Protein Photocaging Lab Book

12/08

Transformation of C0012, I732100, K592009,K5801039,K564013and K1470002 into *E. coli* DH5α

Biobrick number	Gene	Plate number	Well number
C0012	LacI rep + LVA	Plate 2	2N
I732100	LacI rep	Plate 3	8A
K592009	Amilcp Blue	Plate 1	19 E
K5801039	AD GAL4	Plate 1	15P
K564013	+Lacz	Plate 1	8B
K1470002	DBD GAL4	Plate 5	16N

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add 1µL DNA in the cells. Mix gently.
- Incubate 30min on ice.
- Heat shock: 1 min at 42°C.
- Let rest on ice for 5min.
- Add 950µL of SOC medium (warmed) 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.

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Picking single colonies of the plates :

- Plated on another Chloramphenicol plate;
- Inoculated in a liquid medium supplemented with Chloramphenicol (15 ml).

14/08

- Preparing a Glycerol stock of the cells
- Running a miniprep protocol

Conclusion:

Too many cells were used as a start point (10 ml) which resulted in difficulties in obtaining a clear supernatant in the first step in the miniprep protocol. The samples purity aren't reliable . so the protocol to be repeated.

16/08

Miniprep of C0040, C0050, C0053, I13404, I6031(AC) and the parts K564013, K1470002.

Running a second miniprep for the parts.

Results:

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
iOAG1608BB01	Lacz -1				
iOAG1608BB02	Lacz -2				
iOAG1408BB05	DBD Gal4				

Preparation of Materials for Competent cells protocol :

18/08

Digestion Rx for Gene block Dronpa:

- 10ul DNA (200ng);
- 2 ul fast digest buffer;
- 1 ul PstI;
- 1ul EcoRI;

- 6ul water;

-incubate at 37 C for 30 min.
Heat inactivation at 80 C for 5 min.
*note: PstI can't be heat inactivated

Digestion Rx forthe linearized plasmid PsBK3:
20 ul plasmid (500 ng)
3 ul fast digest buffer
1.5 ul PstI
1.5 ul EcoRI
1.5 Alkaline phosphatase fast
2.5 ul water
-incubate at 37 C for 10 min
Heat inactivation at 80 C for 20 min.
*note: PstI can't be heat inactivated

Calculation for the Ligation Rx for inserting Dronpa into the vector PsB1K3.

-Final Concentration for Dronpa in the digestion Rx: 10ng/ul,
Length = 1539 bp.
-Final Concentration for PSB1K3 in the digestion Rx: 16.666 ng/ul,
Length = 2204 bp.

To achieve 1:3 ratio vector : insert ….> use 50 ng vector with 100 ng insert
Ligation Rx:

- 3 ul PSB1K3 digestion rx (50ng);
- 10 ul Dronpa digestion rx (100ng);
- 2 ul T4 DNA ligase buffer (10x);
- 1 ul T4 DNA ligase;
- 4 ul nuclease free water;
- Spin down;
- 10 minutes of incubation;
- Heat inactivation at 65 C for 10 min;
- Chill on ice.

18/08

Generating 100mM conc of the primers stock soln

10mM working solution

PCR RX 1:
Reaction :
T7 RNA polymerase (colony PCR)

FWD	aacacgattaacatcgctaagaacg
REV	cgcgaacgcgaagtcc
Annealing T	51.6 C
Amplicon size	2.655 Kb

Reaction :
Amplifying Dronpa, CARP1 and CARP2 gene blocks

FWD	TTCTGGAATTCGCGGCCGCTTCTAGAG
REV	CGGACTGCAGCGGCCGCTACTAGT
Annealing T	61.9
Amplicon size	1.5 Kb for Dronpa 1.2 for CARP1 1.3 for CARP2

Lacz

FWD	accatgattacggattcactggc
REV	tttttgacaccagaccaactggtaatg
Annealing T	5.37

Amplicon size	3.1 kb

PJ23119

FWD	VF
REV	VR
Annealing T	51.8 C
Amplicon size	To be known

DreamTaq reaction setting/ reaction:
- 25ul Dream taq PCR master mix (2x);
- 2ul FWD primer (10mM working solution);
- 2ul REV primer (10mM working solution);
- Templet DNA 1-10ng;
- Completet the volume till 50 ul using Nuclease free water .

		Tube 1&2
Initial denaturation	95°C	3min
30 cycles	95°C	30s
	51-55	30s
	72°C	3min 30s
final extension	72°C	5min
hold	12°C	∞

PCR Program :Put in the PCR machine and run the following program for Lacz and T7 polymerase.
The following program for other samples.

		Tube 1&2
Initial denaturation	95°C	3min
30 cycles	95°C	30s
	51-61.9	30s
	72°C	1 mins
final extension	72°C	5min
hold	12°C	∞

Competent Cell Protocol II :

- All the work to be done on ice and next to a heater or in the hood.
- From an overnight Culture add 3 ml to each of the three 300 ml liquid medium.
- Shake for 2 hours at 37 C until OD reaches 0.3-0.5.

O.D. Readings for Batches.
Batch 1: 0.350
Batch 2: 0.471
Batch 3: 0.428

- Chill ml Falcon tubes in ice
- Immediatly put the bottels in ice and start deviding them among the falcon tubes
- Centrifuge to harvest cells @3000 rpm for 10 min (4C)
- Remove supernatant and add ml 50 mM Cacl2 to each falcon tube
- Incubate on ice for 30 minutes

- Spin 10 min @3000 rpm (4C)
- Discard the supernatant and Resuspend the pellete in 1 ml of ice cold 50mMCacl2
- Incubate on ice for 30 min
- Add 1 ml glycerol (60%)
- Divide into small eppendorfs
- Store at -80

Transformation Efficiency calculation :
-Thow cells on ice
-add 2ul control DNA (PUC19) to eppindorfs contating cells
-Incubate cells on ice for 30 minutes
-Heat shock cells for 1 minute in 42 C
-Place on ice for 2 min
-Add 900ul SOC medium to the Reaction
-Shake for 30 minutes at 300rpm

Cells	Amount of DNA	Cell count
DH5alpha S(prepared 18/08)	100 ng	860
DH5alpha A	100 ng	46
DH5alpha Batch1 (prepared 20/08_	100 ng	154
DH5alpha Batch 2	100 ng	449
DH5alpha Batch 3	100 ng	123
DH5alpha Batch 4 (prepared 22/8)	50 ng	661

