Logic Circuit Lab book

2017/08/12

Transformation of C0040, C0050, C0053, I13404, I6031 into *E. coli* DH5α - ACB

Aim: Bacterial amplification of the genes needed for our constructs

Protocol:

- Resuspension of the plate lyophilized DNA in 10µL of DNAse free water

Biobrick number	Gene	Plate number	Well number
C0040	TetR	2	2P
C0053	P22C2	2	4F
C0050	HKcI	3	24E
I13404	ecfp	3	14L
I6031	eyfp	2	10H

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add 2µL DNA in the cells. Mix gently. (positive control: 2µL of pUC19)
- Incubate 30min on ice.
- Heat shock: 45s at 42°C.
- Let rest on ice for 5min.
- Add 950µL of LB medium and incubate for 1h at 37°C with shaking.
- Centrifuge for 2min at 5000rpm on a bench centrifuge.
- Resuspend in 100µL sterile water.
- Spread on LB+Chloramphenicol plates:
    - 10%: 10µL of the reaction
    - 90%: 90µL of the reaction
- Incubate overnight at 37°C

Results: colonies counted on 08/14

Gene	Biobrick number	10%		90%	
		Code	Number of colonies	Code	Number of colonies
pUC19	Positive control	iOAC1208BP01	0	iOAC1208BP02	0
TetR	C0040	iOAC1208BP03	0	iOAC1208BP04	2
None	Negative control	iOAC1208BP05	0	iOAC1208BP06	0
P22C2	C0053	iOAC1208BP07	4	iOAC1208BP08	21
HKcI	C0050	iOAC1208BP09	0	iOAC1208BP10	8
eyfp	I6031	iOAC1208BP11	2	iOAC1208BP12	16
ecfp	I13404	iOAC1208BP13	0	iOAC1208BP14	15

Conclusion:

The pUC19 positive control did not work because the cells were grown on Chloramphenicol supplemented media and not on Ampicillin. All the other transformations worked despite a low yield. We can move on with the experiments.

2017/08/15

Liquid culture of *E. coli* DH5α containing of C0040, C0050, C0053, I13404, I6031 - ACB

Aim: Liquid culture to prepare a miniprep.

Protocol:

- Pick 3 single colonies for each plasmid and resuspend in 15 mL LB + Chloramphenicol (25 μg/mL)
- Incubate overnight at 37°C with shaking.

Results: We have growth and can perform the miniprep.

2017/08/16

Miniprep of C0040, C0050, C0053, I13404, I6031 - AG

Aim: Extract plasmid DNA to perform PCR and assemble our constructs.

Protocol: We performed the Thermo Scientific GeneJET Plasmid Miniprep Kit Protocol.

Harvest bacteria

- Centrifugation at 8000 rpm for 2min at room temperature.

Resuspend Cells, Lyse and Neutralize

- Add to the pelleted cells:
    - 250 µL of Resuspension Solution and vortex.
    - 250 µL of Lysis Solution and invert the tube 4-6 times.
    - 350 µL of Neutralization Solution and invert the tube 4-6 times.
    - Centrifuge 5 minutes at 13 000 rpm

Bind DNA

- Transfer the supernatant to the Thermo Scienti c GeneJET Spin Column. Centrifuge 1 minute.

Wash the column

- Add 500 µL of Wash Solution and centrifuge for 30-60 s. × 2 times
- Discard the flow-through.
- Centrifuge empty column for 1 minute at 13 000 rpm

Elute purified DNA

- Transfer the column into a new tube.
- Add 50 µL of Elution Buffer to the column and incubate 2 minutes. Centrifuge 2 minutes.
- Collect the flow-through.

Results: Measurement of DNA concentration using a Nanodrop

- Blank: 1µL of Elution Buffer

- Sample: 1µL for each measurement

Tube Number	Content	BBa	C (ng/µL)	A260/A230	A260/A280
iOAC1608DS01	TetR - 1	C0040	52.7	1.92	1.97
iOAC1608DS02	TetR - 2	C0040	63.6	1.89	1.98
iOAC1608DS03	HKcI - 1	C0050	66.7	1.93	1.95
iOAC1608DS04	HKcI - 2	C0050	56	1.96	1.98
iOAC1608DS05	HKcI - 3	C0050	54.4	1.8	1.93
iOAC1608DS06	P22C2 - 1	C0053	71.2	2.29	1.98
iOAC1608DS07	P22C2 - 2	C0053	84.2	1.89	1.93
iOAC1608DS08	P22C2 - 3	C0053	67.4	2.2	2.02
iOAC1608DS09	Ecfp - 1	I13404	52	1.96	1.99
iOAC1608DS10	Ecfp - 2	I13404	72.9	1.65	1.95
iOAC1608DS11	Ecfp - 3	I13404	52.1	1.93	1.98
iOAC1608DS12	Eyfp - 1	16031	29.5	1.97	2.02

iOAC1608DS13	Eyfp - 2	16031	51.6	2.01	2.01
iOAC1608DS14	Eyfp - 3	16031	45.1	1.93	2.03

Conclusion:
DNA was purified properly and we can move forward with PCR and digestion

Restriction digest of C0040, C0050, C0053, I13404, I6031 - ACB

Aim: Verification of the size of the biobricks.

Protocol:

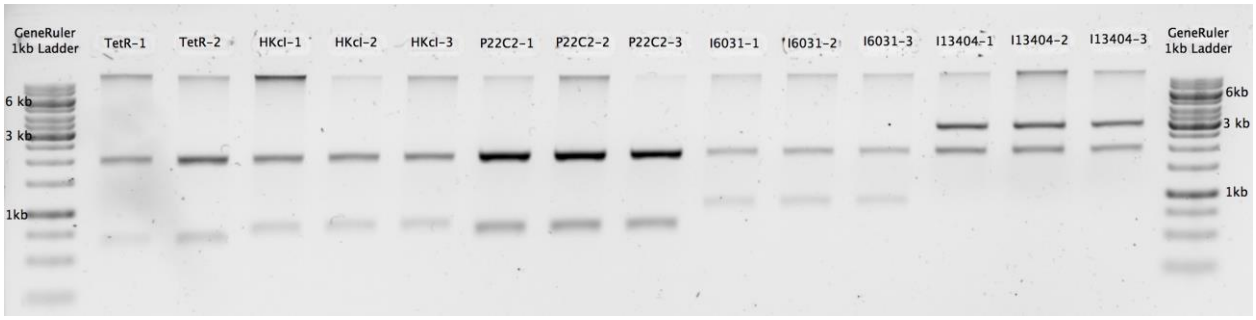
- For each tube mentioned above, prepare the following reaction in 1.5 mL Eppendorfs

	V (µL)
10X Fast Digest Green Buffer	2
DNA	5
Nuclease free water	12
Not I	1

- Incubate 25 min at 37°C

Results: Gel migration 45 min at 100V
Loading :

- Ladder: 2µL GeneRuler 1kb ladder
- Samples: 10µL of the digestion reaction



Expected size:

- Backbone in each well : 2070 bp
- TetR: 685 bp
- HKcI: 769 bp
- P22c2: 712 bp
- I6031: 941 bp
- I13404: 2979 bp

Conclusion:

The plasmids were not fully digested but the biobricks are the expected size.

Transformation of BBa_J04450 & J23100 - OS

Aim: Transformation to extract the backbones for our assembly. J04450 contains backbone pSB4K5 and J23100 contains J61002

Protocol:

- Resuspension of the plate lyophilized DNA in 10µL of DNAse free water

Biobrick number	Gene	Plate number	Well number
J04450	pSB4K5	4	6H
J23100	J61002	4	17D

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add 2µL DNA in the cells. Mix gently. (positive control: 2µL of pUC19)

- Incubate 30min on ice.
- Heat shock: 45s at 42°C.
- Let rest on ice for 5min.
- Add 950µL of LB medium and incubate for 1h at 37°C with shaking.
- Centrifuge for 2min at 5000rpm on a bench centrifuge.
- Resuspend in 100µL sterile water.
- Spread on LB+Kanamycin for pSB4K5 and LB + Ampicillin for J61002 and pUC19 plates:
    - 10%: 10µL of the reaction
    - 90%: 90µL of the reaction
- Incubate overnight at 37°C

Results: Counting of the cells on 08/17

2017/08/17

Resuspension of oligos - ACB

Aim: Make a working solution of our primers

Protocol:

- Centrifuge the tubes for 2 min at 12000rpm.
- Resuspend the DNA in the volume needed for a final concentration of 100 pmol/µL.

