Nanoreactor Lab Book

Project: Nanoreactor
Author: Marina Schreidl
Status: In progress
Entry Created On: 06 May 2026 15:38:18 UTC
Entry Last Modified: 18 Oct 2026 00:12:35 UTC
Export Generated On: 18 Oct 2026 00:15:51 UTC

DIENSTAG, 30.6.2026

Oligo annealing according to Oligo Annealing of:

0.1rc_b_28 SpyTag
0.2_b_32 L2
0.2_b_33 L2rc
0.2_b_34 L3
0.2_b_35 L3rc

Golden Gate according to Golden Gate Assembly of:

0.1rc_b_28 SpyTag
0.2_b_32 L2
0.2_b_33 L2rc
0.2_b_34 L3
0.2_b_35 L3rc

MITTWOCH, 1.7.2026

Trafo according to E. coli Transformation of:

0.1rc_b_28 SpyTag
0.2_b_32 L2
0.2_b_33 L2rc
0.2_b_34 L3
0.2_b_35 L3rc

and 30 ul on plates

Golden Gate according to Golden Gate Assembly of:

0.1_b_27 SpyCatcher
0.3_b_31 LGB4

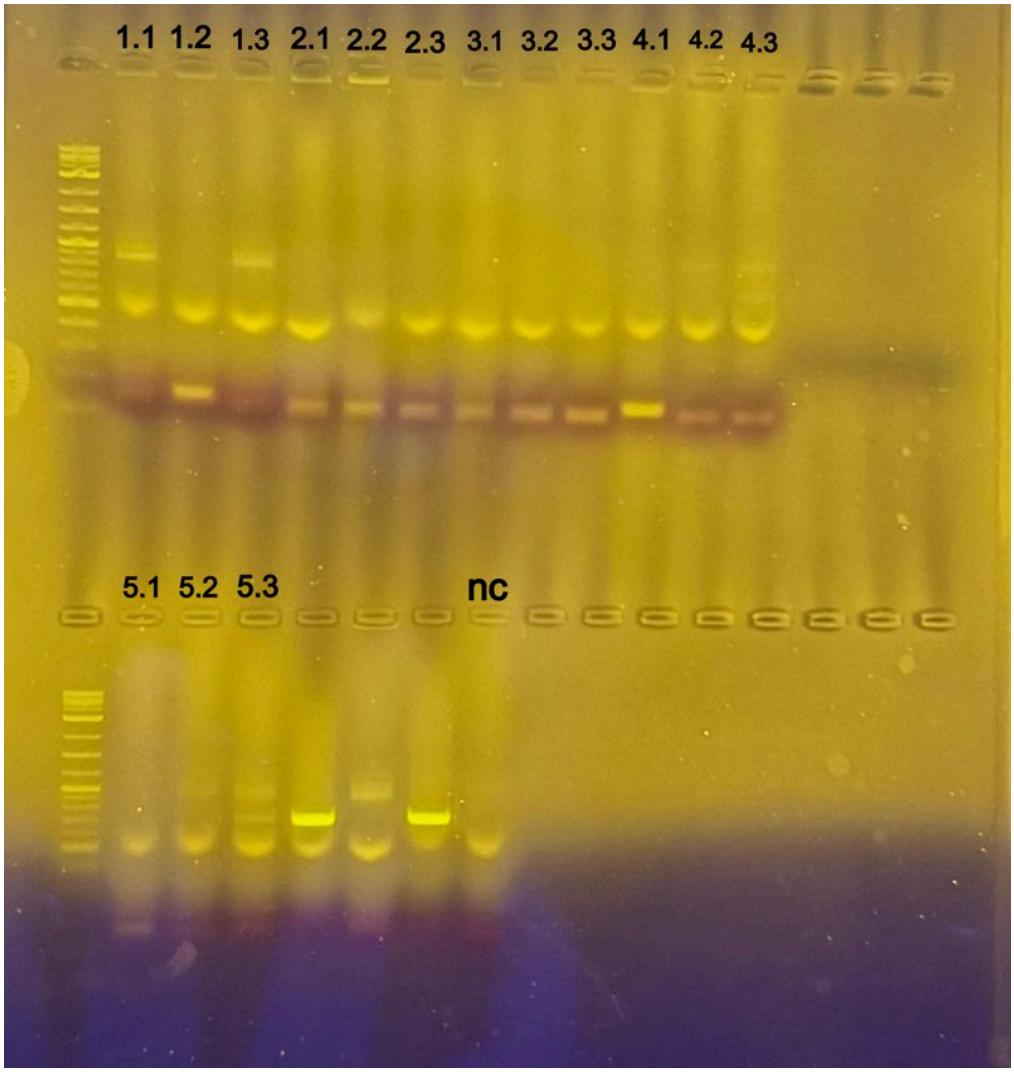
DONNERSTAG, 2.7.2026

cPCR according to Taq colony PCR of:

1. 0.1rc_b_28 SpyTag
2. 0.2_b_32 L2
3. 0.2_b_33 L2rc
4. 0.2_b_34 L3
5. 0.2_b_35 L3rc

with 30 cycles, TM = 54, annealing = 60 s, elongation = 30 s
2 % gel, 130 V and 35 mins

cPCR 02.07.26



Overnight of:

0.1rc_b_28 SpyTag 1.2
0.2_b_32 L2 2.2
0.2_b_33 L2rc 3.3
0.2_b_34 L3 4.2
0.2_b_35 L3rc 5.1 and 5.2

in 3 ml LB

Trafo according to E. coli Transformation of:

0.1_b_27 SpyCatcher
0.3_b_31 LGB4

and 30 ul on plates

XGal wasn't on the plates, redo

FREITAG, 3.7.2026

Miniprep, according to instructions, of overnights:

0.1rc_b_28 SpyTag 1.2
0.2_b_32 L2 2.2
0.2_b_33 L2rc 3.3
0.2_b_34 L3 4.2
0.2_b_35 L3rc 5.1 and 5.2

concentration in table below
5.1 was to bad, redo with better MP colony
5.2 silica membrane was dropped at some point, mayby redo someday

Prep for Sequencing Lvl 0
12µl of plasmid eluated containing 40-100 ng/µl
3 µl of 20uM 69_level0_seq_fw
Economy run in 1.5 Eppis

Sequencing 03.07.

	A	B	C	D	E	F	G	H
1	seq number		construct	ng/ml	plasmid ul	plasmid amount	Water amount	
2	98902308	1	0.1rc_b_28 SpyTag	318.0	3	79.5	9	
3	98902309	2	0.2_b_32 L2	389.8	3	97.45	9	
4	98902310	3	0.2_b_33 L2rc	408.0	2.5	85	9.5	
5	98902311	4	0.2_b_34 L3	249.8	4	83.2666666667	8	
6	98902312	5	0.2_b_35 L3rc 5.2	96.3	12	96.3	0	

redo Trafo according to E. coli Transformation of:

0.1_b_27 SpyCatcher
0.3_b_31 LGB4

and 30 ul on plates

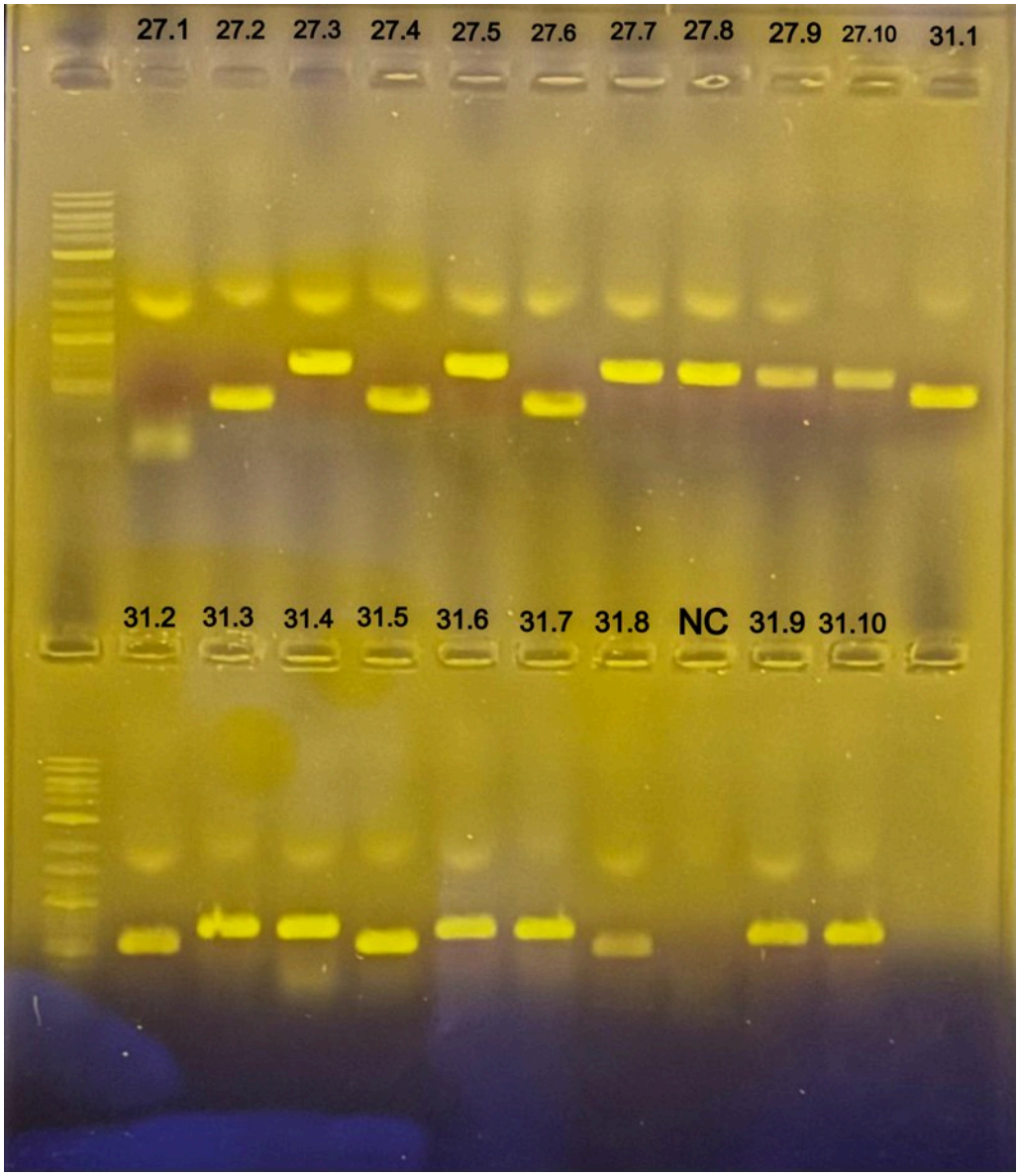
MONTAG, 6.7.2026

cPCR according to Taq colony PCR of:

1. 0.1_b_27 SpyCatcher
2. 0.3_b_31 LGB4

with 30 cycles, TM = 54, annealing = 60 s, elongation = 30 s
1 % gel, 120 V and 30 mins

cPCR 06.07.26



Overnight of:

0.2_b_35 L3rc 5.1 from last cPCR 02.07.
0.1_b_27 SpyCatcher 27.2
0.3_b_31 LGB4 31.5

DIENSTAG, 7.7.2026

Sequencing 03.07. results:

0.1rc_b_28 SpyTag = bad, no insert
0.2_b_32 L2 = correct
0.2_b_33 L2rc = correct
0.2_b_34 L3 = bad
0.2_b_35 L3rc 5.2 = bad

Miniprep, according to instructions, of overnights:

0.2_b_35 L3rc 5.1
0.1_b_27 SpyCatcher 27.2
0.3_b_31 LGB4 31.5

concentration in table below

Prep for Sequencing Lvl 0
12µl of plasmid eluated containing 40-100 ng/µl
3 µl of 20uM 69_level0_seq_fw
Economy run in 1.5 Eppis

Sequencing 07.07.

	A	B	C	D	E	F	G	H
1	seq number		construct	ng/ml	plasmid ul	plasmid amount	Water amount	
2	98902313	1	0.1_b_27 SpyCatcher	264.0	4	88	8	
3	98902314	2	0.3_b_31 LGB4	424.8	2	70.8	10	
4	98902315	3	0.2_b_35 L3rc 5.1	281.5	4	93.8333333333	8	

Golden Gate according to Golden Gate Assembly of:

0.1rc_b_28 SpyTag
0.2_b_34 L3
0.2_b_35 L3rc
0.3_b_64 NlucP

0.3_b_65 CiVCPO

MM: instead of 2.6 ul Bbs1, used 0.52 ul of new Bbs1, difference added in H2O; MM for DNA used for oligos, so 11.5 ul in total

Overnight of:

0.2_b_32 L2 (G)
0.2_b_33 L2rc (G)

forgot Antibiotics, redo

MITTWOCH, 8.7.2026

Trafo according to E. coli Transformation of:

0.1rc_b_28 SpyTag
0.2_b_34 L3
0.2_b_35 L3rc
0.3_b_64 NlucP
0.3_b_65 CiVCPO

and 30 ul on plates

Overnight of:

0.2_b_32 L2 (G) with Antibiotics
0.2_b_33 L2rc (G) with Antibiotics

DONNERSTAG, 9.7.2026

Glycerol stock of:

0.2_b_32 L2 (G) with Antibiotics
0.2_b_33 L2rc (G) with Antibiotics

Sequencing 03.07. results:

0.2_b_35 L3rc 5.1 = bad, as 5.2
0.1_b_27 SpyCatcher 27.2 = correct

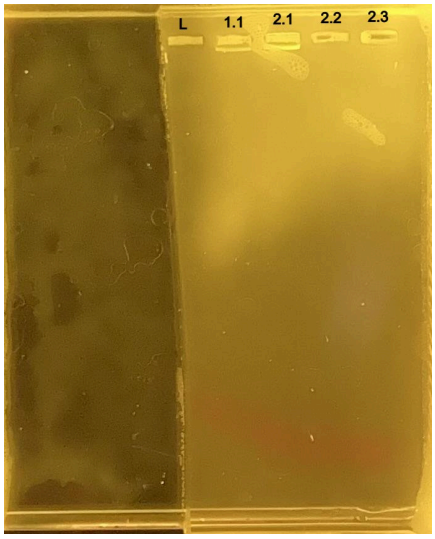
0.3_b_31 LGB4 31.5 = correct

cPCR according to Taq colony PCR of:

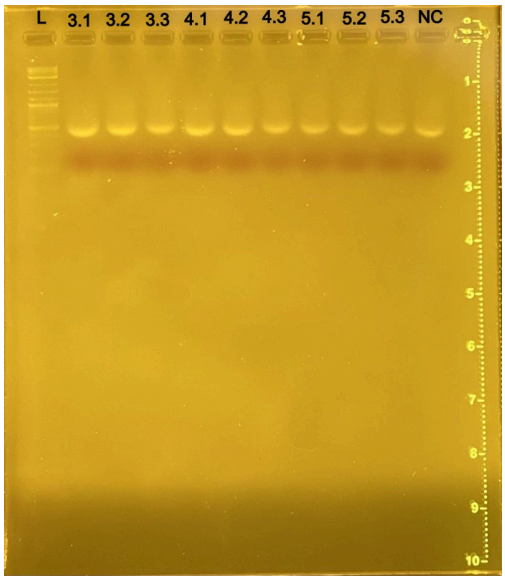
1. 0.3_b_64 NlucP
2. 0.3_b_65 CiVCPO
3. 0.2_b_34 L3
4. 0.2_b_35 L3rc
5. 0.1rc_b_28 SpyTag

with 30 cycles, TM = 54, annealing = 60 s, elongation = 2 mins
DNA = 1 % gel, 130 V and 25 mins
Oligos = 2 % gel, 130 V and 25 mins

cPCR 09.07.



cPCR 09.07.