Transformation efficiency calculation

20/08

Running the Gel from the PCR Rx

Lane 1: Ladder 1KB Thermo

Lane 2: Sample (i) from ACP

Lane 3: Sample (ii) from ACP

Lane 4: T7 pol +ve

Lane 5:T7 pol -ve

Lane 6: LacZ +ve

Lane 7: Lacz -ve

Lane 8 : ladder 1 kb Thermo

Lane 9: J23119 +ve

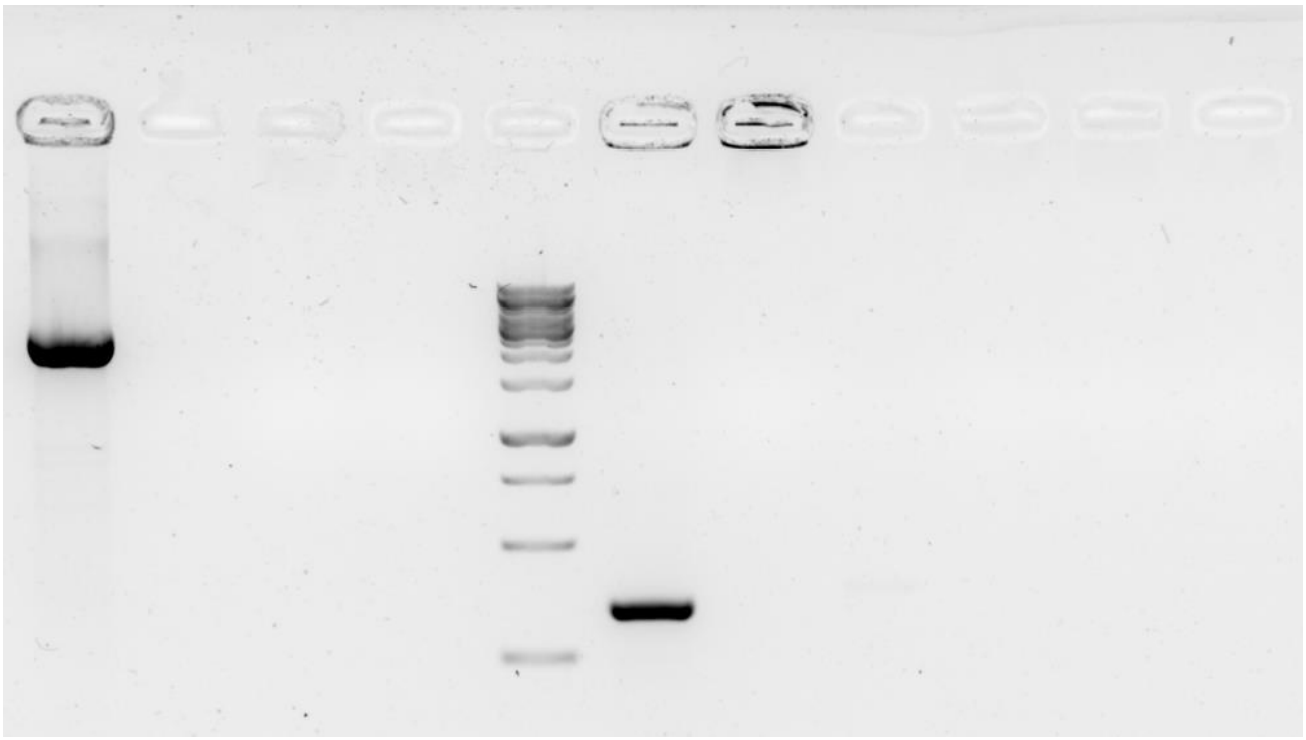
Lane 10: J23119 -ve

Lane 11:Dronpa Geneblock

Lane 12:CARP1 Geneblock

Lane 13: CARP2 Geneblock

Lane 14: Suffix and pre



fix primers

21/08

PCR reactions for adding the linkers

PCR Rx 2:

TetR

FWD	ATGCAGGGTCTCGCAGTGGTAGTAGTGGTAGCAGCTCCAGATTAGATAAAAGTAAAGTGATTAACAGCG
REV	ATGCAGGGTCTCGCTGATGTACCtgaACCCTTAAGGCCGGACCCACTTTCACATTTAAGTTGTTTTTCTAATCC

Rxs

T1	T2	T-ve
Ann 52.6	Ann 70	-ve control

HKCl

FWD	TGCAGGGTCTCGCAGTGGTAGTAGTGGTAGCAGCGTTCAACAGAAAGAGCGTGAAAC
REV	ATGCAGGGTCTCGCTGATGTACCtgaACCCTTAAGGCCTCCGAATCGCTTGTAATCGATTG

Rxs

H1	H2	H-ve
Ann 51.2	Ann 70	-ve control

P22C22

FWD	ATGCAGGGTCTCGCAGTGGTAGTAGTGGTAGCAGCaatacacaattgatgggtgagcg
REV	CAGGGTCTCGCTGATGTACCtgaACCCTTAAGGCCagctactaaagcgtagttttcgtcg

RXs

P1	P2	P-ve

Ann 51.7	Ann 70	-ve control

T7 polymerase

FWD	ATGCAGGGTCTCGCAGTGGTAGTAGTGGTAGCAGCaacacgattaacatcgctaagaacg
REV	ATGCAGGGTCTCGCTGATGTACCtgaACCCTTAAGGCCcgcgaacgcgaagtcc

T1	T2	T-ve
Ann 51.6	Ann 70	-ve control

-for lacz :
As the previous reaction didn't work, 3 different minipreps have been used with the old primer and the new primers

Samples : LAcZ1, Lacz2, Lacz3

FWD	accatgattacggattcactggc
REV	tttttgacaccagaccaactggtaatg

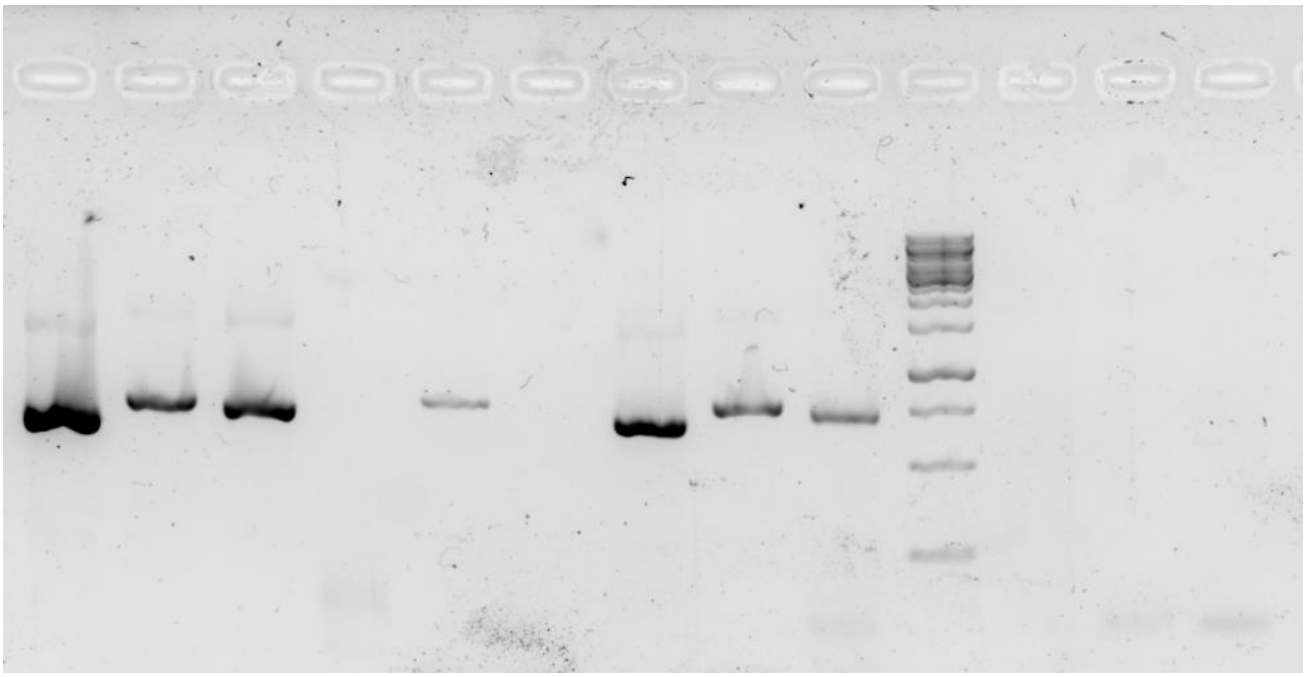
Za1	Zb1	Zc1	Za2	Zb2	Zc2	Z-ve
Ann 53.7	Ann 53.7	Ann 53.7	Ann 70	Ann 70	Ann 70	-ve control