Tube	Volume added (µL)
O.17.031 - AC01	221
O.17.032 - AC02	234
O.17.033 - AC03	274
O.17.034 - AC04	255
O.17.035 - AC05	186
O.17.036 - AC06	337
O.17.037 - AC07	285
O.17.038 - AC08	223
O.17.039 - AC09	275
O.17.040 - AC10	218
O.17.041 - AC11	263
O.17.042 - AC12	272
O.17.043 - AC13	207
O.17.044 - AC14	238

O.17.045 - AC15	290
O.17.046 - AC16	336
O.17.047 - AC17	254
O.17.048 - AC18	247
O.17.049 - AC19	306
O.17.050 - AC20	250
O.17.051 - AC21	243
O.17.052 - AC22	282
O.17.053 - AC23	226
O.17.054 - AC24	235
O.17.055 - AC25	248
O.17.056 - AC26	229
O.17.057 - AC27	230
O.17.058 - AC28	229

- Vortex the tubes
- Dilute 10 times (for a final concentration of 10 pmol/µL): 10µL of DNA with 90µL of water.

PCR of C0040, C0050, C0053, I13404, I6031 - ACB

Aim: Amplify the fragments in order to perform Golden Gate assembly of our fragments

Protocol:

- On ice, Set the following reactions in 0.2mL PCR tubes using 2X DreamTaq Master Mix

Tube number	**eyfp**	**1**	**P_{lacI}**	**2**	**ecfp**	**3**	**Term-P_{bad}**	**4**	**Term**	**5**
2X Master Mix		25		25		25		25		25
Forward primer	AC07	2,5	AC11	2,5	AC03	2,5	AC15	2,5	AC19	2,5
Reverse primer	AC08	2,5	AC12	2,5	AC04	2,5	AC16	2,5	AC20	2,5
DNA template	eyfp-1	3,39	eyfp-1	3,39	ecfp-1	1,92	ecfp-1	1,92	ecfp-1	1,92
Water		16,61		16,61		18,08		18,08		18,08
Total		**50,00**		**50,00**		**50,00**		**50,00**		**50,00**
T m	**51°C**		**47,9°C**		**49,9°C**		**45,3°C**		**46,4°C**	

Tube number	**TetR**	**6**	**HKcl**	**7**	**P22C2-pAC004**	**8**	**P22C2-pAC003**	**9**
2X Master Mix		**25**		**25**		**25**		**25**
Forward primer	**AC21**	**2,5**	**AC17**	**2,5**	**AC13**	**2,5**	**AC23**	**2,5**
Reverse primer	**AC22**	**2,5**	**AC18**	**2,5**	**AC14**	**2,5**	**AC24**	**2,5**

DNA template	**TetR-1**	**1,90**	**HKcl-1**	**1,50**	**P22C2-2**	**1,19**	**P22C2_2**	**1,19**
Water		**18,10**		**18,50**		**18,81**		**18,81**
Total		**50,00**		**50,00**		**50,00**		**50,00**
T m	**48°C**		**47,9°C**		**51,6 °C**		**51,6 °C**	

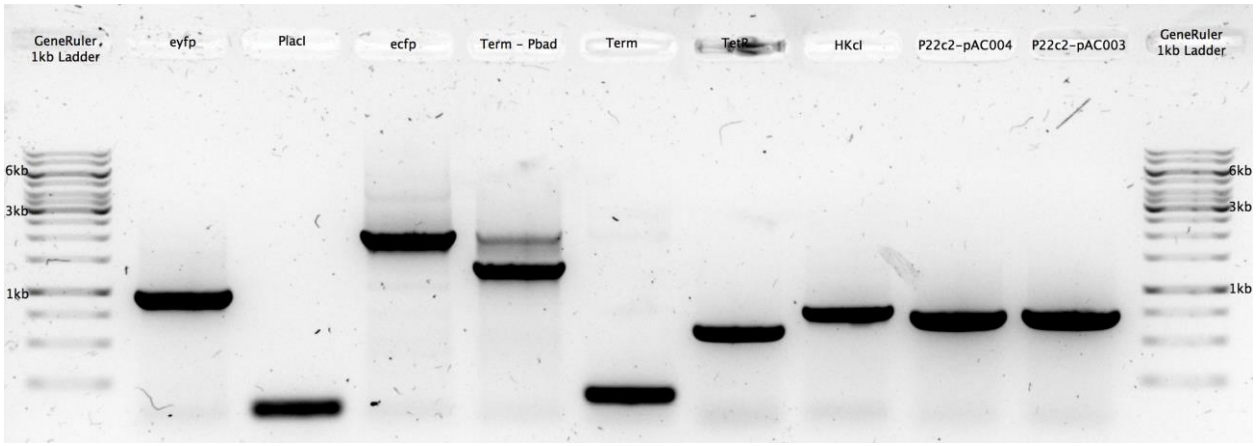
- Put in the PCR machine and run the following program

Initial denaturation	95°C	3min
30 cycles	95°C	30s
	Tm	30s
	72°C	2min
final extension	72°C	5min
hold	12°C	∞

Results: 1% agarose gel migration at 100V for 20min

Loading:

- Ladder: 2µL of 100bp plus DNA Ladder
- Sample: 5µL of sample



	Theoretical size (bp)	Observed size (bp)
eyfp	977	~1000
PlacI	117	~100
ecfp	2128	~2000
Term - Pbad	1416	~1500
Term	184	~200
TetR	621	~600
HKcl	801	~750
P22c2-PAC004	744	~750
P22c2-pAC003	748	~750

Conclusion: The PCR worked and we obtained the desired products.

Liquid culture of pSB4K5 & J6031 - OS

Aim: Miniprep

Protocol: Pick a colony and grow in LB + Kanamycin or LB + Ampicillin overnight at 37°C.
2017/08/18

Miniprep of pSB4K5 and J6031 - OS

Aim: Extract plasmid DNA to perform PCR and assemble our constructs.

Protocol: We performed the Thermo Scientific GeneJET Plasmid Miniprep Kit Protocol.
Harvest bacteria

- Centrifugation at 8000 rpm for 2min at room temperature.

Resuspend Cells, Lyse and Neutralize

- Add to the pelleted cells:
    - 250 µL of Resuspension Solution and vortex.
    - 250 µL of Lysis Solution and invert the tube 4-6 times.
    - 350 µL of Neutralization Solution and invert the tube 4-6 times.
    - Centrifuge 5 minutes at 13 000 rpm

Bind DNA

- Transfer the supernatant to the Thermo Scienti c GeneJET Spin Column. Centrifuge 1 minute.

Wash the column

- Add 500 µL of Wash Solution and centrifuge for 30-60 s. × 2 times
- Discard the flow-through.
- Centrifuge empty column for 1 minute at 13 000 rpm

Elute purified DNA

- Transfer the column into a new tube.
- Add 50 µL of Elution Buffer to the column and incubate 2 minutes. Centrifuge 2 minutes.
- Collect the flow-through.

Results: Measurement of DNA concentration using a Nanodrop

- Blank: 1µL of Elution Buffer

- Sample: 1µL for each measurement

Tube Number	Content	BBa	C (ng/µL)	A260/A230	A260/A280
	pSB4K5-A		93.8		
	pSB4K5-B		148.7		
	pSB4K5-C		123.4		
	J61002-A		231.5		
	J61002-B		290.8		
	J61002-C		230.3		

Conclusion:
DNA was purified properly and we can move forward with PCR.

PCR of pSB4K5, J6031 & RiboJ - ACB

Aim: Amplify the fragment to proceed with Golden Gate Assembly of my plasmids.