Overnight of:

0.2_b_34 L3 4.1 from 02.07.
0.1_b_27 SpyCatcher 27.2 (G) with Antibiotics from 06.07.
0.3_b_31 LGB4 31.5 (G) with Antibiotics from 06.07.

FREITAG, 10.7.2026

Glycerol stock of:

0.1_b_27 SpyCatcher 27.2 (G) with Antibiotics
0.3_b_31 LGB4 31.5 (G) with Antibiotics

Miniprep, according to instructions, of overnights:

0.2_b_34 L3 4.1

concentration in table below

Prep for Sequencing Lvl 0
12µl of plasmid eluated containing 40-100 ng/µl

3 µl of 20uM 69_level0_seq_fw
Economy run in 1.5 Eppis

Sequencing 10.07.

	A	B	C	D	E	F	G	H
1	seq number		construct	ng/ml	plasmid ul	plasmid amount	Water amount	
2	98902324	1	0.2_b_34 L3 4.1	397.6	2	66.2666666667	10	

DIENSTAG, 14.7.2026

Sequencing 10.07. results:

0.2_b_34 L3 4.1 = correct, but turned from 3x into 4x(GSSS) Linker

new construct 0.2_b_67 L4 with that

Golden Gate according to Golden Gate Assembly of:

0.1rc_b_28 SpyTag
0.2_b_34 L3
0.2_b_35 L3rc
0.3_b_64 NlucP
0.3_b_65 CiVCPO

Overnight of:

0.2_b_34 L3 4.1 (G) with Antibiotics for 0.2_b_67 L4 stock

MITTWOCH, 15.7.2026

Trafo according to E. coli Transformation of:

0.1rc_b_28 SpyTag
0.2_b_34 L3
0.2_b_35 L3rc
0.3_b_64 NlucP
0.3_b_65 CiVCPO

0.5_a_30 NCS
0.3_a_18 Mx

1h incubation and 30 ul on plates

DONNERSTAG, 16.7.2026

cPCR according to Taq colony PCR of:

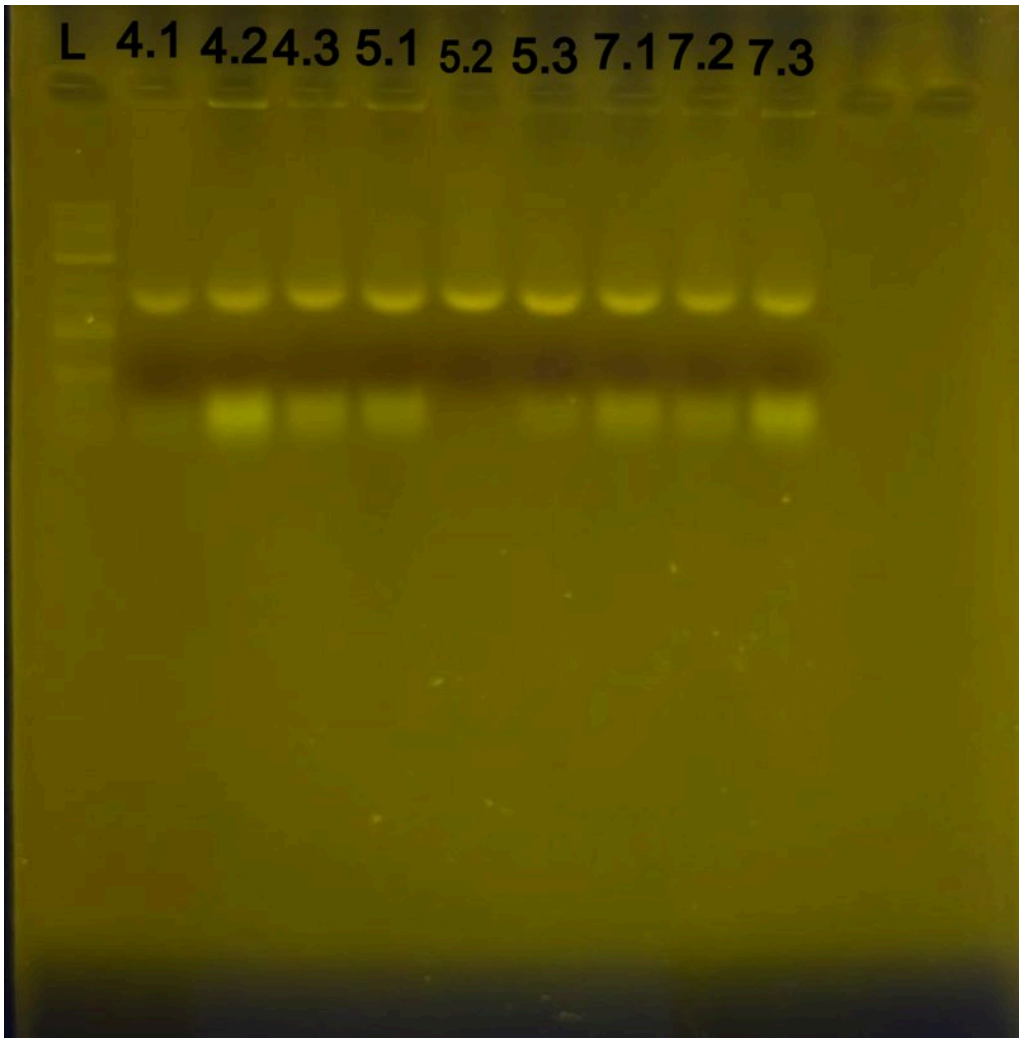
1. 0.1rc_b_28 SpyTag
2. 0.2_b_34 L3
3. 0.2_b_35 L3rc
4. 0.3_b_64 NlucP
5. 0.3_b_65 CiVCPO