FWD	ATGCAGGGTCTCGCAGTGGTAGTAGTGGTAGCAGCaccatgattacggattcactggc
REV	ATGCAGGGTCTCGCTGATGTACCtgaACCCTTAAGGCCtttttgacaccagaccaactggtaatg

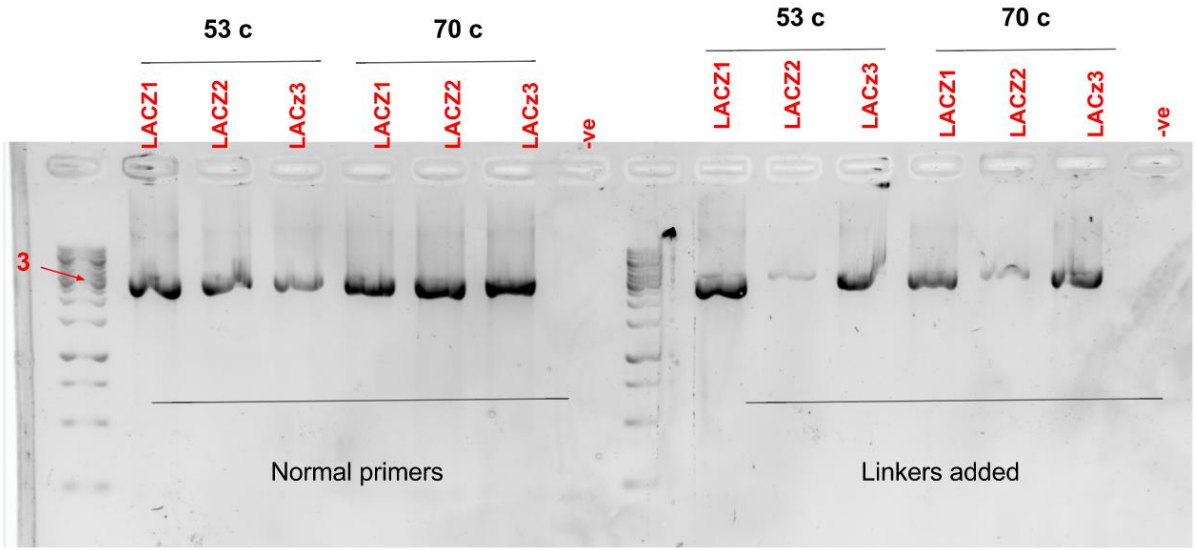
LZa1	LZb1	LZc1	LZa2	LZb2	LZc2	LZ-ve
Ann 53.7	Ann 53.7	Ann 53.7	Ann 70	Ann 70	Ann 70	-ve control

PCR program

The results Repressors and T7pol:

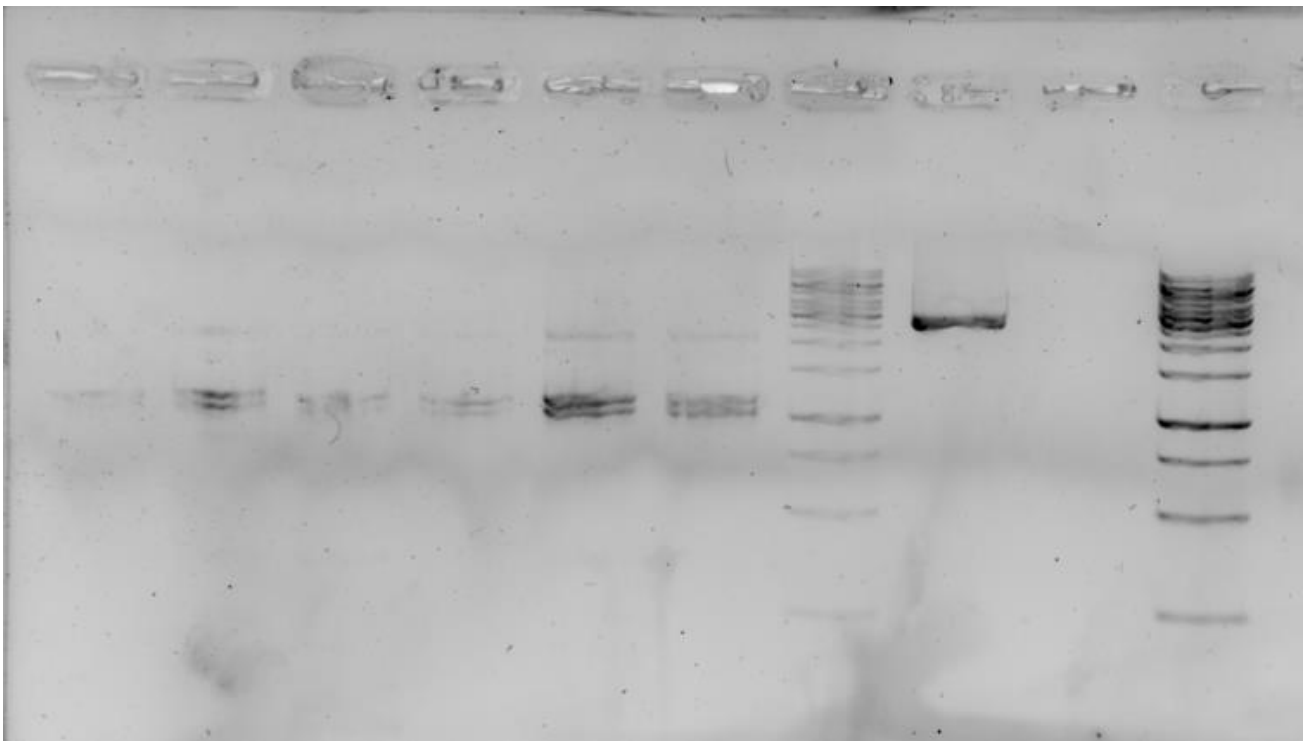


The lacZ



PCR Rx 3:

Colony Pcr of the dronpa transformation and repeating the T7 polymerase program



23/08

-rerunning the PCR products of the colonies from Dronpa ligation

--Miniprep from Dronpa ligation Colonies (only for 2 samples) D1, D2 :

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
-

Sample	Conc	260/280	260/230
D1	71.2 ng/ul		
D2	41.7 ng/ul		

-Picking Colonies from the transformation of the Part (T7+mrfp), and making a glycerol stock and an overnight culture (colony 1 and 2).

24/08

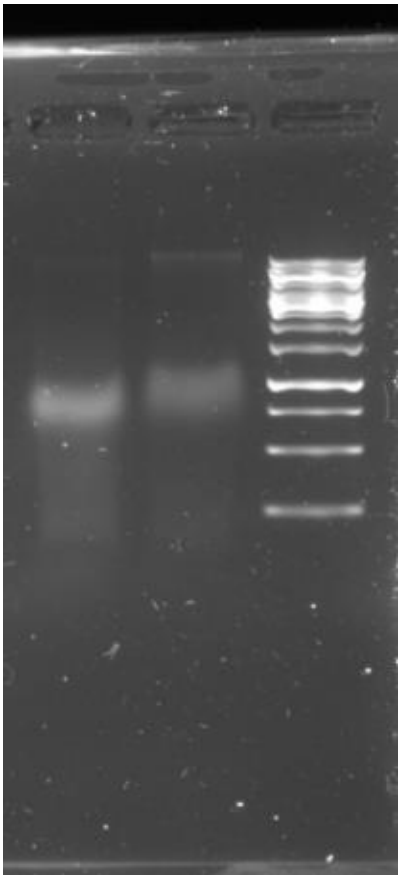
Restriction Digestion for D1, D2 samples with NotI
Recommended Protocol for Digestion
Protocol for Fast Digestion of Different DNA 1.