Protocol:

- Dilute the template DNA 100 times.
- On ice, Set the following reactions in 0.2mL PCR tubes using 2X DreamTaq Master Mix

Tube number	**J61002**	**1**	**pSB4K5**	**2**	**RiboJ**	**3**
2X Master		**25**		**25**		**25**

Mix						
Forward primer	**AC25**	**2,5**	**AC17**	**2,5**	**AC13**	**2,5**
Reverse primer	**AC26**	**2,5**	**AC18**	**2,5**	**AC14**	**2,5**
DNA template	**J61002**	**0.83**	**pSB4K5**	**0.81**	**RiboJ**	**1**
Water		**19.57**		**19.19**		**1919**
Total		**50,00**		**50,00**		**50,00**
T m	**49°C**		**56.9°C**		**46.8°C**	

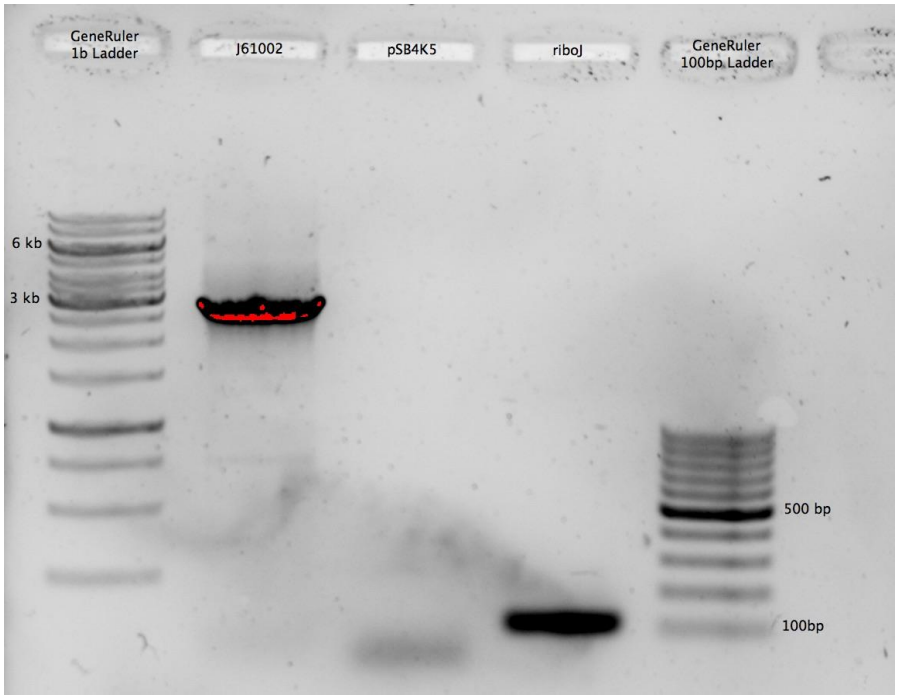
- Put in the PCR machine and run the following program

		Tube 1&2	**Tube 3**
Initial denaturation	95°C	3min	3min
30 cycles	95°C	30s	30s
	Tm	30s	30s
	72°C	3min	1min
final extension	72°C	5min	5min
hold	12°C	∞	∞

Results: 1% agarose gel migration at 100V for 20min

Loading:

- Ladder: 2µL of 100bp plus DNA Ladder



- Sample: 5µL of sample

Results:

J61002 and the RiboJ were properly amplified but pSB4K5 did not work.

2017/08/19

PCR of pSB4K5, pJFR1- ACB

Aim: Amplify the fragment to proceed with Golden Gate Assembly of my plasmids.

Protocol:

- Dilute the template DNA 100 times.
- On ice, Set the following reactions in 0.2mL PCR tubes using 2X DreamTaq Master Mix

Tube number	**pJFR1**	**1**	**pSB4K5**	**2**
2X Master Mix		**25**		**25**
Forward primer	**AC05**	**2.5**	**AC01**	**2,5**
Reverse primer	**AC06**	**2.5**	**AC02**	**2,5**
DNA template	**J61002**	**0.55**	**pSB4K5**	**0.81**
Water		**19.45**		**19.19**
Total		**50,00**		**50,00**
T m	**52.2° C**		**56.9° C**	

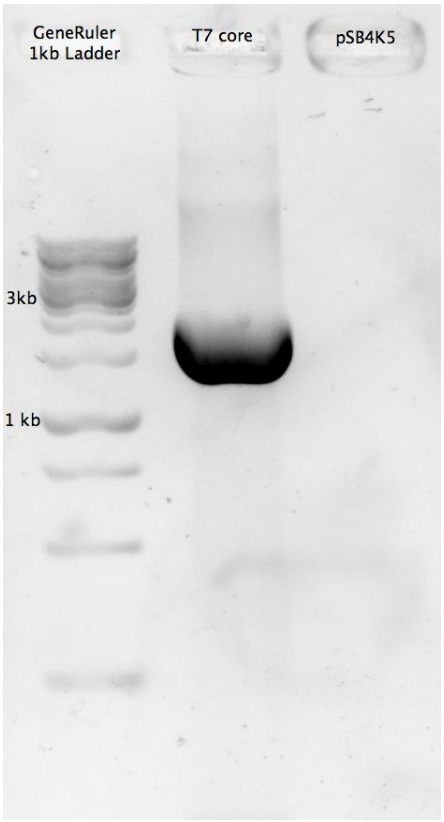
- Put in the PCR machine and run the following program

		Tube 1&2
Initial denaturation	95°C	3min
30 cycles	95°C	30s
	Tm	30s
	72°C	3min 30s
final extension	72°C	5min
hold	12°C	∞

Results: 1% agarose gel migration at 100V for 20min

Loading:

- Ladder: 2µL of 1kb DNA Ladder



- Sample: 5µL of sample

Results:

T7 core was properly amplified but pSB4K5 did not work. It is probably due to a problem in the design of the primers so I will order new ones.

DpnI restriction digest of the previous PCR apmlifications and PCR purifications - ACB

Aim: Make sure the Template DNA in the PCR is degrated.

Protocol:

- For each PCR reaction that worked, mix the following reaction :

	V(µL)
Buffer 10X	2
PCR reaction	5
DpnI	1
Water	12
Total Volume	20

- Incubate 20 min at 37°C
- Proceed with the PCR purification (QIAquick PCR Purification Kit)
    - Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
    - Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.
    - To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
    - Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.
    - Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
    - Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
    - Centrifuge for 1 min at 17,900 x g.

Results: Measurement of the concentration on the NanoDrop:

- Blank: 1µL of DNA free water
- Sample: 1µL

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230
iOC1908DS01	eyfp	53.6	1.62	0.68
iOC1908DS02	PlacI	21.6	1.65	0.6

iOC1908DS03	ecfp	15.3	1.78	1.03
iOC1908DS04	Pbad	10.4	1.83	1.39
iOC1908DS05	Term	7.9	1.76	1.15
iOC1908DS06	TetR	14.3	1.8	1.01
iOC1908DS07	HKcI	13.4	1.81	1.25
iOC1908DS08	P22c2-4	13.9	1.83	1.16
iOC1908DS09	P22c2-3	14.5	1.8	1.35
iOC1908DS10	J61002	11.3	1.76	1.15
iOC1908DS11	RiboJ	4.7	1.84	0.79

2017/08/21

Resuspension of oligos - ACB

Aim: Make a working solution of our primers

Protocol:

- Centrifuge the tubes for 2 min at 12000rpm.
- Resuspend the DNA in the volume needed for a final concentration of 100 pmol/µL.

Tube	Volume added (µL)
O.17.081 - AC29	347
O.17.082 - AC30	184
O.17.083 - AC31	780
O.17.084 - AC32	921
O.17.085 - AC33	853

- Vortex the tubes
- Dilute 10 times (for a final concentration of 10 pmol/µL): 10µL of DNA with 90µL of water.

Golden Gate Assembly of J61002 and RiboJ - ACB

Aim: Assembly of the promoter expression plasmid.

Protocol:

- Set up the following reaction in a 0.2mL PCR tube

Part	**Tube number**	**size (bp)**	**Ratio**	**n (pmol)**	**c (ng/µL)**	**m (ng)**	**V (µL)**

J61002		2948	1	0,02	11,3	36,436232	3,22
RiboJ		75	10	0,2	4,7	9,276758	1,97
T4 ligase							1,00
BsaI							1,00
10X T4 buffer							2,00
10X BsaI Buffer							2,00
Water							8,80
pAC001							**20,00**

- Incubate in a PCR machine with the following program

  Repeat steps 1 & 2 80 times

1) 37°C for 5 min
2) 16°C for 1 min
3) 37°C for 10 min

Results: Will be seen in the following Transformation results.