6. 0.5_a_30 NCS
7. 0.3_a_18 Mx

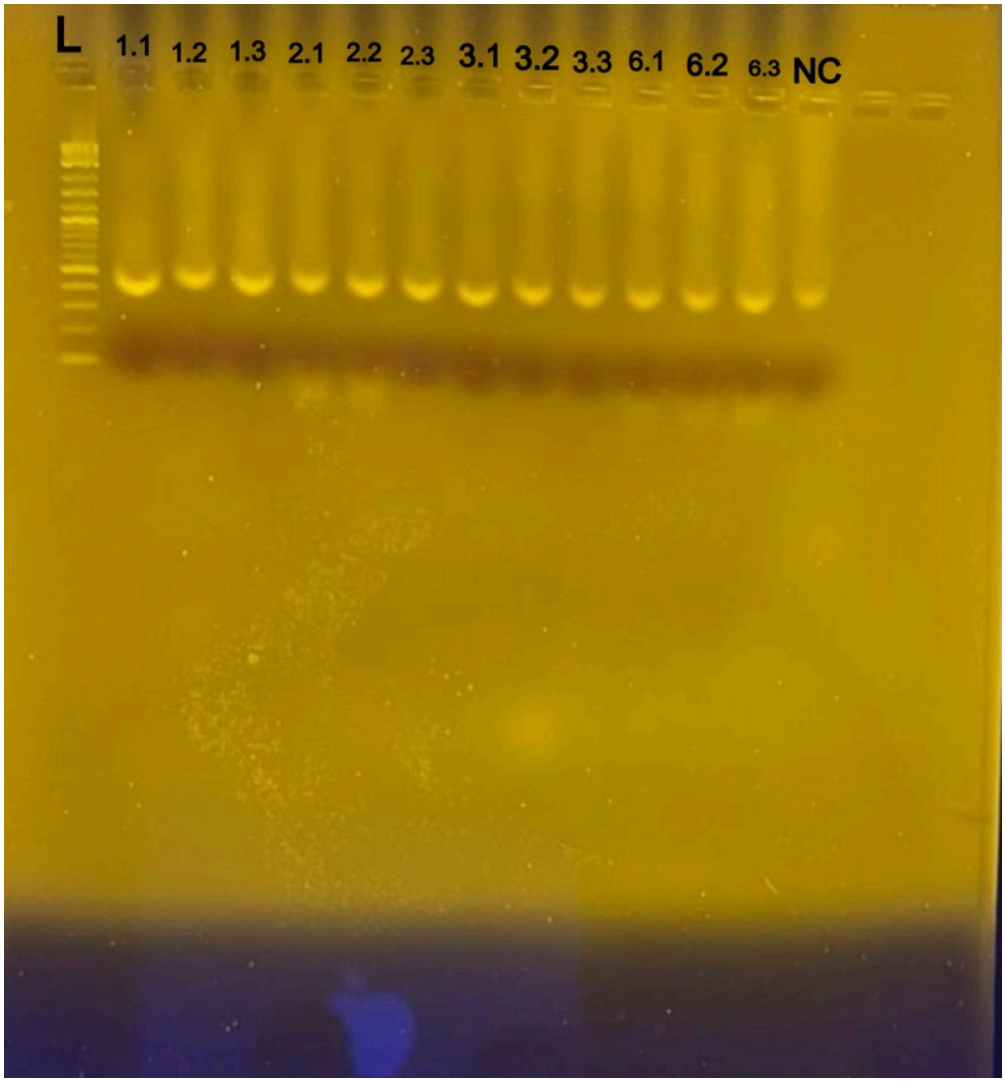
with 30 cycles, TM = 54, annealing = 60 s, elongation = 2 mins
DNA = 1 % gel, 120 V and 25 mins
Oligos = 2 % gel, 120 V and 25 mins

didn't fucking work

cPCR 16.07. 1% 1



cPCR 16.07. 2% 1



redo that shit

redo cPCR according to Taq colony PCR of:

2% Gel:

1. 0.1rc_b_28 SpyTag
2. 0.2_b_34 L3
3. 0.2_b_35 L3rc
4. 0.5_a_30 NCS

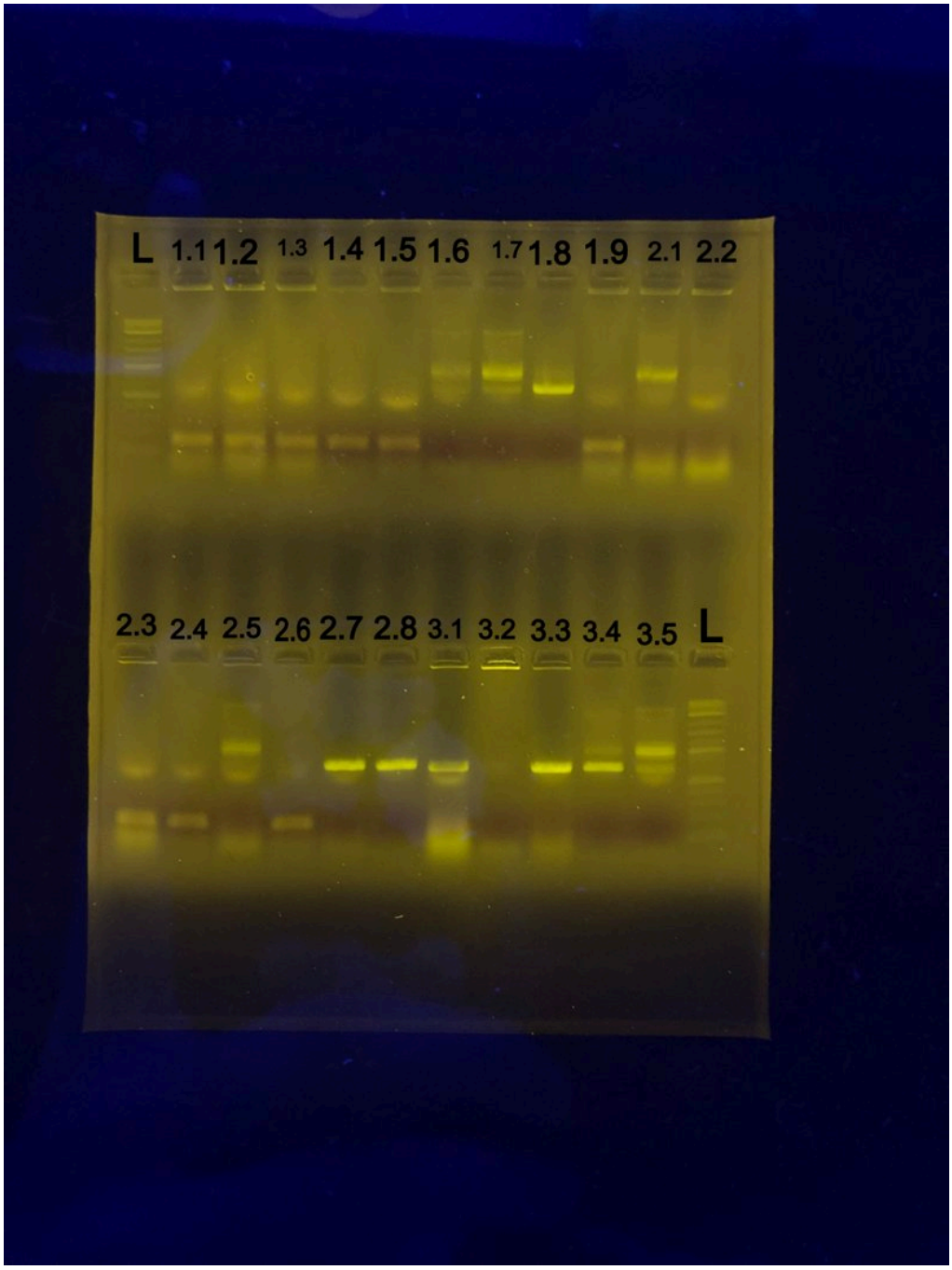
1% Gel:

1. 0.3_b_64 NlucP
2. 0.3_b_65 CiVCPO
3. 0.3_a_18 Mx

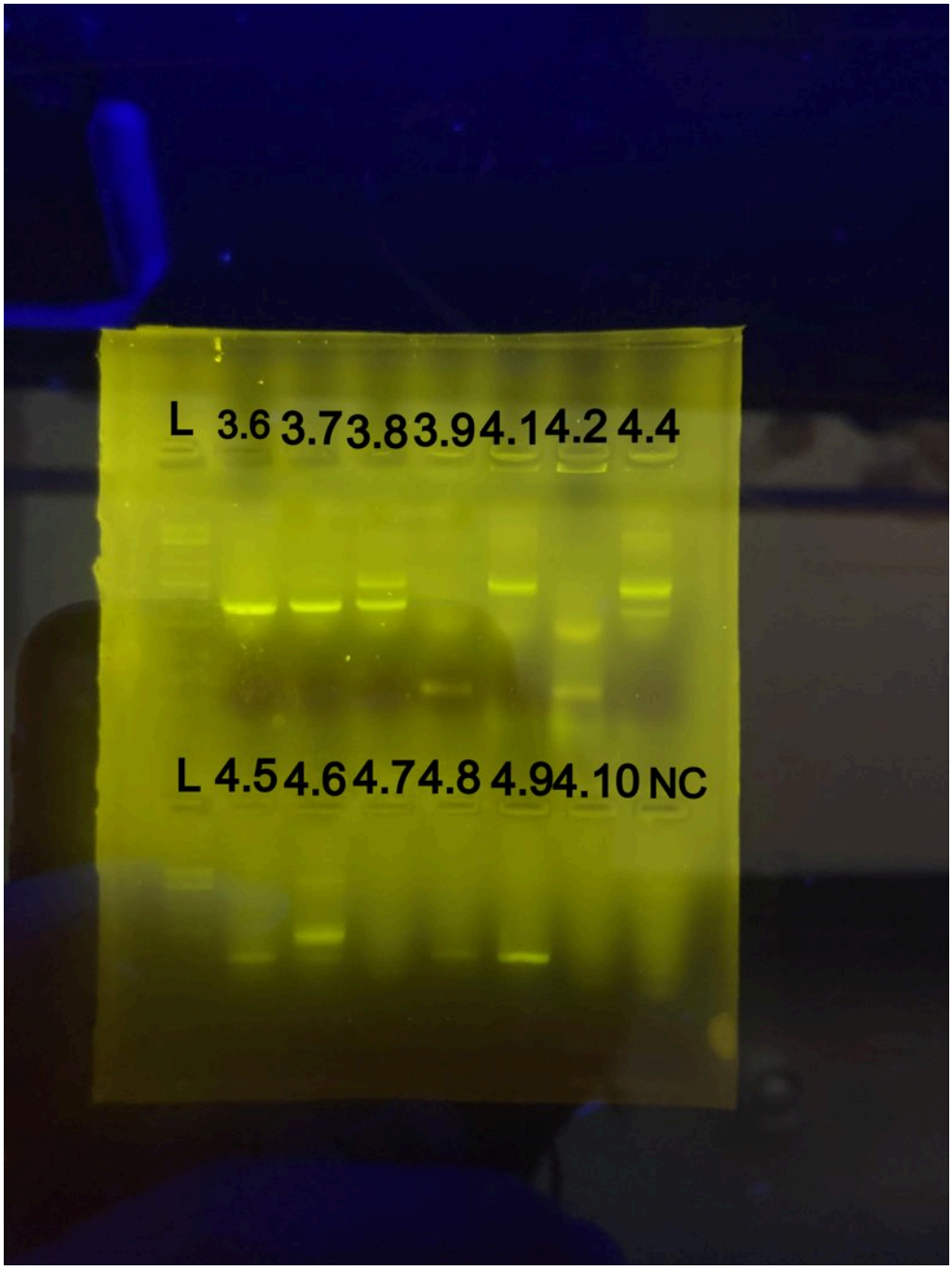
with 30 cycles, TM = 54, annealing = 60 s, elongation = 2 mins
DNA = 1 % gel, 130 V and 20 mins
Oligos = 2 % gel, 130 V and 20 mins

colonies from masterplate + where possible more, max. 10

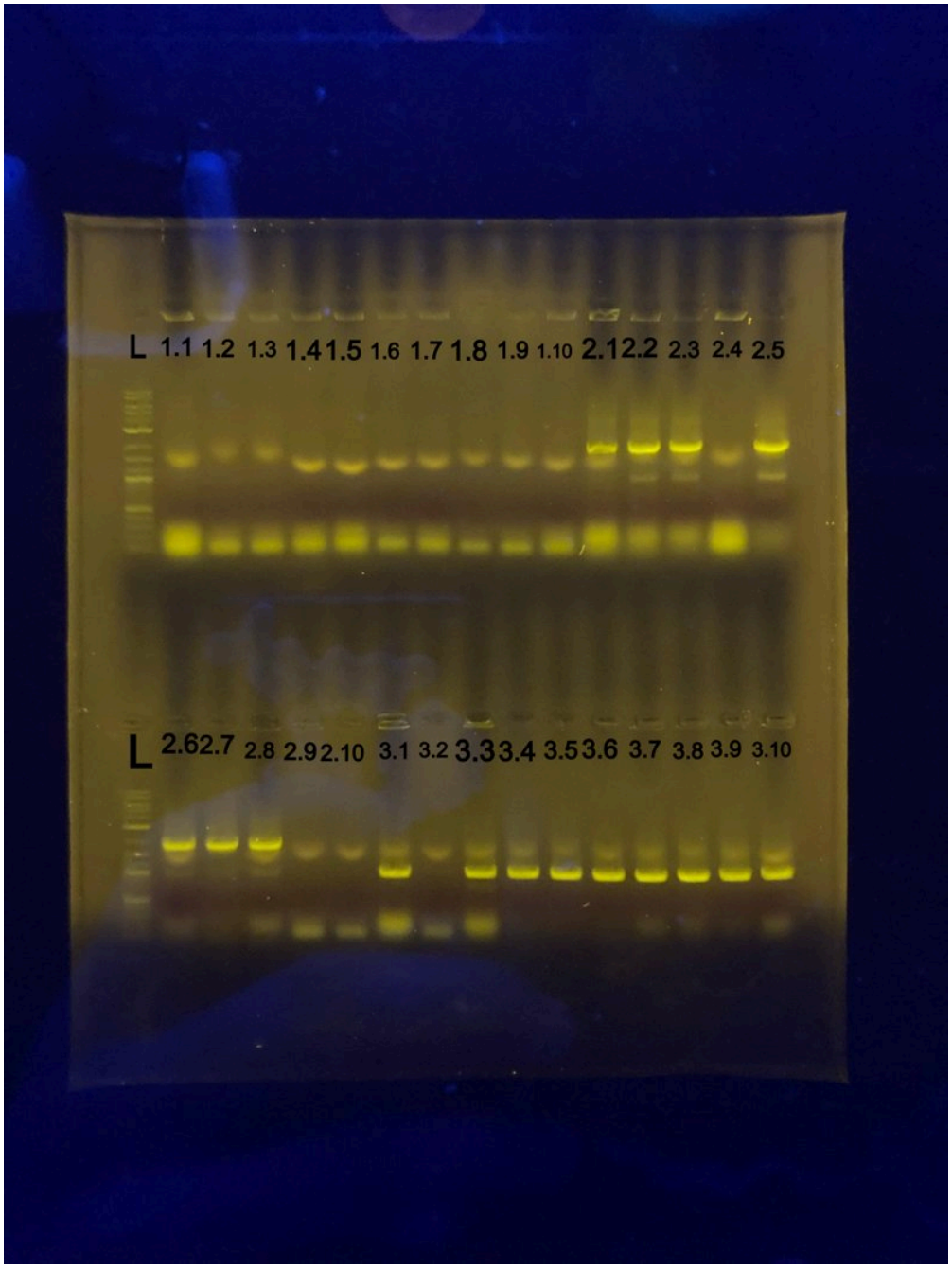
cPCR 16.07. 2% 2



cPCR 16.07. 2% 3



cPCR 16.07. 1% 2



0.3_b_64 NlucP: no band, redo

Overnight of:

0.1rc_b_28 SpyTag 1.1
0.1rc_b_28 SpyTag 1.3
0.2_b_34 L3 2.4
0.2_b_34 L3 2.6
0.2_b_35 L3rc 3.9
0.5_a_30 NCS 4.2

0.3_b_65 CiVCPO 2.3
0.3_b_65 CiVCPO 2.7
0.3_a_18 Mx 3.4
0.3_a_18 Mx 3.5
0.3_a_18 Mx 3.6

FREITAG, 17.7.2026

redo cPCR according to Taq colony PCR of:

0.3_b_64 NlucP

with 30 cycles, TM = 54, annealing = 60 s, elongation = 60 s
DNA = 1 % gel, 120 V and 15 mins

10 new colonies

cPCR 17.07.2026



Miniprep, according to instructions, of overnights:

0.1rc_b_28 SpyTag 1.3
0.2_b_34 L3 2.6
0.2_b_35 L3rc 3.9
0.3_b_65 CiVCPO 2.7

0.5_a_30 NCS 4.2
0.3_a_18 Mx 3.4

concentration in table below

Prep for Sequencing Lvl 0
12µl of plasmid eluated containing 40-100 ng/µl
3 µl of 20uM 69_level0_seq_fw
Economy run in 1.5 Eppis

Sequencing 17.07.

	A	B	C	D	E	F	G	H
1	seq number		construct	ng/ml	plasmid ul	plasmid amount	Water amount	
2	98902330	1	0.3_b_65 VCPO 2.7	352.6	3	88.15	9	
3	98902331	2	0.3_a_18 Mx 3.4	277.2	3	69.3	9	
4	98902332	3	0.1_b_28 spy tag 1.3	258.9	3	64.725	9	
5	98902333	4	0.2_b_34 L3 2.6	248.5	3	62.125	9	
6	98902334	5	0.2_b_35 L3rc 3.9	291.2	3	72.8	9	
7	98902335	6	0.5_a_30 NCS 4.2	287.3	3	71.825	9	

DIENSTAG, 21.7.2026

Sequencing 17.07. results:

0.1rc_b_28 SpyTag 1.3 = correct
0.2_b_34 L3 2.6 = correct
0.2_b_35 L3rc 3.9 = correct
0.3_b_65 CiVCPO 2.7 = correct, 1 basemutation -> 1 aa mutation

0.5_a_30 NCS 4.2 = correct
0.3_a_18 Mx 3.4 = correct

Golden Gate according to Golden Gate Assembly of:

1.1_b_088 LGB4L3rcST
1.3_b_087 SCL3QT

Overnight of:

0.1rc_b_28 SpyTag 1.3 (G) with Antibiotics
0.2_b_34 L3 2.6 (G) with Antibiotics
0.2_b_35 L3rc 3.9 (G) with Antibiotics
0.3_b_65 CiVCPO 2.7 (G) with Antibiotics

0.3_b_64 NlucP 9

MITTWOCH, 22.7.2026

Trafo according to E. coli Transformation of:

1.1_b_088 LGB4L3rcST
1.3_b_087 SCL3QT

Miniprep, according to instructions, of overnights:

0.3_b_64 NlucP 9

concentration in table below

Prep for Sequencing Lvl 0
12µl of plasmid eluated containing 40-100 ng/µl
3 µl of 20uM 69_level0_seq_fw
Economy run in 1.5 Eppis

Sequencing 22.07.

	A	B	C	D	E	F	G	H
1	seq number		construct	ng/ml	plasmid ul	plasmid amount	Water amount	
2	98902336	1	0.3_b_64 NlucP 9	256.7	3	64.175	9	

DONNERSTAG, 23.7.2026

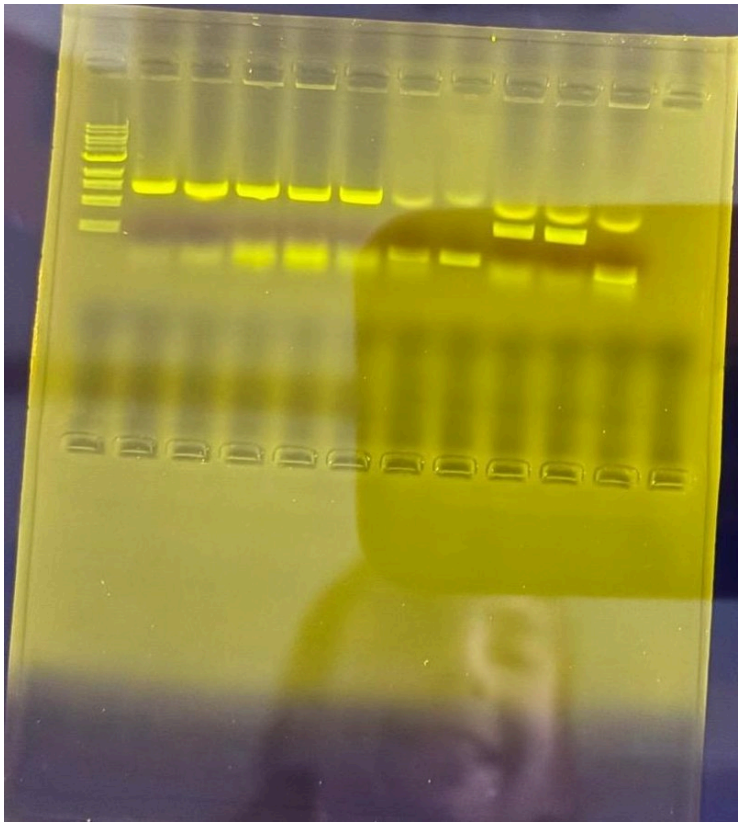
cPCR according to Taq colony PCR of:

1. 1.3_b_087 SCL3QT
2. 1.1_b_088 LGB4L3rcST

with 30 cycles, TM = 57, annealing = 60 s, elongation = 90 s
DNA = 1 % gel, 120 V and 25 mins

no blue colonies, xgal worked on other plates, 5 colonies picked per plate

cPCR 23.07. 1



NC was on other gel

Glycerol stock of:

0.1rc_b_28 SpyTag 1.3 (G) with Antibiotics
0.2_b_34 L3 2.6 (G) with Antibiotics
0.2_b_35 L3rc 3.9 (G) with Antibiotics
0.3_b_65 CiVCPO 2.7 (G) with Antibiotics

Overnight of:

1.3_b_087 SCL3QT 1.4
1.1_b_088 LGB4L3rcST 2.4

FREITAG, 24.7.2026

Sequencing 22.07. results:

0.3_b_64 NlucP 9 = not correct

Miniprep, according to instructions, of overnights:

1.3_b_087 SCL3QT 1.4
1.1_b_088 LGB4L3rcST 2.4

concentration in tabel below

Prep for Sequencing Lvl 1
E.coli night seq
3 µl of 20uM oSN_17 fw
3 µl of 20uM oSN_18 rv

Sequencing 24.07.