Combine the following reaction components at room temperature in the order indicated:

Water, nuclease-free	15 µL
10X FastDigest or 10X FastDigest Green Buffer	2 µL
DN A	(0.5-1 µg/µL)
FastDigest enzyme	1 µL 1

Mix gently and spin down.
3. Incubate at 37°C in a heat block or water thermostat for 30 min (plasmid DNA), for 10 min (genomic DNA), or for 5 min (PCR product).

Inactivate the enzyme by heating for 5 min at 80°C.
4. If the FastDigest Green Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel.
Result :



Comment :
There is a smear in the gel instead of 2 clear bands .. it's very doubtful that the gene was inserted correctly in the plasmid

- The PCR product from the previous day should be purified and sequenced

MiniPrep protocol for the samples C1, C2 (T7+mrfp)
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
-

Sample	Conc	260/280	260/230
C1	35 ng/ul	2.06	1.77
C2	54 ng/ul	1.99	2.13

25/08/17

Redoing the PCR rx

PCR Rx 4.

26/08

Making a gel for visualization of the PCR (1%)

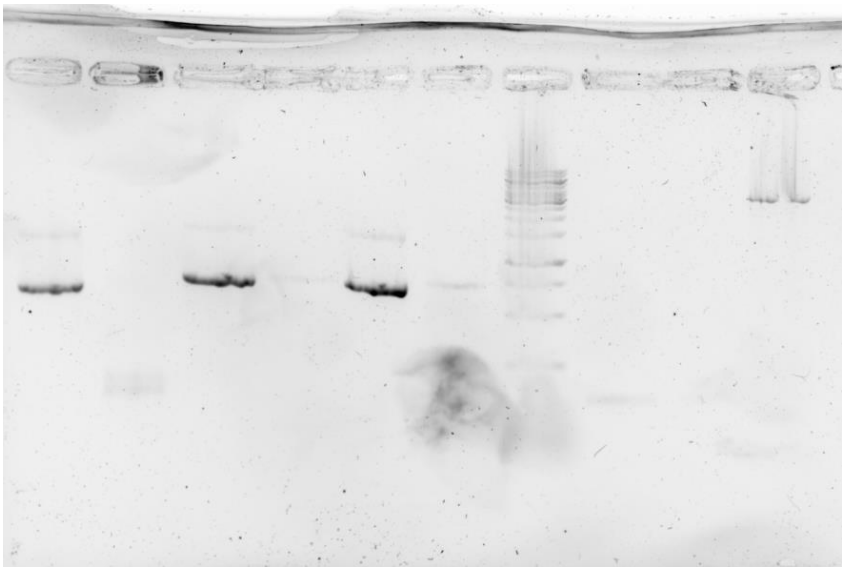
Lane1:TetR+ve

Lane2:TetR-ve

Lane3:HKC:I+ve

Lane4:HKCI-ve

Lane5 :P22C2+ve
Lane6:P22C2-ve
Lane7:Ladder
Lane8:T7pol+ve
Lane9:T7pol-ve
Lane10:Lacz+ve
Lane11:Lacz-ve



Comment on gel :
I can proceed with PCR purification only with samples of TetR ,HKCI and P22C2 but not T7pol and Lacz

Starting a transformation :

- The dronpa ligation in a DH5alpha strain
- The T7+mrfp in a BL21 strain

Transformation protocol

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add 1µL DNA in the cells. Mix gently.
- Incubate 30min on ice.
- Heat shock: 1 min at 42°C.
- Let rest on ice for 5min.
- Add 950µL of SOC medium (warmed) 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

PCR purification Protocol :

Aim: Get the DNA ready for Golden Gate Assembly

Protocol:(QIAquick PCR Purification Kit)
Samples to be Purified :
TetR, HKCI, P22c2 from PCR rx 4
Lacz samples from PCR Rx 2
T7 polymerase from Rx 3

- 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 m**icrocentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Sample	conc	260/280	230/260
LinDTetR	44.1 ng/ul	1.88	2.08
LinDHKCI	56.1 ng/ul	1.92	2.04
LinDP22C2	56.8 ng/ul	1.90	2.23
LinDT7polymerase	30.6ng/ul	2.02	1.89

LinDLacz	94.3ng/ul	1.88	2.13

Digestion Rx for Gene block mApple:
10ul DNA (200ng)
2 ul fast digest buffer
1 ul PstI
1ul EcoRI
6ul water
-incubate at 37 C for 30 min
Heat inactivation at 80 C for 5 min
*note: PstI can't be heat inactivated

Digestion Rx forthe linearized plasmid PsB1C3:
20 ul plasmid (500 ng)
3 ul fast digest buffer
1.5 ul PstI
1.5 ul EcoRI
1.5 Alkaline phosphatase fast
2.5 ul water
-incubate at 37 C for 10 min
Heat inactivation at 80 C for 20 min
*note: PstI can't be heat inactivated

Calculation for the Ligation Rx for inserting into the vector PsB1C3

-Final Concentration for mApple in the digestion Rx: 10ng/ul
Length = 1701 bp
-Final Concentration for PsB1C3 in the digestion Rx: 16.666 ng/ul
Length = 2070 bp
To achieve 1:3 ratio vector : insert ….> use 50 ng vector with 100 ng insert

Ligation Rx (T4 DNA ligase Thermo Fischer)

3 ul PSB1C3 digestion rx (50ng)

10 ul mAppel digestion rx (100ng)
2 ul T4 DNA ligase buffer (10x)
1 ul T4 DNA ligase
4 ul nuclease free water

- Spin down
- Over night incubation
- Heat inactivation at 65 C for 10 min
- Chill on ice

Out of the Rx .. doing a transformation with 2 ul after 10 min in room temp

27/08/17

Heat inactivation of the overnight digestion reaction 65C for 10 minutes

- Transformation of the mApple ligation part and the linearized Backbone as a control
- Transformation of the Dronpa ligation part and the linearized Backbone as a control

- Pick colony from the previous ligation (colony PCR)
- Redoing the PCR for D1, LinDT7pol,LInDLacz and Colony 1 of the t7+ mrfp

28/8/17

Making a gel to visualize the previous PCR RX.
Plasmid miniprep.
Protocol:
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

-PCR reaction with the xbaI primer amplifying dronpa ..
List of RX: DF DR
XbaIsuffix
XbaIVR

29/8/17

Visualize gel result on a gel.
Repeat ligation reaction using HC T4 Dna ligase.
Dronpa digestion rx + PSB3C5.

2 reactions : one 10 min incubation and 1 over night ligation + transformation of old mApple ligation into linearized PSB1C.
PCR purification Protocol.

Protocol:(QIAquick PCR Purification Kit)
Samples to be Purified
Lacz samples from PCR Rx
T7 polymerase from Rx
Dronpa part amplified using specific primers
Dronpa Part with extra XbaI cutting site

- 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 m**icrocentrifuge tube.
- Add 50µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 17,900 x g.