2017/08/22

Transformation of the Golden Gate assembly product - ACB

Aim: Amplify the product of the Golden Gate assembly

Protocol:

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add the following amount of DNA in each tube

Tube	V DNA (µL)
pAC001	4µL of Golden Gate reaction
J61002	2 µL of J61002 miniprep
Negative control	No DNA

- Incubate 30min on ice.
- Heat shock: 45s at 42°C.
- Let rest on ice for 5min.
- Add 950µL of LB medium and incubate for 1h at 37°C with shaking.
- Centrifuge for 2min at 5000rpm on a bench centrifuge.
- Resuspend in 100µL sterile water.
- Spread on LB + Ampicillin plates:
    - 10%: 10µL of the reaction
    - 90%: 90µL of the reaction
- Incubate overnight at 37°C

Results:

	10%	90%

Content	Tube number	Number of colonies	Tube number	Number of colonies
pAC001	iOAC2308PD01	4	iOAC2308PD02	7
J61002	iOAC2308PD03	285	iOAC2308PD04	~1000
Nothing	iOAC2308PD05	0	iOAC2308PD06	0

2017/08/23

PCR purification of T7 core PCR - ACB

Aim: Get the DNA ready for Golden Gate Assembly

Protocol:

Proceed with the PCR purification (QIAquick PCR Purification Kit)

- Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
- Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.
- To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.
- Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Results

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230

iOAC2308DS02	T7 core	141.9	1.89	2.24

Resuspend the tube i.g.011 - G-block P6- ACB

Aim: Prepare the DNA to amplify them

Protocol:

- Centrifuge 3000xg for 1min.
- Add 25µL of DNAse free water.
- Vortex quickly.
- Incubate at 50°C for 20 min.
- Vortex quickly & centrifuge quickly.
- Dilute 1µL of this reaction in 9 µL of DNAse free water: tube iOAC2308DS01.

PCR of i.g.17.011 - G-block P6 & P12- ACB

Aim: Prepare the DNA for Assembly.

Protocol:

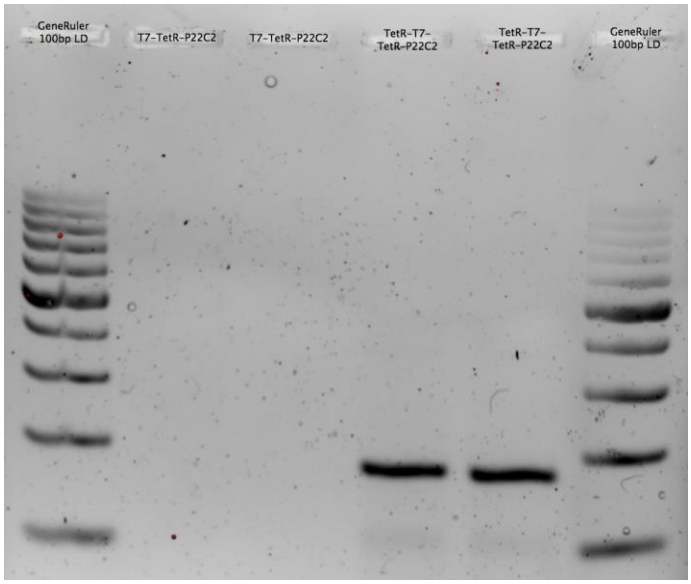
- Set the following reactions in 0.2 mL PCR tubes

		1	**2**		**3**	**4**
Q5 2X Master Mix		**25**	**25**		**25**	**25**
Forward primer	**AC29**	**2.5**	**2.5**	**AC31**	**2.5**	**2.5**
Reverse primer	**AC30**	**2.5**	**2.5**	**AC30**	**2.5**	**2.5**

DNA template		**1**	**1**		**1**	**1**
Water		**19**	**19**		**19**	**19**
Total			**50,00**		**50,00**	
T m	**63°C**			**66°C**		

- Run the following program in a PCR machine:

Initial denaturation	98°C	30s
30 cycles	98°C	10s
	Tm	15s
	72°C	15s
final extension	72°C	2min
hold	12°C	∞



Results: 2% agarose gel. Migration for 40min at 100V.
Conclusion: The PCR worked for the extension of the promoter but the simple amplification did not.

Liquid culture of DH5 α + pAC001 - ACB

Aim: Liquid culture for miniprep

Protocol:

- Pick 3 single colonies and resuspend in 10 mL LB+Amp
- Grow overnigh at 37°Ct under shaking conditions

2017/08/24

Miniprep of pAC001 - ACB

Aim: Extract plasmid DNA to perform PCR and assemble our constructs.

Protocol: We performed the Thermo Scientific GeneJET Plasmid Miniprep Kit Protocol.

Harvest bacteria

- Centrifugation at 8000 rpm for 2min at room temperature.

Resuspend Cells, Lyse and Neutralize

- Add to the pelleted cells:
    - 250 µL of Resuspension Solution and vortex.
    - 250 µL of Lysis Solution and invert the tube 4-6 times.
    - 350 µL of Neutralization Solution and invert the tube 4-6 times.
    - Centrifuge 5 minutes at 13 000 rpm

Bind DNA

- Transfer the supernatant to the Thermo Scienti c GeneJET Spin Column. Centrifuge 1 minute.

Wash the column

- Add 500 µL of Wash Solution and centrifuge for 30-60 s. × 2 times
- Discard the flow-through.
- Centrifuge empty column for 1 minute at 13 000 rpm

Elute purified DNA

- Transfer the column into a new tube.
- Add 50 µL of Elution Buffer to the column and incubate 2 minutes. Centrifuge 2 minutes.
- Collect the flow-through.

Results: Measurement of DNA concentration using a Nanodrop

- Blank: 1µL of Elution Buffer
- Sample: 1µL for each measurement

Tube Number	Content	Strain	C (ng/µL)	A260/A230	A260/A280
iOAC2408DS01	pAC001	A	49.6	1.99	1.73
iOAC2408DS02	pAC001	B	92.4	1.93	1.69
iOAC2408DS03	pAC001	C	101.3	2.01	1.86

Resuspension of G-Block P3, P4 - ACB

Aim: Prepare the DNA to amplify them

Protocol:

- Centrifuge 3000xg for 1min.
- Add 25µL of DNAse free water.
- Vortex quickly.
- Incubate at 50°C for 20 min.
- Vortex quickly & centrifuge quickly.
- Dilute 1µL of this reaction in 9 µL of DNAse free water.

PCR of P3, P4, P6 P9, P10 - ACB

Aim: Amplify the fragments in order to perform Golden Gate assembly of our fragments

Protocol:

- On ice, Set the following reactions in 0.2mL PCR tubes using 2X DreamTaq Master Mix

Tube number	**P6**	**1**	**P3**	**2**	**P9**	**3**	**P4**	**4**	**P10**	**5**
2X Master Mix		25		25		25		25		25
Forward primer	AC29	2,5	AC29	2,5	AC33	2,5	AC29	2,5	AC33	2,5
Reverse primer	AC30	2,5	AC30	2,5	AC30	2,5	AC30	2,5	AC30	2,5
DNA template	P6	1	P3	1	P3	1	P4	1	P4	1
Water		19		19		19		19		19
Total		**50,00**		**50,00**		**50,00**		**50,00**		**50,00**
T m	**63°C**		**63°C**		**62°C**		**63°C**		**62°C**	

- Put in the PCR machine and run the following program

Initial denaturation	98°C	30s
30 cycles	98°C	5s
	Tm	10s
	72°C	15S
final extension	72°C	2min

hold	12°C	∞

Results: 1% agarose gel migration at 100V for 20min

Loading:

- Ladder: 2µL of 100bp plus DNA Ladder
- Sample: 5µL of sample

Conclusion: The PCR worked and we obtained the desired products.

2017/08/25

PCR purification of P3, P4, P6, P9, P10 - ACB

Aim: Get the DNA ready for Golden Gate Assembly

Protocol:

Proceed with the PCR purification (QIAquick PCR Purification Kit)

- Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
- Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.
- To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.
- Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Results

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230
iOAC2508DS01	P6 - PCR	110.7	1.73	1.03
iOAC2508DS02	P3 - PCR	97.2	1.88	2.16
iOAC2508DS03	P9 - PCR	116.2	1.86	2.04
iOAC2508DS04	P4 - PCR	80.8	1.73	1.74
iOAC2508DS0	P10 - PCR	170.4	1.71	1.01

5				

Double-digest of the promoters & J61002 - ACB

Aim: Digest for ligation of the promoters in J61002

Protocol:

- Set the reaction in 1.5mL Eppendorf tubes:

	J61002-A	J61002-B	J61002-C	P6	P3	P9	P4	P10
10x Cutsmart	5	5	5	5	5	5	5	5
EcoRI	1	1	1	1	1	1	1	1
SpeI	1	1	1	1	1	1	1	1
Water	23	33	33	33	33	33	33	33
DNA	20	10	10	10	10	10	10	10
Total	50	50	50	50	50	50	50	50

- Incubate 1h at 37°C.
- Heat Inactivation: 65°C for 20min, 80°C for 20min.