	A	B	C	D	E	F	G	H
1	seq number		construct	ng/ml	plasmid ul	plasmid amount	Water amount	
2		1	1.3_b_087	394.7	3	98.675	9	
3		2	1.1_b_088	153.2	5	63.8333333333	7	

DIENSTAG, 28.7.2026

Sequencing 22.07. results:

1.3_b_087 SCL3QT = correct
1.1_b_088 LGBL3rcST = correct

Overnight of:

1.3_b_087 SCL3QT 1.4 (G) with Antibiotics
1.1_b_088 LGBL3rcST 2.4 (G) with Antibiotics

MITTWOCH, 29.7.2026

Golden Gate according to Golden Gate Assembly of:

1.1_b_090_CiVCPO
2_b_089 LGB_L3rc_ST + SC_L3_Qt

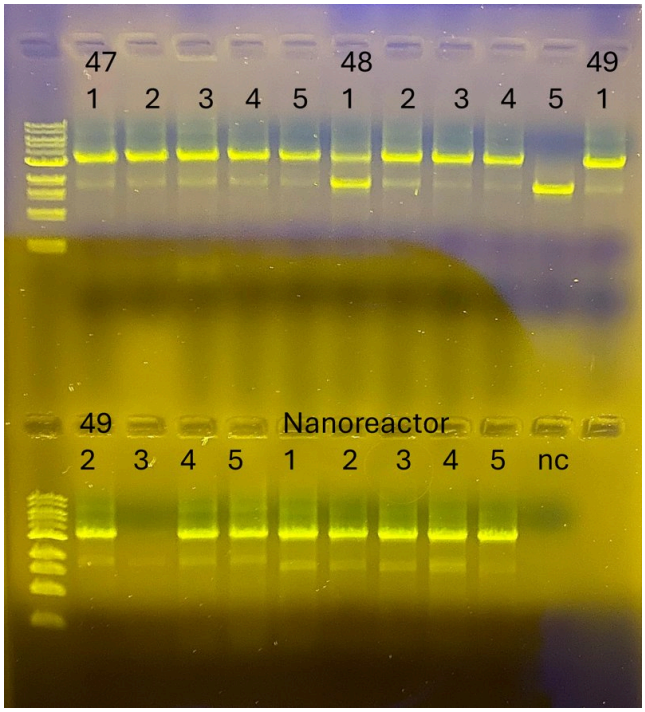
Glycerol stock of:

1.3_b_087 SCL3QT 1.4 (G) with Antibiotics
1.1_b_088 LGBL3rcST 2.4 (G) with Antibiotics

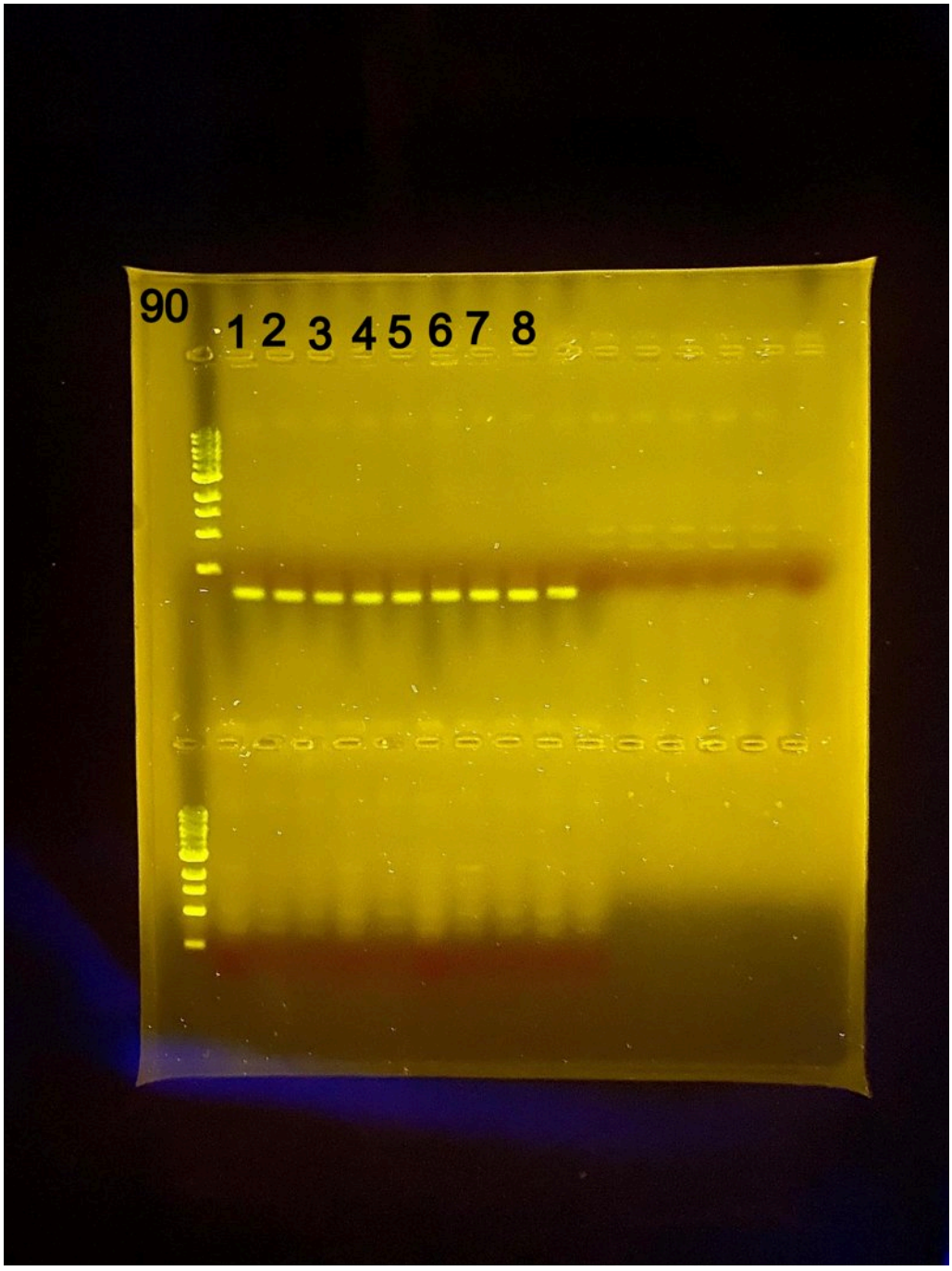
FREITAG, 31.7.2026

Nightseq 2_b_089 LGB_L3rc_ST + SC_L3_Qt von Masterplate Nr. 5 genommen

cPCR 31.07.



cPCR 31.07.



Nightseq 2_b_089 LGB_L3rc_ST + SC_L3_Qt von Masterplate Nr. 5 genommen

Sequencing 31.07.1

	A	B	C	D	E
1	seq number		construct	ng/ml	
2	11150468	1	2_b_089 fw	547.4	
3	11150469	2	2_b_089 rv	547.4	

MITTWOCH, 5.8.2026

Sequencing 31.07. results:

2_b_089 LGB_L3rc_ST + SC_L3_Qt Nr. 5 = correct

Overnight of:

2_b_089 LGB_L3rc_ST + SC_L3_Qt Nr. 5 (G) with Antibiotics

DONNERSTAG, 6.8.2026

Glycerol stock of:

2_b_089 LGB_L3rc_ST + SC_L3_Qt Nr. 5 (G) with Antibiotics

DIENSTAG, 18.8.2026

Golden Gate according to Golden Gate Assembly of:

0.3_b_64 NlucP
0.4_b_067 L3rc

MITTWOCH, 19.8.2026

Trafo according to E. coli Transformation of:

1. 0.4_b_067 L3rc
2. 0.3_b_64 NlucP
3. ScURA3 Marker Plasmid (DK)