Sample	conc	260/280	230/260
T7 pol with linker	61	1.86	2.40
Lacz with linkers	45.5	1.89	2.25
Dronpa amplified with DF DR	101.6	1.69	1.16
Dronpa + XbaI cutting site	129	1.8	1.72

30/08/17

*Transformation of the overnight ligation reactions
-PSB3C5+dronpa part
-PSB3C5+mApple part

*TA cloning of dronpa and mApple :

Inserting Dronpa and Mapple in T vector
ADDING 3' A OVERHANG TO A PCR PRODUCT
Procedure
Purify the PCR product. Before adding the overhangs it is very important to remove all the Proofreading DNA Polymerase (Pfu) by purifying the PCR product carefully

(e.g. with a commercial PCR purification kit or phenol extraction and DNA precipitation); since the proofreading activity of DNA Polymerase will degrade the A overhangs, creating blunt ends again.

Prepare Taq DNA polymerase reaction mix for a typical 20 - 50 µl reaction:

	Final Concentration
Purified PCR product	100ng (5ul gene block)
dNTP (10 mM)	0.2 mM
PCR Buffer with Mg (10x)	1x (1.5 mM MgCl2)
Taq DNA Polymerase (5 U/µl)	1U
ddH2O	Complete reaction till 20

1. Incubate 20 min at 72 °C.

Set up ligation reaction in PGEM vector

PCR Purify the 2 samples

	PGEM+Mapple	PGEM+Dron	PGEM+ve	PGEM-ve
PCR product	8ul	8ul	-	-
2X Rapid Ligation Buffer, T4 DNA Ligase	10ul	10ul	5	5
pGEM Vector (50ng)	1ul	1ul	1	1
T4 DNA Ligase (3 Weiss units/µl)	1ul	1ul	1	1
Control insert	-	-	2ul	-

DNA				
nuclease-free water	-	-	Up to 10ul	Up to 10ul

-Ligation for Over night at 4 C

Restriction digestion reactions for both mApple and Dronpa gene blocks to be ligated into PSB3C5

mApp 303 Dron 304

Buffer (3.1) 10x	ECORI(50% efficiency)	PstI (100% efficiency)	100 ng DNA	Water
2ul	2ul	1 ul	5 ul	Up to 20 ul

305 Restriction digestion reaction for Dronpa gene blocks to be ligated into J61002

Buffer (3.1)10x	XbaI(75% efficiency)	PstI (100% efficiency)	100 ng DNA	Water
2ul	1.5	1 ul	5 ul	Up to 20 ul

301 Restriction digestion reaction for J61002 part

Buffer (2.1)10x	SpeI(100% efficiency)	PstI (75% efficiency)	DNA	Water
2ul	1 ul	1.5 ul	5 ul (1000ng)	Up to 20 ul

306 Restriction digestion reaction for(PCR purified product amplified with xbaI cutting site)

Buffer (3.1)10x	XbaI(75% efficiency)	PstI (100% efficiency)	DNA	Water
2ul	1.5 ul	1 ul	7.7 ul (1000ng)	Up to 20 ul

302 Restriction digestion reaction for PsB1C3 part

Buffer (2.1)10x	ECORIHF	PstI	DpnI	DNA	Water
3ul	1.5	1.5 ul	1ul	10 ul (250 ng)	Up to 30 ul

- Incubate for 2 hrs at 37 C
  *for the J61002 part AP was added after reaction completion and incubated for 10 min then heat inactivation for 20 min at 80 C
- Clean up digestion by 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 30 at 13000 rpm. 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 13000 rpm Discard flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more for 1 min ro 13000 rpm
- Place each QIAquick column in a clean **1.5mL m**icrocentrifuge tube.
- Add 20µL water heated to 50°C. Incubate 1 min at 50°C.
- Centrifuge for 1 min at 13000 rpm.

Sample	conc	260/280	230/260

301 (J6002 pst1 +speI)	26 ng/ul		
302 (Psb1c3 ecoRI +pst1)	165.1ng/ul	1.16	0.67
303 (mapp EcoRI +pst1)	162.6	1.50	1.12
304 (Dron EcoRI +pst1)	5 ng/ul	2.53	0.45
305 (Dron pst1+xba1)	99.2 ng/ul	1.32	0.71
306 (PcrxbDron pst1+xba1)	18.1ng/ul	2.23	0.92

**IN RED : RESULTS that don't make any sense (the measuring was repeated for 2 times)

Ligation reaction

310: Dronpa gb+ J6 (305+301)

insert	vector	10x ligation buffer	DNA ligase	water
10ul	2ul	2ul	1ul	Up to 20ul

311:Dronpa xb + J6 (306+301)

insert	vector	10x ligation buffer	DNA ligase	water
6ul	2ul	2ul	1ul	Up to 20ul

312: Dronpa gb+PSBC3 (305+302)

insert	vector	10x ligation buffer	DNA ligase	water
10ul	3ul	2ul	1ul	Up to 20ul

313:Dronpa gb +PSB3C5 (305+already digested by Sasha).

insert	vector	10x ligation buffer	DNA ligase	water
10ul	4ul	2ul	1ul	Up to 20ul

314: mAppleGB +PSBC3 (303+302)

insert	vector	10x ligation buffer	DNA ligase	water
10ul	3ul	2ul	1ul	Up to 20ul

315:mApple full +PSB3C5 (303 + a RDtaken from Sasha).

insert	vector	10x ligation buffer	DNA ligase	water
10ul	4ul	2ul	1ul	Up to 20ul

Overnight ligation reaction at 16 C
31/08/17
Heat inactvation of the ligation reactions 65C for 10 min

- Transformation of the following ligation reaction
- - 310
- -311
- -312
- -313
- 314
- 315
- The 4 ligation reaction from the pGEM T vector

- colony Pcr for 20 colonies from the plate PsB1k3+ Dronpa.

-Sending Parts for sequencing.

1/09

Visualization of the gel.
Picking colonies and spreading them on plates (and preparing over night cuture).

02/09/17

- Visulaisation of the parts under the right/emission excitation.
- Preparing glycerol stock.

Miniprep protocol.
The results.
Mp: mini prep C3/C5: in plasmid PSB1C3/5 J6: under the anderson

no:	Assigned name	Conc ng/ul	260/280	260/230
1	MPC3mAp	51.7	1.63	1.87
2	MPC3Dron1	206.2 105.1	1.14 1.39	1.17 0.94
3	MPJ6Dx1	56.6	1.97	1.28
4	MPJ6Dx2	53.6	2.0	1.85
5	MPJ6Dx3	44.3	2.11	1.39
6	MPJ6Dx4	42.0	2.07	1.99
7	MPJ6Dx6	182.4 182.3	1.34 0.89	0.73 1.0
8	MPJ6Dx7	41.0 38.1	1.69 0.55	1.24 0.91
9	MPJ6Dx8	94.1 18.0	1.69 1.85	-4.06 1.27
10	MPJ6Dx9	175.3 180.0	0.98 0.95	0.94 1.95

11	MPJ6Dx10	34.3	1.83	1.17
12	MPJ6Dx11	77.3 61.2	0.65 1.44	0.94 0.93
13	MPJ6Dx12	27.1	1.87	1.12
14	MPJ6Dx13	47.0	1.88	1.04
15	MPJ6Dx14	68.6 111.6	0.58 0.67	1.25 0.92
16	MPJ6Dx15	57.5	1.88	1.62
17	MPJ6Dx16	44.7	1.93	2.20
18	MPJ6Dx17			
19	MPJ6Dx18	108.0 229.0	1.01 0.92	2.11 1.70
20	MPJ6Dx19	25.8	2.02	0.94
21	MPJ6Dx20	55.7	1.22	0.65
22	MPJ6DF1	30.0	1.84	0.93
23	MPJ6DF2	40.2	2.07	1.38
24	MPJ6DF3	25.4	1.91	1.42
25	MPJ6DF4	35.7	1.73	1.34
26	MPJ6DF5	21.8	2.16	1.01
27	MPPgemM2	66.1	1.96	1.57
28	MPPgemM3	373.1	1.90	2.20
29	MPPgemD5	58.4	1.82	1.39
30	MPPgemD7	415.7	1.89	2.1
31	MPPgemD11	373.7	1.88	2.22
32	MPPgemD8	441	1.89	2.22
33	MPC5M1	289.3	1.89	2.11
34	MPC5M2	318	1.91	2.02

35	MPC5M5	183.9 214.4	1.16 1.42	2.73 6.46
36	MPC5M7	 158.4	1.73	1.71
37	MPC3Dron2	52.5	1.90	1.29

Transformation of the GFP part for Paul
Location plate 1 well 5i (cam resistant)
Transformation of t7+mrfp in BL21.