PCR purification of the promoter PCR - ACB

Aim: Get the DNA ready for Assembly

Protocol:

Proceed with the PCR purification (QIAquick PCR Purification Kit)

- Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
- Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.

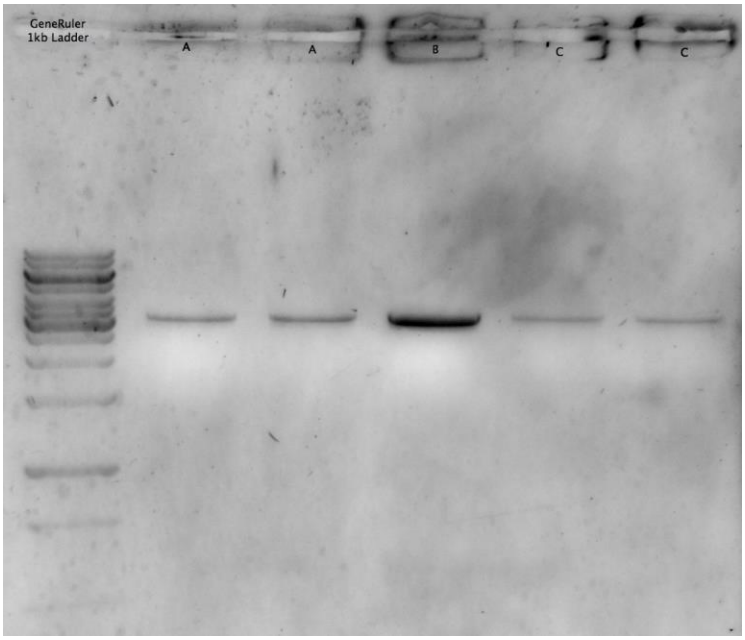
- To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.
- Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Results

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230
iOAC2508DS06	P6 - digest	11.6	1.90	1.63
iOAC2508DS07	P3 - digest	20.6	1.92	1.35
iOAC2508DS08	P9 - digest	11.6	1.93	1.3
iOAC2508DS09	P4 - digest	11.1	2.02	2.20
iOAC2508DS10	P10 - digest	11.0	2.08	2.30

Gel purification of J61002 - ACB

Aim: Get the DNA ready for Assembly



Protocol:

- Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene.
- Place a QIAquick spin column in a provided 2 ml collection tube.
- To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60 s.
- Discard flow-through. Place the QIAquick column back into the same tube.
- To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 30–60 s.
- Discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min.

- Place QIAquick column in a clean 1.5 ml microcentrifuge tube.
- To elute DNA, add 50 µl water to the center of the QIAquick membrane and centrifuge the column for 1 min.

Results:

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230

iOAC2508DS11	J61002-A	3.0	2.69	0.03
iOAC2508DS12	J61002-B	26.8	1.70	0.09
iOAC2508DS13	J61002-C	21.8	1.67	0.17

Ligation of J61002 with P3, P4, P6, P9, P10 - ACB

Aim: Assemble of the reporter plasmids with the promoters.

Protocol:

- Set the following reaction in 0.2mL PCr strips:

Part	**Control**	**P3**	**P4**	**P6**	**P9**	**P10**
pAC001	2,79	2,79	2,79	2,79	2,79	2,79
Insert	0,00	4,86	4,01	3,63	4,86	5,58
T4 ligase	1,00	1,00	1,00	1,00	1,00	1,00
10X T4 buffer	2,00	2,00	2,00	2,00	2,00	2,00
Water	14,21	9,35	10,2	10,58	9,35	8,63
Final product	**20**	**20**	**20**	**20**	**20**	**20**

- Incubate overnight at 16°C
- Heat inactivation 20 min at 80°C

2017/08/26

PCR of P2, P5, P8, P11 - ACB

Aim: Amplify the fragments in order to perform Golden Gate assembly of our fragments

Protocol:

- On ice, Set the following reactions in 0.2mL PCR tubes using 2X DreamTaq Master Mix

Tube number	**P2**	**1**	**P8**	**2**	**P5**	**3**	**P11**	**4**
2X Master Mix		25		25		25		25
Forward primer	AC29	2,5	AC32	2,5	AC29	2,5	AC31	2,5
Reverse primer	AC30	2,5	AC30	2,5	AC30	2,5	AC30	2,5
DNA template	P2	1	P2	1	P5	1	P5	1
Water		19		19		19		19
Total		**50,00**		**50,00**		**50,00**		**50,00**
T m	**63°C**		**65°C**		**63°C**		**66°C**	

- Put in the PCR machine and run the following program

Initial	98°C	30s

denaturation		
30 cycles	98°C	5s
	Tm	15s
	72°C	20s
final extension	72°C	2min
hold	12°C	∞

Results: 1% agarose gel migration at 100V for 20min

Loading:

- Ladder: 2µL of 100bp plus DNA Ladder
- Sample: 5µL of sample

Conclusion: The PCR worked and we obtained the desired products.

PCR purification of P2, P5, P8, P11, P12 - ACB

Aim: Get the DNA ready for Assembly

Protocol:

Proceed with the PCR purification (QIAquick PCR Purification Kit)

- Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
- Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.
- To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.
- Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.

- Centrifuge for 1 min at 17,900 x g.

Results

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230
iOAC2608DS01	P2-PCR	91.4	1.83	2.07
iOAC2608DS02	P8-PCR	78.1	1.88	2.26
iOAC2608DS03	P5-PCR	123.8	1.73	1.10
iOAC2608DS04	P11-PCR	65.0	1.83	1.87
iOAC2608DS05	P12-PCR	68.4	1.84	1.81

Digestion of P2, P5, P8, P11, P12 - ACB

Aim: Digest for ligation of the promoters in J61002

Protocol:

- Set the reaction in 1.5mL Eppendorf tubes:

	P2	P5	P8	P11	P12
10x Cutsmart	5	5	5	5	5
EcoRI	1	1	1	1	1
SpeI	1	1	1	1	1

Water	33	33	33	33	33
DNA	10	10	10	10	10
Total	50	50	50	50	50

- Incubate 1h at 37°C.
- Heat Inactivation: 65°C for 20min, 80°C for 20min.

PCR purification of the digested P2, P5, P8, P11, P12 - ACB

Aim: Get the DNA ready for Assembly

Protocol:

Proceed with the PCR purification (QIAquick PCR Purification Kit)

- Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
- Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.
- To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.
- Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Results

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230
iOAC2608DS06	P2-digest	12.5	1.97	1.95
iOAC2608DS07	P5-digest	11.9	2.02	1.93

iOAC2608DS08	P8-digest	13.4	1.99	1.559
iOAC2608DS09	P11-digest	9.3	1.90	1.54
iOAC2608DS10	P12-digest	8.2	2.12	1.35

Ligation of J61002 with P2, P5, P8, P11, P12 - ACB

Aim: Assemble of the reporter plasmids with the promoters.

Protocol:

- Set the following reaction in 0.2mL PCr strips:

Part	**Control**	**P2**	**P5**	**P8**	**P11**	**P12**
pAC001	2,79	2,79	2,79	2,79	2,79	2,79
Insert	0,00	3,17	3,33	3,73	6,44	7,9
T4 ligase	1,00	1,00	1,00	1,00	1,00	1,00
10X T4 buffer	2,00	2,00	2,00	2,00	2,00	2,00
Water	14,21	11,04	10,88	10,48	7,77	6,31
Final product	**20**	**20**	**20**	**20**	**20**	**20**

- Incubate overnight at 16°C
- Heat inactivation 20 min at 80°C

Transformation of J61002-P3, J61002-P4, J61002-P6, J61002-P9, J61002-P10 - ACB

Aim: Verify the validity of the ligation reaction.

