[eGFP]	x 8 =	20 ul
12.5 ul	MM TAQ 2x	x 8 =	100 ul
7.5 ul	RNase free H2O	x 8 =	60 ul

Negative controls: primers no colonies.
Positive control: cc52 plasmid with its primers. Used cc52 c1 7/8 76.8 ng/ul 0.5 ul.

PCR cycling conditions

1.	94 C	5 min	
2.	94 C	1 min	
3.	56 C	1 min	
4.	72 C	1 min 15 sec	Back to 2. x 29
5.	72 C	5 min	
6.	4 C	hold	

Results
Ran on a 1 % agarose gel for 40 min at 100 V.
From left to right:
CC52 colonies 1 to 5, negative, positive, Smartladder, CC54 colonies 1 to 5.



The bands on the gel are not clear enough for a colony PCR. The PCR did work, because the positive control worked. The plasmids are not in the cells.

23.09.2014
Janna
Electroporation of different combinations with dCsgB
Made the following combinations:
1. dCsgB with GG50 camR
2. dCsgB with GG51 camR

3. dCsgB+CC52 with CC54 camR+kanR
4. dCsgB+CC54 with CC52 camR+kanR
5. dCsgB+CC54 with GG50 camR+kanR
6. dCsgB+CC54 with GG51 camR+kanR
7. dCsgB with MilliQ
8. dCsgB+CC54 with MilliQ kanR
9. dCsgB+CC52 with MilliQ camR

I used GG50 colony 4 (97.6 ng/ul), GG51 colony 1 (177.3 ng/ul), CC54 c2 (38 ng/ul) and CC52 c7 (133.3 ng/ul). Used the electroporation protocol.
Resulting plates:

dCsgB	with GG50	kan 100 ul	some growth
		cam conc.	growth
	with GG51	kan 100 ul	some growth
		cam conc.	growth
	with MilliQ	no ant. 100 ul	much growth
		kan 100 ul	some growth
		cam 100 ul	no growth
dCsgB+CC54	with CC52	kan+cam conc.	growth
	with GG50	kan+cam conc.	growth
	with GG51	kan+cam conc.	growth
	with MilliQ	kan+cam 100 ul	no growth
		kan 100 ul	much growth
		cam 100 ul	no growth
dCsgB+CC52	with CC54	kan+cam conc.	some growth
	with MilliQ	kan+cam 100 ul	much growth
		kan 100 ul	no growth
		cam 100 ul	no growth

25.09.2014
Janna

Colony PCR of the dCsgB constructs

Did a colony PCR of the dCsgB cells to test if all the wanted plasmids were present in the cells. Used the following combinations and used the same 6 colonies for both ep rows within each combination:

CC52+CC54
1. primers FW[CsgBA] and RV[CsgBA]; 6 x colony, negative control.
2. primers FW[eGFP] and RV[eGFP]; 6 x colony, negative control, positive control.

CC54+GG50
3. primers FW[Rham] and RV[CsgA GG]; 6 x colony, negative control.
4. primers FW[eGFP] and RV[eGFP]; 6 x colony, negative control, positive control.

CC54+GG51
5. primers FW[Rham] and RV[CsgA:HIS GG]; 6 x colony, negative control.
6. primers FW[eGFP] and RV[eGFP]; 6 x colony, negative control, positive control.

GG50
7. primers FW[Rham] and RV[CsgA GG]; 6 x colony, negative control.

GG51
8. primers FW[Rham] and RV[CsgA:HIS GG]; 6 x colony, negative control.

Master Eps:
ME CC54 (3 x 8 = 24 eps)

2.5 ul	FW[eGFP]	x 24 =	60 ul (5 uM)	3 ul (20 uM)
2.5 ul	RV[eGFP]	x 24 =	60 ul (5 uM)	3 ul (20 uM)
12.5 ul	MasterMix TAQ 2x	x 24 =	300 ul	-
7.5 ul	H2O RNase free	x 24 =	180 ul	294 ul

ME CC52 (1 x 8 = 8 eps)

2.5 ul	FW[CsgBA]	x 8 =	20 ul (5 uM)
2.5 ul	RV[CsgBA]	x 8 =	20 ul (5 uM)
12.5 ul	MasterMix TAQ 2x	x 8 =	100 ul
7.5 ul	H2O RNase free	x 8 =	60 ul

ME GG50 (2 x 8 = 16 eps)

2.5 ul	FW[Rham]	x 16 =	40 ul (5 uM)	2 ul (20 uM)
2.5 ul	RV[CsgA GG]	x 16 =	40 ul (5 uM)	-
12.5 ul	MasterMix TAQ 2x	x 16 =	200 ul	-
7.5 ul	H2O RNase free	x 16 =	120 ul	158 ul