13/09

Restriction digestion.

RX : cut with ECORI And Pst1
- fast digest thermofischer enzymes
(2ug DNA in each rx) in each.

Sample	Volume added
MPC3mAp	20
MPC3Dron1	20
MPJ6Dx1	20
MPJ6Dx2	20
MPJ6Dx3	20
MPJ6Dx4	20
MPJ6Dx6	20
MPJ6Dx7	20

MPJ6Dx8	20
MPJ6Dx9	20
MPJ6Dx10	20
MPJ6Dx11	20
MPJ6Dx12	20
MPJ6Dx13	20
MPJ6Dx14	20
MPJ6Dx15	20
MPJ6Dx16	20
MPJ6Dx17	20
MPJ6Dx18	20
MPJ6Dx19	20
MPJ6Dx20	20
MPJ6DF1	20
MPJ6DF2	20
MPJ6DF3	20
MPJ6DF4	20
MPJ6DF5	20
MPPgemM2	20
MPPgemM3	20
MPPgemD5	20
MPPgemD7	20
MPPgemD11	20
MPPgemD8	20
MPC5M1	20
MPC5M2	20

MPC5M5	20
MPC5M7	20
MPC3Dron2	20

RX cut with ECORI and BSAI

- Digest with NEB enzymes
  - Digest in CutSmart® Buffer at 37°C.

Sample	Volume added
MPC3mAp	15
MPC3Dron1	15
MPJ6Dx1	15
MPJ6Dx2	15
MPJ6Dx3	15
MPJ6Dx4	15
MPJ6Dx6	15
MPJ6Dx7	15
MPJ6Dx8	15
MPJ6Dx9	15
MPJ6Dx10	15
MPJ6Dx11	15
MPJ6Dx12	15
MPJ6Dx13	15
MPJ6Dx14	15
MPJ6Dx15	15
MPJ6Dx16	15

MPJ6Dx17	15
MPJ6Dx18	15
MPJ6Dx19	15
MPJ6Dx20	15
MPJ6DF1	15
MPJ6DF2	15
MPJ6DF3	15
MPJ6DF4	15
MPJ6DF5	15
MPPgemM2	15
MPPgemM3	15
MPPgemD5	15
MPPgemD7	15
MPPgemD11	15
MPPgemD8	15
MPC5M1	15
MPC5M2	15
MPC5M5	15
MPC5M7	15
MPC3Dron2	15

Runnng gels .

14/09

PCR Rx to generate the 3 outputs (promoter t7 with single operator)

Using 3 templets

-t7-p22c2-tetr (for p22c2 output)

-t7 HkcI Tetr (for HkcI output)

-t7-tetr-p22c2 (for tetr output

RXI

Template -t7-p22c2-tetr (for p22c2 output) 1ng/ul

FWD	AC29	GGTAGTCCTTCTGGAATTCG	Tm	54.1
REV	AG21	ttcgctactagtcagcggaacagcgattaaag	Tm	64.8

ANN: 49.1

RX2:

-t7 HkcI Tetr (for HkcI output)1ng/ul

FWD	AC29	GGTAGTCCTTCTGGAATTCG	Tm	54.1
REV	AG20	ttcgctactagtcagagctgaacttatggttcac	Tm	63.7

ANN: 49.1

RX3:

-t7-tetr-p22c2 (for tetr output) 1ng/ul

FWD	AC29	GGTAGTCCTTCTGGAATTCG	Tm	54.1
REV	AG22	ttcgctactagtgaaggagtgtgatctctatcac	Tm	62.9

ANN: 49.1

15/09

Sending the samples for sequencing

18/09

Repeating the PCRs with a Phusion polymerase

n.	input	Primers	Melting temp	Annealing temp	Amplicon size

1	C0040 (tetR)	AG09/AG10	67.9/72.4	70.9	642 +70 bp
2	-ve ctrl				
3	C0050 (HKcI)	AG11/AG12	64.2/68.9	67.2	708+70 bp
4	-ve ctrl				
6	C0053 (PP22c2)	AG13/AG14	67/64.8	67.8	739 +70bp
5	-ve ctrl				
7	K564013 (lacz)	AG07/AG08	68.1/69.4	71.1	3100 +70
8	-ve ctrl	AG07/AG08			
9	BBa_I2032 (T7 pol)	AG03/AG04	66.1/67.6	66.1	2655 +70
10	-ve ctrl	AG03/AG04			
11	t7-p22c2-tetr (for p22c2 output)	Fwd: AC29 REV: AG21	60.6/74.6	60.6	
12	-ve	Fwd: AC29 REV: AG21			
13	t7 HkcI Tetr (for HkcI output)	Fwd: AC29 REV: AG20	60.6/72.1	60.6	
14	-ve	Fwd: AC29 REV: AG20			
15	t7-tetr-p22c2 (for tetr output)	Fwd: AC29 REV: AG22	60.6/70.9	60.6	
16	-ve	AC29/AG22			
17	Geneblock dronpa	AG15/suffix	63.3/71.8	66.7	1.5
18	-ve ctrl	AG15/suffix	63.3/71.8	66.7	1.5

- 10mM dNTPs 1ul
- Phusion DNA Polymerase 0.5ul
- 5x Phusion HF Buffer 10ul
- Nuclease-Free Water up to 35.5
- 1ng/ul template 1ul
- 10uM Fwd primer 1ul
- 10uM Rev Primer 1ul

Master mix for 20 rx
20 ul DNTPs
10ul Phusion DNA polymerase
710ul water
200 ul 5x Phusion HF buffer

47 ul MM/Rx

Extension time : 30 sec/ 1kb
30 Sec (for tetR, P22C2,HKCl)
60 sec (lacz and t7 polymerase)
60 sec for dronpa

Reactions (1-6)

Initial denaturation	**98°C**	**30 s**
Denaturation	**98°C**	**10 s**
Annealing	**67.2-70.9**	**30s**
Extension	**72°C**	**30s**
	34 cycel	
final extension	**72°C**	**5min**
hold	**12°C**	**∞**

Reactions (11-16)

Initial denaturation	**98°C**	**30 s**
Denaturation	**98°C**	**10 s**
Annealing	**60.6**	**5s**
Extension	**72°C**	**5s**
	34 cycel	
final extension	**72°C**	**5min**
hold	**12°C**	**∞**

Rxs 7-10, 17& 18

Initial denaturation	**98°C**	**30 s**
Denaturation	**98°C**	**10 s**
Annealing	**66.1-71.1**	**30s**
Extension	**72°C**	**60s**
	34 cycel	
final extension	**72°C**	**5min**
hold	**12°C**	**∞**

19/09/17

Pcr purification of Samples.
Clean up digestion by 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 30 at 13000 rpm. 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 13000 rpm Discard flow-through and place the QIAquick column back in the same tube.