Protocol:

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add the following amount of DNA in each tube

Tube	V DNA (µL)
Reactions	5µL of ligation reaction
Negative control	No DNA

- Incubate 30min on ice.
- Heat shock: 45s at 42°C.
- Let rest on ice for 5min.
- Add 950µL of LB medium and incubate for 1h at 37°C with shaking.
- Centrifuge for 2min at 5000rpm on a bench centrifuge.
- Resuspend in 100µL sterile water.
- Spread on LB + Ampicillin plates:
    - 10%: 10µL of the reaction
    - 90%: 90µL of the reaction
- Incubate overnight at 37°C

Results: Observation of the growth on 27/08/2017

Content	90%		10%	
	Plate number	Colonies	Plate number	Colonies
pAC001+P3	iOAC2608PD01	4	iOAC2608PD02	1
pAC001+P4	iOAC2608PD0	19	iOAC2608PD0	1

	3		4	
pAC001+P6	iOAC2608PD05	17	iOAC2608PD06	4
pAC001+P9	iOAC2608PD07	22	iOAC2608PD08	4
pAC001+P10	iOAC2608PD09	92	iOAC2608PD10	4
Control	iOAC2608PD11	0	iOAC2608PD12	0

2017/08/27

Digestion of pSB4K5 with NotI - ACB

Aim: Linearize the plasmid prior to PCR.

Protocol:

- Set the following reaction in a 1.5mL Eppendorf tube (template: tube pSB4K5 clone B)

	V(µL)
Template	10
Not I	1
Cutsmart Buffer 10x	5
Water	34
	50

- Incubate 1h at 37°C
- Heat inactivation: 80°C for 20min.

Overnight culture of the DH5α-J61002-P3, DH5α-J61002-P4, DH5α-J61002-P6, DH5α-J61002-P9, DH5α-J61002-P10 - ACB

Aim: Liquid culture for miniprep

Protocol: For each reaction:

- Pick 3 single colonies and resuspend in 10 mL LB+Amp
- Grow overnigh at 37°Ct under shaking conditions

Transformation of ligation products: J61002-P2, J61002-P5, J61002-P8, J61002-P11, J61002-P12 - ACB

Aim: Verify the validity of the ligation reaction.

Protocol:

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add the following amount of DNA in each tube

Tube	V DNA (µL)
Reactions	5µL of ligation reaction
Negative control	No DNA

- Incubate 30min on ice.
- Heat shock: 45s at 42°C.
- Let rest on ice for 5min.
- Add 950µL of LB medium and incubate for 1h at 37°C with shaking.
- Centrifuge for 2min at 5000rpm on a bench centrifuge.
- Resuspend in 100µL sterile water.
- Spread on LB + Ampicillin plates:
    - 10%: 10µL of the reaction
    - 90%: 90µL of the reaction
- Incubate overnight at 37°C

Results: Observation of the growth on 27/08/2017

Content	90%		10%	
	Plate number	Colonies	Plate number	Colonies
pAC001+P2	iOAC2708DS01	46	iOAC2708DS02	1

pAC001+P5	iOAC2708DS03	56	iOAC2708DS04	2
pAC001+P8	iOAC2708DS05	74	iOAC2708DS06	5
pAC001+P11	iOAC2708DS07	41	iOAC2708DS08	0
pAC001+P12	iOAC2708DS09	14	iOAC2708DS10	4
Control	iOAC2708DS11	0	iOAC2708DS012	0

2017/08/28

Miniprep of J61002-P3, J61002-P4, J61002-P6, J61002-P9, J61002-P10 - ACB

Aim: Extract plasmid DNA to perform PCR and assemble our constructs.

Protocol: We performed the Thermo Scientific GeneJET Plasmid Miniprep Kit Protocol.

Harvest bacteria

- Centrifugation at 8000 rpm for 2min at room temperature.

Resuspend Cells, Lyse and Neutralize

- Add to the pelleted cells:
    - 250 µL of Resuspension Solution and vortex.
    - 250 µL of Lysis Solution and invert the tube 4-6 times.
    - 350 µL of Neutralization Solution and invert the tube 4-6 times.
    - Centrifuge 5 minutes at 13 000 rpm

Bind DNA

- Transfer the supernatant to the Thermo Scienti c GeneJET Spin Column. Centrifuge 1 minute.

Wash the column

- Add 500 µL of Wash Solution and centrifuge for 30-60 s. × 2 times
- Discard the flow-through.
- Centrifuge empty column for 1 minute at 13 000 rpm

Elute purified DNA

- Transfer the column into a new tube.
- Add 50 µL of Elution Buffer to the column and incubate 2 minutes. Centrifuge 2 minutes.
- Collect the flow-through.

Results: Measurement of DNA concentration using a Nanodrop

- Blank: 1µL of Elution Buffer
- Sample: 1µL for each measurement

Tube Number	Content	C (ng/µL)	A260/A230	A260/A280
iOAC2808DS01	P3-A	105.3	1.91	1.97
iOAC2808DS02	P3-B	263.8	2.04	2.28
iOAC2808DS03	P3-C	227.1	1.67	0.86
iOAC2808DS04	P4-A	72.5	1.93	1.81
iOAC2808DS05	P4-B	251.8	1.73	0.94
iOAC2808DS06	P4-C	174.4	1.69	0.81
iOAC2808DS07	P6-A	184.9	1.94	1.64
iOAC2808DS08	P6-B	117.6	1.92	2.03
iOAC2808DS09	P6-C	173.3	1.76	1.10
iOAC2808DS10	P9-A	183.7	1.79	1.07
iOAC2808DS11	P9-B	69.6	1.92	1.48
iOAC2808DS12	P10-A	168.9	1.76	0.96
iOAC2808DS13	P10-B	277.3	1.59	0.70

iOAC2808DS14	P10-C	278.4	1.80	1.04

PCR of pSB4K5 - ACB

Aim: Amplify the fragments in order to perform Golden Gate assembly of our fragments

Protocol:

- On ice, Set the following reactions in 0.2mL PCR tubes using 2X DreamTaq Master Mix

Tube number	**Undigested**	**1**	**Digested**	**2**
2X Phusion Master Mix		25		25
Forward primer	AC1’	2,5	AC1’	2,5
Reverse primer	AC2’	2,5	AC2’	2,5
DNA template	P2	1	P2	1
Water		19		19
Total		**50,00**		**50,00**
T m	**56.**		**56.**	

7°C **7°C**

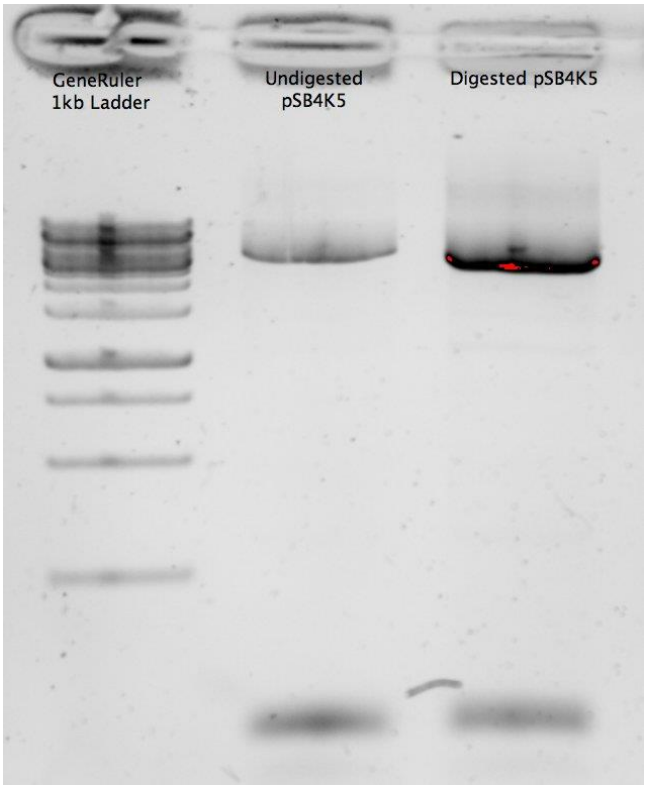
- Put in the PCR machine and run the following program

Initial denaturation	98°C	1min
30 cycles	98°C	10s
	Tm	10s
	72°C	2min
final extension	72°C	min
hold	12°C	∞

Results: 1% agarose gel migration at 100V for 20min

Loading:

- Ladder: 2µL of 100bp plus DNA Ladder



- Sample: 5µL of sample

Conclusion: The PCR worked and we obtained the desired products.