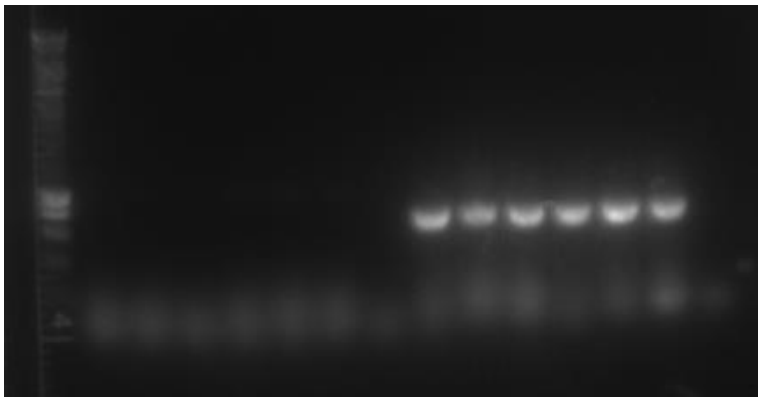
ME GG51 (2 x 8 = 16 eps)

2.5 ul	FW[Rham]	x 16 =	40 ul (5 uM)	2 ul (20 uM)
2.5 ul	RV[CsgA:HIS GG]	x 16 =	40 ul (5 uM)	-
12.5 ul	MasterMix TAQ 2x	x 16 =	200 ul	-
7.5 ul	H2O RNase free	x 16 =	120 ul	158 ul

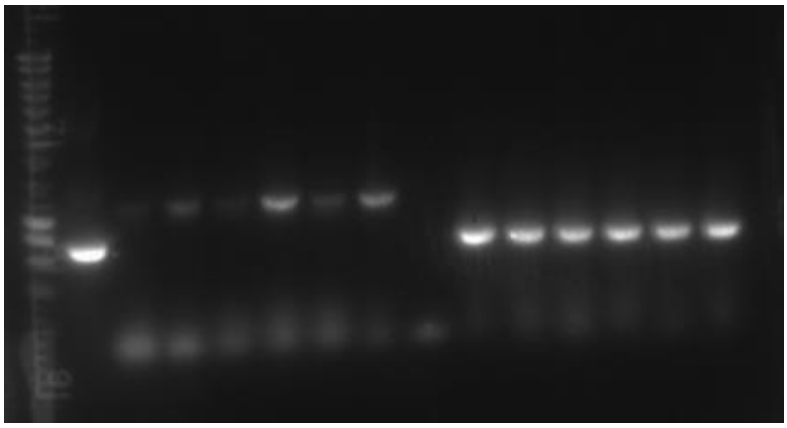
PCR cycling program

1.	94 C	5 min	
2.	94 C	1 min	
3.	62 C	1 min	
4.	72 C	1 min	Back to 2. x 29
5.	72 C	5 min	
6.	4 C	hold	

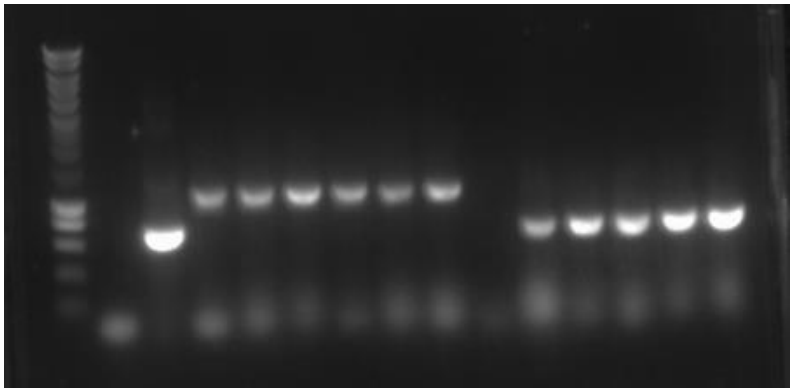
26.09.2014
Loaded the PCR samples of 25.09.2014 on gel.



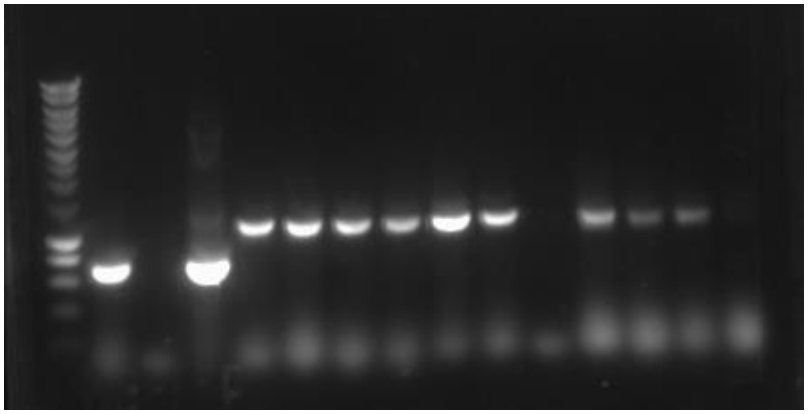
From left to right:
1. SmartLadder
2. to 8. CC52+CC54 tested for CC52
9. to 15. CC52+CC54 tested for CC54 (except positive control)