Centrifuge the QIAquick column once more for 1 min ro 13000 rpm.

Place each QIAquick column in a clean 1.5mL microcentrifuge tube.

Add 35µL water heated to 50°C. Incubate 1 min at 50°C.

Centrifuge for 1 min at 13000 rpm.

Golden gate

Golden Gate into Backbone 23

	1	2	3	4	5
Part	**TetR**	**HKcl**	**P22c2**	**T7**	**LacZ**
Backbone 23	**2.34**	**2.34**	**2.34**	**2.34**	**2.34**
Insert	**6.74**	**4.59**	**7.76**	**3.49**	**9.01**
T4 ligase	1	1	1	1	1
BsaI	1	1	1	1	1
10X T4 buffer	2	2	2	2	2
10X BsaI Buffer	2	2	2	2	2
Water	**4.92**	**7.07**	**3.9**	**8.17**	**2.65**
Total	**20**	**20**	**20**	**20**	**20**

Golden Gate into Backbone 4

	6	7	8	9	10
Part	**TetR**	**HKcl**	**P22c2**	**T7**	**LacZ**

Backbone 4	**1.71**	**1.71**	**1.71**	**1.71**	**1.71**
Insert	**6.74**	**4.59**	**7.76**	**3.49**	**9.01**
T4 ligase	1	1	1	1	1
BsaI	1	1	1	1	1
10X T4 buffer	2	2	2	2	2
10X BsaI Buffer	2	2	2	2	2
Water	**5.55**	**7.7**	**4.53**	**8.8**	**3.28**
Total	**20**	**20**	**20**	**20**	**20**

Golden Gate into Backbone 6

	11	**12**	**13**	**14**	**15**
Part	**TetR**	**HKcl**	**P22c2**	**T7**	**LacZ**
Backbone 6	**2.18**	**2.18**	**2.18**	**2.18**	**2.18**
Insert	**6.74**	**4.59**	**7.76**	**3.49**	**9.01**
T4 ligase	1	1	1	1	1
BsaI	1	1	1	1	1
10X T4 buffer	2	2	2	2	2
10X BsaI Buffer	2	2	2	2	2
Water	**5.08**	**7.23**	**4.06**	**8.33**	**2.81**
Total	**20**	**20**	**20**	**20**	**20**

Restriction digestion reactions for mApple to be ligated into 1c31210

Buffer	ECORI(	PstI	200 ng DNA	Water
3ul	1ul	1 ul	10 ul	15

Restriction digestion reactions for pSB1C3-R0010-sfGFP

Buffer FD 10x	ECORI	PstI	Fast Ap	300 ng DNA	Water
3ul	1ul	1 ul	1ul	5ul	19

Restriction digestion reactions for Dronpa to be ligated into J23110

Buffer	xbaI(	PstI	200 ng DNA	Water
3ul	1ul	1 ul	20	5

2PCR clean up

Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
Place the sample in the column & centrifuge for 30 at 13000 rpm. 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 13000 rpm Discard flow-through and place the QIAquick column back in the same tube.

Centrifuge the QIAquick column once more for 1 min ro 13000 rpm

Place each QIAquick column in a clean 1.5mL microcentrifuge tube.

Add 20 µL water heated to 50°C. Incubate 1 min at 50°C.

Centrifuge for 1 min at 13000 rpm.

311:Dronpa xb + J6 (306+301)

insert	vector	10x ligation buffer	DNA ligase	water
6ul	2ul	2ul	1ul	Up to 20ul

:mApple full +(Gfp vector) (

insert	vector	10x ligation buffer	DNA ligase	water
20ul	4ul	2ul	1ul	Up to 30ul

Overnight ligation reaction at 16 C.

20/09/2017

By ACB <3

Transformation of the ligation reaction M1 (Chlo)

Transformation of the ligation reaction Dx (Amp)

Transformation of the golden gate results :

1-15 (Amp)

21/09/2017

Transformation of t7+mrfp plasmid into Bl21

Checking transformation results from yeterday.

PCR RX :

Preparing the parts (TetR, P22c2,HKCl, T7 , lac z) to be inserted in mApple

Repeating the PCRs with a Phusion polymerase

n.	input	Primers	Melting temp	Annealing temp
1	C0040 (tetR)	AG27 / AG28	67.9/72.4	70.9
2	-ve ctrl			
3	C0050 (HKcI)	AG29/ AG30	64.2/68.9	67.2
4	-ve ctrl			
6	C0053 (PP22c2)	AG31/AG32	67/64.8	67.8
5	-ve ctrl			
7	K564013 (lacz)	AG25/AG26	68.1/69.4	71.1

8	-ve ctrl			
9	BBa_I2032 (T7 pol)	AG23/AG24	66.1/67.6	66.1
10	-ve ctrl			

- 10mM dNTPs 1ul
- Phusion DNA Polymerase 0.5ul
- 5x Phusion HF Buffer 10ul
- Nuclease-Free Water up to 35.5
- 1ng/ul template 1ul
- 10uM Fwd primer 1ul
- 10uM Rev Primer 1ul

Master mix for 12 rx
12 ul DNTPs
6 ul Phusion DNA polymerase
426 ul water
120 ul 5x Phusion HF buffer

Reactions (1-6)

Initial denaturation	**98°C**	**30 s**
Denaturation	**98°C**	**10 s**
Annealing	**67.2-70.9**	**30s**
Extension	**72°C**	**30s**
	34 cycel	
final extension	**72°C**	**5min**
hold	**12°C**	**∞**

Reactions (11-16)

Initial denaturation	**98°C**	**30 s**
Denaturation	**98°C**	**10 s**
Annealing	**60.6**	**5s**
Extension	**72°C**	**5s**
	34 cycel	
final extension	**72°C**	**5min**
hold	**12°C**	**∞**

Rxs 7-10, 17& 18

Initial denaturation	**98°C**	**30 s**
Denaturation	**98°C**	**10 s**
Annealing	**66.1-71.1**	**30s**
Extension	**72°C**	**60s**
	34 cycel	
final extension	**72°C**	**5min**
hold	**12°C**	**∞**

Results

Gel by Phusion polymerase generation of parts to be inserted in mAPPLE :

22/09

Inoculating colonies in preparation for miniprep
Culturing the part sent from ETH Zurich

23/09

Restriction digestion reaction
(for the pgem vector)
For mapple part
For the gfp plasmid

Pst1 and ,ECORI 30ul reaction

Restriction digestion reactions for mApple to be ligated into pSB1C3-R0010-sfGFP

Buffer	ECORI(	PstI	200 ng DNA	Water
2ul	1ul	1 ul	10 ul	1

Restriction digestion reactions for pSB1C3-R0010-sfGFP

Buffer FD 10x	ECORI	PstI	Fast Ap	500 ng DNA	Water
3ul	1ul	1 ul	1ul	10ul	14

Restriction digestion reactions for mApplein Pgem (27/28)

to be ligated into pSB1C3-R0010-sfGFP

Buffer	ECORI(	PstI	DNA	Water
3ul	1ul	1 ul	20 ul (1ug) 27 10ul (3ug) 28	15

Pcr clean up for the HKCI and P22c22 for mApple (the pcr products)
Clean up for digestion results for mApple and pSB1C3-R0010-sfGFP

Gel extraction for mapple in pgem (no correct bands in the gel)

Inserting mApple in T vector (Second time)

*Adding the 3' overhang to the geneblock:

	Final Concentration
Mapple (gene block)	100ng (5ul gene block)
Master mix dream taq 2x	5 ul