PCR purification of pSB4K5 - ACB

Aim: Get the DNA ready for Assembly

Protocol:

Proceed with the PCR purification (QIAquick PCR Purification Kit)

- Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
- Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.
- To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.

- Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Results

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230
iOAC2808DS15	pS4K5	31.3	1.83	2.98
iOAC2808DS16	pSB4K5	63.1	1.81	2.47

Golden Gate Assembly of pAC002 - ACB

Aim: Assembly of the promoter expression plasmid.

Protocol:

- Set up the following reaction in a 0.2mL PCR tube

Part	**Tube number**	**size (bp)**	**Ratio**	**n (pmol)**	**c (ng/µL)**	**m (ng)**	**V (µL)**	
pSB4K5		3419	1	0,04	63,1	84,5150188	1,34	o
ecfp		2096	2	0,08	15,3	103,6240928	6,77	o
T7 core		2121	2	0,08	141,9	104,8600328	0,74	o
eyfp		941	2	0,08	53,6	46,52366	0,87	

						48		
T4 ligase				0		0	1,00	
DpnI				0		0	0,50	
BsaI				0		0	1,00	
10X T4 buffer				0		0	2,00	o
10X BsaI Buffer				0		0	2,00	o
Water				0		0	3,78	o
pAC002		**8577**					**20,00**	

- Incubate in a PCR machine with the following program:
    - 37°C for 10min
    - Repeat 80 times
        - 16°C for 5min
        - 37°C for 5min Stop repeat
    - 37°C for 10 min
    - 65°C for 10 min
    - 80°C for 10min

Results: Will be seen in the following Transformation results.

Overnight culture of the DH5α-J61002-P2, DH5α-J61002-P5, DH5α-J61002-P8, DH5α-J61002-P11, DH5α-J61002-P12 - ACB

Aim: Liquid culture for miniprep

Protocol: For each reaction:

- Pick 3 single colonies and resuspend in 10 mL LB+Amp
- Grow overnigh at 37°Ct under shaking conditions

2017/08/29

Miniprep of J61002-P2, J61002-P5, J61002-P8, J61002-P11, J61002-P12 - ACB

Aim: Extract plasmid DNA to perform PCR and assemble our constructs.

Protocol: We performed the Thermo Scientific GeneJET Plasmid Miniprep Kit Protocol.

Harvest bacteria

- Centrifugation at 8000 rpm for 2min at room temperature.

Resuspend Cells, Lyse and Neutralize

- Add to the pelleted cells:
    - 250 µL of Resuspension Solution and vortex.
    - 250 µL of Lysis Solution and invert the tube 4-6 times.
    - 350 µL of Neutralization Solution and invert the tube 4-6 times.
    - Centrifuge 5 minutes at 13 000 rpm

Bind DNA

- Transfer the supernatant to the Thermo Scienti c GeneJET Spin Column. Centrifuge 1 minute.

Wash the column

- Add 500 µL of Wash Solution and centrifuge for 30-60 s. × 2 times
- Discard the flow-through.
- Centrifuge empty column for 1 minute at 13 000 rpm

Elute purified DNA

- Transfer the column into a new tube.
- Add 50 µL of Elution Buffer to the column and incubate 2 minutes. Centrifuge 2 minutes.
- Collect the flow-through.

Results: Measurement of DNA concentration using a Nanodrop

- Blank: 1µL of Elution Buffer
- Sample: 1µL for each measurement

Tube Number	Content	C (ng/µL)	A260/A230	A260/A280
iOAC2908DS01	P2-A	57.0	1.83	1.65
iOAC2908DS02	P2-B	137.3	1.83	1.37
iOAC2908DS03	P2-C	196.4	1.80	1.28
iOAC2908DS04	P5-A	83.9	1.83	1.50
iOAC2908DS05	P5-B	211.2	1.88	1.42
iOAC2908DS06	P5-C	109.1	1.82	1.35
iOAC2908DS07	P8-A	198.8	1.74	0.91
iOAC2908DS08	P8-B	194.9	1.98	2.01
iOAC2908DS09	P8-C	307.7	2.04	2.17
iOAC2908DS10	P11-A	190.7	1.97	2.10
iOAC2908DS11	P11-B	40.9	0.93	0.36
iOAC2908DS12	P11-C	220.6	1.99	2.27
iOAC2908DS13	P12-A	131.3	1.91	2.04

iOAC2908DS14	P12-B	91.8	1.95	1.83
iOAC2908DS15	P12-C	169.7	1.86	1.80

Transformation of pAC002 - ACB

Aim: Amplify the product of the Golden Gate assembly

Protocol:

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add the following amount of DNA in each tube

Tube	V DNA (µL)
GG reaction (pAC002)	4µL of Golden Gate reaction
Negative control	No DNA

- Incubate 30min on ice.
- Heat shock: 45s at 42°C.
- Let rest on ice for 5min.
- Add 950µL of LB medium and incubate for 1h at 37°C with shaking.
- Centrifuge for 2min at 5000rpm on a bench centrifuge.
- Resuspend in 100µL sterile water.
- Spread on LB + Ampicillin plates:
    - 10%: 10µL of the reaction
    - 90%: 90µL of the reaction
- Incubate overnight at 37°C

Results:

There was no growth.

2017/08/30

Resuspension of P1 g-block - ACB

Aim: Prepare the DNA to amplify them

Protocol:

- Centrifuge 3000xg for 1min.
- Add 25µL of DNAse free water.
- Vortex quickly.
- Incubate at 50°C for 20 min.
- Vortex quickly & centrifuge quickly.
- Dilute 1µL of this reaction in 9 µL of DNAse free water.

PCR of P1 - ACB

Aim: Amplify

Protocol:

- Set the following reaction in a PCR strip:
- On ice, Set the following reactions in 0.2mL PCR tubes using 2X DreamTaq Master Mix

Tube number	**P1**	**1**	**P7**	**2**
2X Phusion Master Mix		25		25
Forward primer	AC29	2,5	AC32	2,5
Reverse primer	AC30	2,5	AC30	2,5

DNA template	P1	1	P1	1
Water		19		19
Total		**50, 00**		**50, 00**
T m	**63° C**		**63° C**	

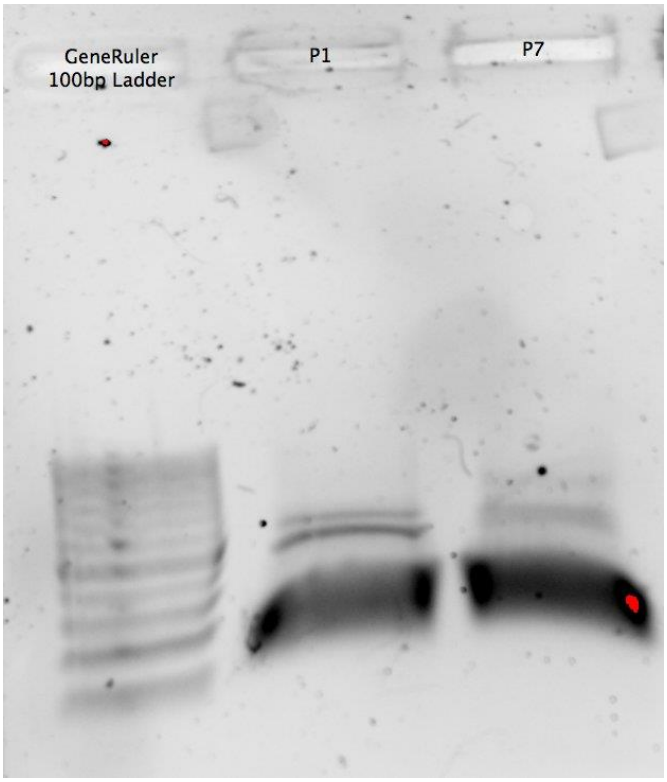
- Put in the PCR machine and run the following program

Initial denaturation	98°C	30S
30 cycles	98°C	5s
	Tm	15s
	72°C	20s
final extension	72°C	2 min
hold	12°C	∞

Results: 1% agarose gel migration at 100V for 20min

Loading:

- Ladder: 2µL of 100bp plus DNA Ladder
- Sample: 5µL of sample



Results: The PCR worked.