From left to right:
1. SmartLadder
2. CC52+CC54 tested for CC54 positive control
3. to 9. CC54+GG50 tested for GG50
10. to 15. CC54+GG50 tested for CC54

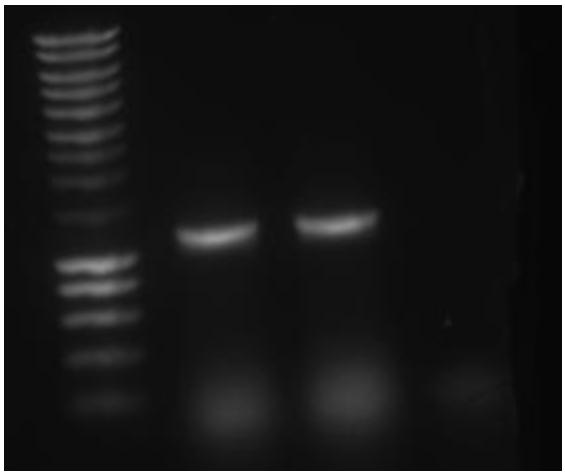


From left to right:
1. SmartLadder
2. and 3. CC54+GG50 tested for CC54 negative and positive control
4. to 10. CC54+GG51 tested for GG51
11. to 15. CC54+GG51 tested for CC54



From left to right:

1. SmartLadder
2. to 4. CC54+GG51 colony 6, negative, positive
5. to 11. GG50
12. to 15. GG51 (first part)



1. SmartLadder
2. GG51 colony 5
3. GG51 colony 6
4. GG51 negative control

All plasmids are present, except for CC52. Probably a case of wrong primers or wrong annealing temperature. As colony 1 came from dCsgB+CC52 added CC54, it should contain both and therefore we worked with this colony.

02.10.2014
Joan

Prepare Curli samples for eGFP assay

Samples to test are “delta csgB” strains containing:

- CC54 + CC50 col 4
- CC54 + CC51 col 1
- CC54 + CC52 col 1
- CC54 alone col3

Aliquots of each culture (OD = 0.55) were transferred into a 96 well-plate; induced with Rhamnose (0.25 or 0.5%) and grown O/N at 37 degrees.

03.10.2014
Joan

eGFP assay - Plate reader experiment

Excitation and Emission wavelengths: 488 and 508nm respectively

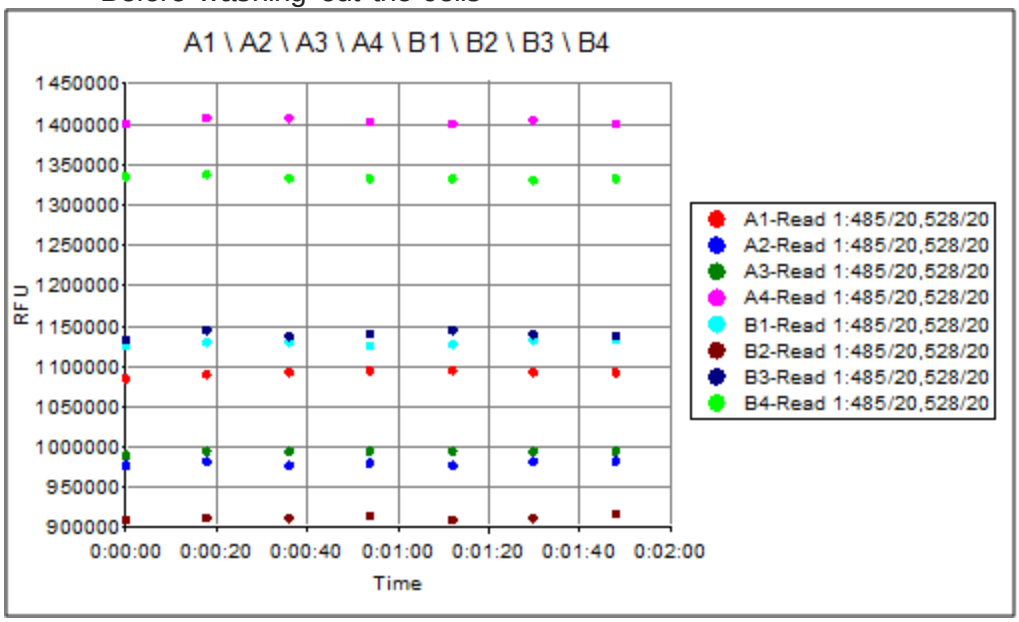
Different readouts were performed:

- Before washing out the cells

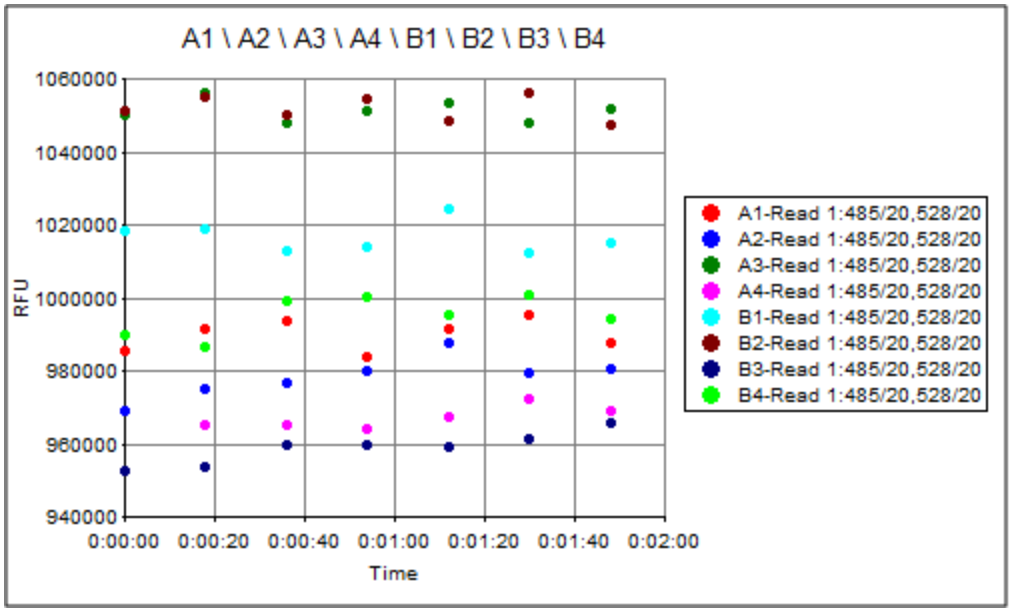
- After washing twice the wells (with a total volume of 300uL LB) and adding 200uL LB
- After washing two more times (4 in total) the wells by pipetting up and down the content of the wells. Add in the end 200uL LB
- After washing again

Results:
CC54 alone shows more fluorescence before washing out the cells.
Once washed, some curli forming cultures start emitting clearly more fluorescence than the CC54 alone (neg control). However, some cultures that should produce curli show less fluorescence than the negative control, indicating that these cultures produced no curli

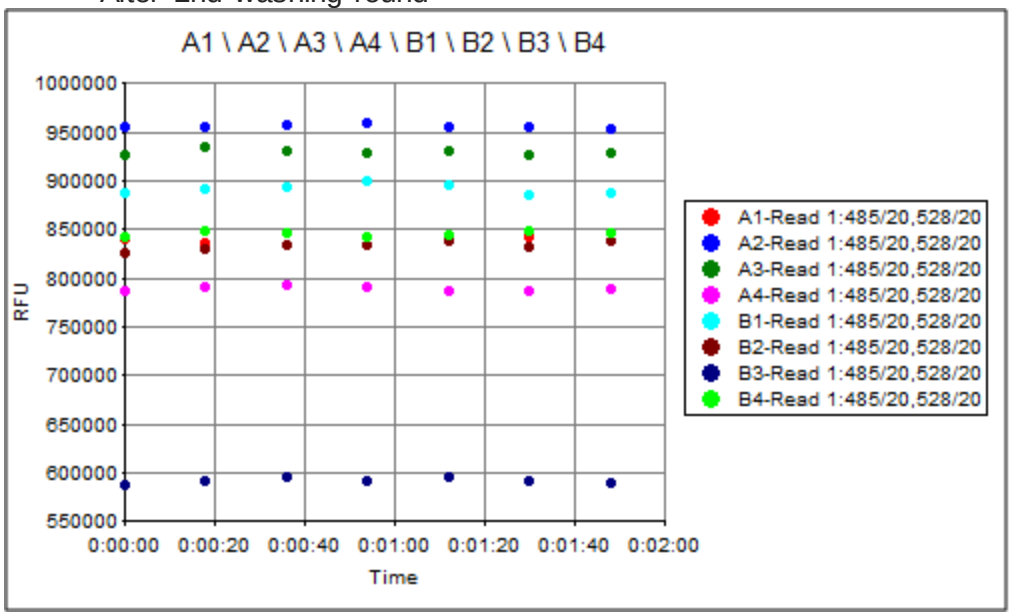
- Before washing out the cells



- After 1st washing round



- After 2nd washing round



- After 3rd washing round