***Incubate 30 min at 72 °C.**

*Set up ligation reaction in PGEM vector

	PGEM+Mapple	PGEM+ve	PGEM-ve
PCR product	8ul	-	-
2X Rapid Ligation Buffer, T4 DNA Ligase	10ul	5	5
pGEM Vector (50ng)	1ul	1	1
T4 DNA Ligase (3 Weiss units/µl)	1ul	1	1
Control insert DNA	-	2ul	-
nuclease-free water	-	Up to 10ul	Up to 10ul

-Ligation for Overnight at 4 C

Plasmid miniprep for the Golden gates plasmids GG.01-GG.15 and the part

24/09

Results of the plasmid miniprep :

Glycerol stock	Plasmid	Conc ng/ul	260/280	260/230
AG.A2.2309.GG1.01	GG1.01	177	1.91	2.33
AG.A2.2309.GG1.02	GG1.02	58.3	2.03	2.1
AG.A2.2309.GG1.03	GG1.03	89.6	1.91	1.91
AG.A2.2309.GG1.04	GG1.04	19.2	2.24	2.21
AG.A2.2309.GG1.05	GG1.05	96.2	1.93	2.63
AG.A2.2309.GG1.06	GG1.06	86.9	1.93	2.64
AG.A2.2309.GG1.07	GG1.07	31.4	2.08	3.11
AG.A2.2309.GG1.08	GG1.08	98.7	1.94	2.4
AG.A2.2309.GG1.09	GG1.09	32.5	2.09	2.74
AG.A2.2309.GG1.10	GG1.10	17.6	2.08	8.43
AG.A2.2309.GG1.11	GG1.11	73.9	1.97	2.15
AG.A2.2309.GG1.12	GG1.12	48.6	1.93	2.36
AG.A2.2309.GG1.13	GG1.13	55.1	1.91	1.62

AG.A2.2309.GG1.14	GG1.14	33	2.09	1.6
AG.A2.2309.GG1.15	GG1.15	67	1.95	2.07
AG.C3.2309 top 10	gusA	155.4	1.91	2.18
AG.C3.2309.gfp	C3-R0010-sfgfp	33	1.98	2.12

Performing the following transformations:
GG1.05 ,GG1.010 and GG1.15 into JF1 (plated on Ampicillin plates)
Gfp vector+mApple, gfp vector into DH5alpha (plated on Chloramphenicol plates)

The Pgem transformations:
Pgem+mApple (from 1st reaction)
Pgem+mApple (from 2nd reaction)
Pgem +ve and -ve controls

**Pgem vectors are plated on Ampicillin plates +X-gal

Transformation protocol

- Thaw competent cells on ice for 30min
- Aliquot 50µL in 1.5mL Eppendorf tubes.
- Add 1µL DNA in the cells. Mix gently.
- Incubate 30min on ice.
- Heat shock: 1 min at 42°C.
- Let rest on ice for 5min.
- Add 950µL of SOC medium (warmed) 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+Antibiotic plates:
    - 10%: 10µL of the reaction
    - 90%: 90µL of the reaction
- Incubate overnight at 37°C

25/09

Sending the Golden gates results to sequencing:

8 FC6960 G1.15 325454 VR	1 FC6846 G1.15 325453 VF2
9 FC6961 G1.14 325454 VR	2 FC6847 G1.14 325453 VF2
10 FC6962 G1.13 325454 VR	3 FC6848 G1.13 325453 VF2
11 FC6963 G1.12 325454 VR	4 FC6849 G1.12 325453 VF2
12 FC6964 G1.11 325454 VR	5 FC6850 G1.11 325453 VF2
13 FC6965 G1.10 325454 VR	6 FC6851 G1.10 325453 VF2
14 FC6966 G1.09 325454 VR	7 FC6852 G1.09 325453 VF2

Checking transformation results

Experimenting lacz.

26 /09

Sample no:	Primers	Tm	Ann	Extension time
	AG18/AG19	56.0/55.4	50.4	2 mins

Diagnostics of the mApple colonies .

27/09

PcR reactions to determine the colonies with the lacz

Results from mApple diagnostics.

Results from lacz diagnostics.

Subculture of the right colonies.

28/09

Plasmid miniprep

Bugbusters was used for protein extraction.

Protein concentration were measured on Nanodrop.

10/10

	6	7	8	9	10
Part	**TetR**	**HKcI**	**P22c2**	**T7**	**LacZ**
Backbone 4	**1.71**	**1.71**	**1.71**	**1.71**	**1.71**
Insert	**6.74**	**4.59**	**7.76**	**3.49**	**9.01**
T4 ligase	1	1	1	1	1
BsaI	1	1	1	1	1
10X T4 buffer	2	2	2	2	2
10X BsaI Buffer	2	2	2	2	2
Water	**5.55**	**7.7**	**4.53**	**8.8**	**3.28**
Total	**20**	**20**	**20**	**20**	**20**

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The D23D4D6 lac z experiment

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transformations

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Native gel for Dronpa lacz constructs to determine the concentration

Check transformation results

Restriction digestion of another c3 vector (gel purification) and c3 gfp (with AP)

- Making a new ligation reaction
-

Order primers for the assembly of Dronpa mApple fusion

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- Make a dilution of G.05 G.10 G.15
  Run a native gel
- The results in a GFP filter

Coomasie staining of the proteins :

Check the transformation results (of t7+mrfp in G1.09)
Pick the colonies that shows both red and green

Transformations in a DH5 alpha of
CARp 3 Carp4 (amp resistance for sasha)
P10 and p30 (Cam resistance for alma)
Plong in c3
P short in C3
T long in c3
T short in c3
T3 long in c3
T3 short in c3

Plong in c3 gfp
P short in C3gfp
T long in c3 gfp
T short in c3 gfp
T3 long in c3 gfp
T3 short in c3 gfp

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- Checking transformations results (only carp 3 and carp 4 worked)

  Comment : for the pcr products maybe the restriction digestion wasn't done appropriatry due to the buffer (repeat)

- Colony pcr for (t7 pol in D6 gusAin D6)
- Run a gel
- Pick the right colonies
- Mutagensis in the mAPP
- (varaiations of DMSo and mgcl2 conc)
- Run a gel
  Purification restriction digestion

- Gel
- Ligation

-make glycerol stock of the over night cultures
lacz in wt
Lacz in mut 1 lacz in mut 2
T7 polemrase in dronpa wt
T7 polemrase in dronpa mut 1 + mrfp

Plasmid miniprep
lacz in wt
Lacz in mut 1 lacz in mut 2
T7 polemrase in dronpa wt
T7 polemrase in dronpa mut 1 + mrfp

Pcr using phusion (for mApp gene block)

AG25	Lacz for mA FWD	ATGCAGGGTCTCACAGCaccatgattacggattcactggc
AG26	Lacz for mA REV	ATGCAGGGTCTCAGGCCtttttgacaccagaccaactggtaatg

AG07	fwd Lacz with linkers and BsaI RS	ATGCAGGGTCTCGCAGTGGTAGTAGTGGTAGCAGCaccatgattacggattcactggc
AG08	rev Lacz with linkers and BsaI RS	ATGCAGGGTCTCGCTGATGTACCtgaACCCTTAAGGCCtttttgacaccagaccaactggtaatg

mA fwd Dronpa Rev	AG25 AG08	Ann
Dronpa fwd mA REV	AG26 AG07	Ann

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Arrival of rsTag RFP primers

- Miniprep of the overnight culture of the strain with rsTag RFP :
  -Pcr amplification of rsTag RFP and Lacz in preparation for the Gibson assembly :

AG45 /AG46
AG47/AG48

AG49/ AG 50