Golden Gate Assembly of pAC002

Aim: Assembly of the backbone to test the promoters

Protocol:

- Set the following reaction in 0.2 mL PCR strips:

Part	Tube number	size (bp)	Ratio	n (pmol)	c (ng/µL)	m (ng)	V (µL)	
pSB4K5	iOAC2808DS15	3419	1	0,04	63,1	84,5150188	1,34	
ecfp	iOAC1908DS03	2096	2	0,08	15,3	103,6240928	6,77	
T7	iOAC2308	2121	2	0,08	141,9	104,8600	0,74	

core	DS02					328		
eyfp	iOAC1908 DS01	941	2	0,08	53,6	46,5236648	0,87	
T4 ligase				0		0	1,00	
DpnI						0	0,50	
BsaI				0		0	1,00	
10X T4 buffer				0		0	2,00	
10X BsaI Buffer				0		0	2,00	
Water				0		0	3,78	
pAC002		**8577**					**20,00**	

- Incubate in a PCR machine with the following cycle
    - 37°C for 10min
    - Repeat 80 times
        - 16°C for 5min
        - 37°C for 5min Stop repeat
    - 37°C for 10 min
    - 65°C for 10 min
    - 80°C for 10min

Results: Transformation into E. coli

2017/08/31

PCR purification of overnight - ACB

Aim: Get the DNA ready for Assembly

Protocol:

Proceed with the PCR purification (QIAquick PCR Purification Kit)

- Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
- Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.
- To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.
- Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Results

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230
iOAC3108DS01	P1	25.6	1.94	1.99
iOAC3108DS02	P7	29.1	1.94	1.93

Digestion of P1 & P7 with EcoRI and SpeI

Aim: Digest for ligation of the promoters in J61002

Protocol:

- Set the reaction in 1.5mL Eppendorf tubes:

	P1	P7
10x Cutsmart	5	5
EcoRI	1	1
SpeI	1	1
Water	33	33
DNA	10	10
Total	50	50

- Incubate 1h at 37°C.
- Heat Inactivation: 65°C for 20min, 80°C for 20min.

PCR purification of digestion - ACB

Aim: Get the DNA ready for Assembly

Protocol:

Proceed with the PCR purification (QIAquick PCR Purification Kit)

- Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
- Place the sample in the column & centrifuge for 30s at 17,900 x g. Discard the flow-through and place the QIAquick column back in the same tube.
- To wash, add 750µL Buffer PE to the QIAquick column and centrifuge for 1min at 17,900 x g. Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 17,900 x g.
- Place each QIAquick column in a clean 1.5mL microcentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Results

Tube Number	Gene	C (ng/µL)	A260/A280	A260/A230
iOAC3108DS03	P1 digested	4.7	1.97	0.66
iOAC3108DS04	P7 digested	2.5	2.33	0.57

Ligation of P1 & P7 into J61002 - ACB

Aim: Assemble of the reporter plasmids with the promoters.

Protocol:

- Set the following reaction in 0.2mL PCr strips:

Part	**Control**	**P1**	**P7**
pAC001	2,79	2,79	2,79
Insert	0,00	3,17	3,33
T4 ligase	1,00	1,00	1,00
10X T4 buffer	2,00	2,00	2,00
Water	14,21	11,04	10,88
Final product	**20**	**20**	**20**

- Incubate overnight at 16°C
- Heat inactivation 20 min at 80°C

Transformation of the ligation of P1 & P7 & the Overnight Golden Gate Assembly - ACB

Aim: Amplify the product of the Golden Gate assembly

Protocol:

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add the following amount of DNA in each tube

Tube	V DNA (µL)
GG reaction (pAC002)	4µL of Golden Gate reaction
Ligation reaction	5µL of ligation
Negative control	No DNA

- Incubate 30min on ice.
- Heat shock: 45s at 42°C.
- Let rest on ice for 5min.
- Add 950µL of LB medium and incubate for 1h at 37°C with shaking.
- Centrifuge for 2min at 5000rpm on a bench centrifuge.
- Resuspend in 100µL sterile water.
- Spread on LB + Ampicillin plates:
    - 10%: 10µL of the reaction
    - 90%: 90µL of the reaction
- Incubate overnight at 37°C

Results:

We got cells on the P7 and golden gate plates and none on the controle or P1 plates.

2017/09/01

Overnight culture of pAC002 and plasmid pP7 - ACB

Aim: Culture for Miniprep.

Protocol:

- Pick colonies on the plates
- Resuspend in 10mL of LB + Ampicillin for pP7 and LB + Kanamycin for pAC002

2017/09/02

Miniprep of pAC002 and pP7

Aim: Extract plasmid DNA.

Protocol: We performed the Thermo Scientific GeneJET Plasmid Miniprep Kit Protocol.
Harvest bacteria

- Centrifugation at 8000 rpm for 2min at room temperature.

Resuspend Cells, Lyse and Neutralize

- Add to the pelleted cells:
    - 250 µL of Resuspension Solution and vortex.
    - 250 µL of Lysis Solution and invert the tube 4-6 times.
    - 350 µL of Neutralization Solution and invert the tube 4-6 times.
    - Centrifuge 5 minutes at 13 000 rpm

Bind DNA

- Transfer the supernatant to the Thermo Scienti c GeneJET Spin Column. Centrifuge 1 minute.

Wash the column

- Add 500 µL of Wash Solution and centrifuge for 30-60 s. × 2 times
- Discard the flow-through.
- Centrifuge empty column for 1 minute at 13 000 rpm

Elute purified DNA

- Transfer the column into a new tube.
- Add 50 µL of Elution Buffer to the column and incubate 2 minutes. Centrifuge 2 minutes.
- Collect the flow-through.

Results: Measurement of DNA concentration using a Nanodrop

- Blank: 1µL of Elution Buffer
- Sample: 1µL for each measurement

Tube Number	Content	C (ng/µL)	A260/A230	A260/A280
iOAC0209DS01	pP7-A	66.6	1.96	1.72
iOAC0209DS02	pP7-B	59.6	1.91	1.62
iOAC0209DS03	pP7-C	80.5	1.93	1.72
iOAC0209DS04	pAC002-A	112.7	1.95	2.03
iOAC0209DS05	pAC002-B	130.5	1.96	2.08

PCR of pAC002 - ACB

Aim: Amplification pAC002 for Golden Gate Assembly

Protocol:

- On ice, set the following reaction in a 0.2mL PCR strips.

Tube number	**1**	**2**
2X Phusion MM	25	25
AC2'	2,5	2,5
AC10	2,5	2,5
DNA template	1	1
Water	19	19
Total	**50,00**	**50,00**

- Put in the PCR machine and run the following program

Initial denaturation	98°C	30s
30 cycles	98°C	10s
	58.2	30s
	72°C	4min
final extension	72°C	10min
hold	12°C	∞

Results: 1% agarose gel migration at 100V for 20min
Loading:

- Ladder: 2µL of 100bp plus DNA Ladder

- Sample: 5µL of sample

The PCR did not work

Digestion of pAC002 with XhoI - ACB

Aim: Amplification pAC002 for Golden Gate Assembly

Protocol:

2017/09/03

Golden Gate Assembly of pAC003, pAC004, pAC005 - ACB

Aim: Assembly of the repressor plasmids

Protocol:

- Set the following reaction in a 0.2mL tube:

- Run the following program in a PCR machine:

Ligation of P1 into pAC001 - ACB

Aim: Assembly of the expression plasmid.

Protocol:

- Set the following reaction in a 0.2 mL Tube

- Incubate overnight at 16°C

2017/09/06

Transformation of pP1 & pAC003,pAC004, pAC005 in *E. coli* DH5 alpha - VP

Aim: transform the DNA into e.coli dh5alpa

Protocol:

I did the standard transformation protocol only I added 5microL of PACOO1 L1 and L2 instead of 1microL and I added 4 microL of PACOO3, PACOO4, PACOO5 instead of 1microL

2017/09/16

PCR of pAC002 - ACB

Aim:

Protocol:

Results:

2017/09/17

PCR purification of pAC002 - ACB

Aim:

Protocol:

Results:

Golden Gate Assembly of pAC003, pAC004 & pAC005- ACB

Aim:

Protocol:

Results:

2017/09/18

Transformation of the Golden Gate assembly - ACB

Aim:

Protocol:
5µL of reaction

Results: The reaction didn't work

2017/09/19

Transformation of the Golden Gate assembly - ACB

Aim:

Protocol:
10µL of reaction

Results: The reaction didn’t work.