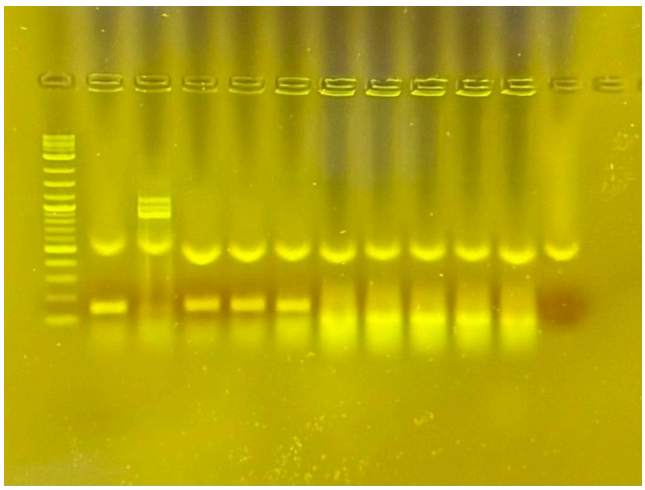
DONNERSTAG, 20.8.2026

cPCR according to Taq colony PCR of:

1. 0.4_b_067 L3rc
2. 0.3_b_64 NlucP

with 30 cycles, TM = 54, annealing = 60 s, elongation = 60 s
DNA = 1 % gel, 120 V and 25 mins

cPCR 20.08.



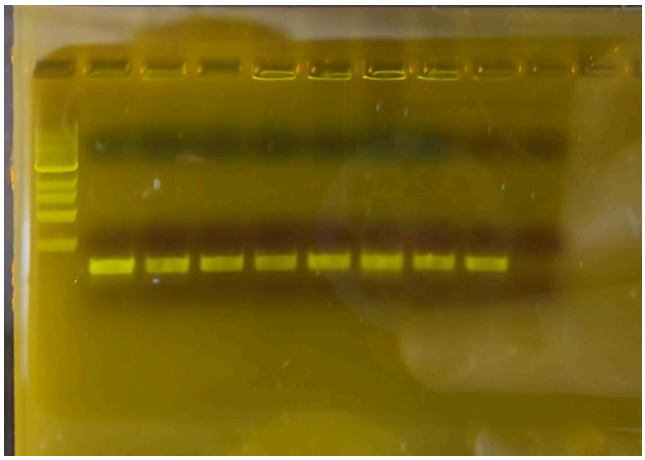
Trafo of ScURA3 Marker Plasmid (DK) didn't work, redo Monday

FREITAG, 21.8.2026

nIghtseq von 0.4_b_067L3rc mit fw primer. Code: 11 150 485

Taq colony PCR of 1.1_b_090_CiVCPO:

cPCR 21.08.26



MONTAG, 24.8.2026

Trafo according to E. coli Transformation of:

1. ScURA3 Marker Plasmid (DK)

use 1 ul of DNA

Golden Gate according to Golden Gate Assembly of:

0.3_b_64 NlucP

with new 15 nM Stock, 5 ul backbone

DIENSTAG, 25.8.2026

Trafo according to E. coli Transformation of:

0.3_b_64 NlucP

Sequencing 21.08. results:

0.4_b_067L3rc = not correct, used wrong primer, redo wednesday

Overnight of:

ScURA3 Marker Plasmid (DK)

MITTWOCH, 26.8.2026

Miniprep, according to instructions, of overnights:

ScURA3 Marker Plasmid (DK)

Miniprep 26.08.

	A	B
1	construct	ng/l
2	ScURA3	22.4

PCR for Gibson, according to Q5 PCR, of :

ScURA3 Marker Plasmid
Lvl2 Destination BB

cPCR according to Taq colony PCR of:

1. 0.3_b_64 NlucP

with 30 cycles, TM = 54, annealing = 60 s, elongation = 60 s
DNA = 1 % gel, 120 V and 25 mins

Gibson according to Gibson Assembly :

ScURA3 Marker Plasmid PCR Product
Lvl2 Destination BB PCR Product

FREITAG, 28.8.2026

Level 2 yeast transformation of: S. cerevisiae Transformation

Lvl2_b_089

Trafo according to E. coli Transformation of:

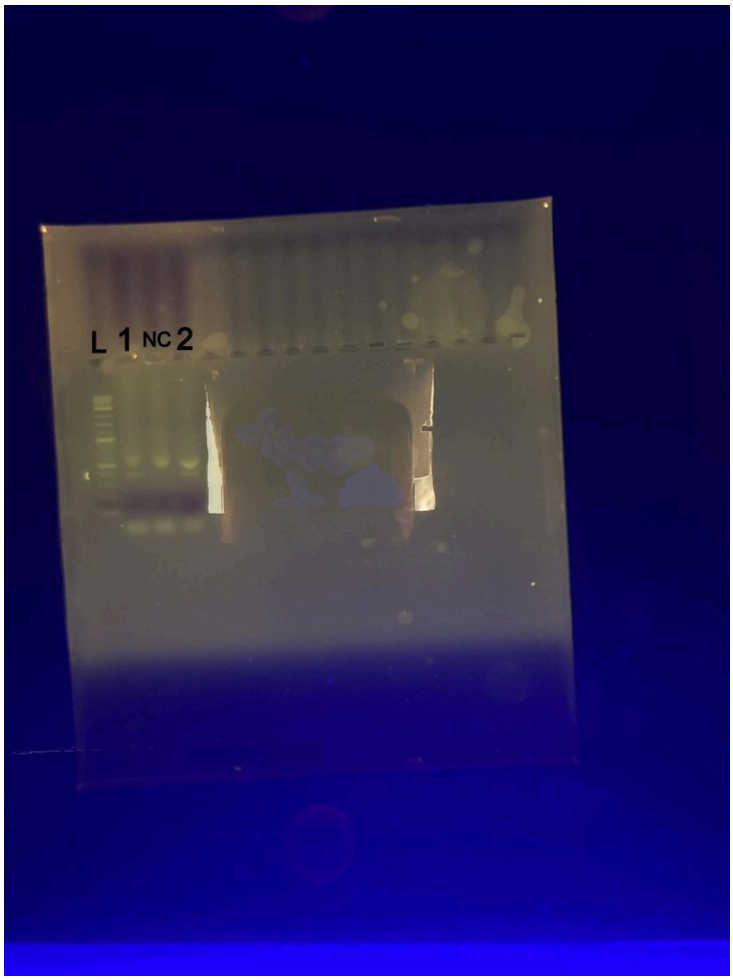
Gibson product Lvl_2_bb URA

FREITAG, 4.9.2026

Taq colony PCR of:

Lvl_2_bb URA

cPCR 04.09.



DIENSTAG, 8.9.2026

Overnight of:

Lvl_2_bb URA

MITTWOCH, 9.9.2026

Miniprep, according to instructions, of overnights:

Lvl_2_bb URA

Full Plasmid Sequencing of:

Lvl_2_bb URA

FREITAG, 11.9.2026

Sequencing results:

Lvl_2_bb URA = sequencing wasn't possible, reads too bad

MONTAG, 14.9.2026

Order by GenScript:

NLucP pESC-Ura
SpyCatcher + Enc_Qt pESC-His
Mx pESC-His
Qt pESC-His
ciVCPO + TP_Mx pESC-His
ciVCPO + TP_Qt pESC-His

DIENSTAG, 29.9.2026

Yeast trafo according to S. cerevisiae Transformation of:

1. 2_b_089 LGB_L3rc_ST + SC_L3_Qt and NLucP pESC-Ura
2. SpyCatcher + Enc_Qt pESC-His and NLucP pESC-Ura
3. Mx pESC-His and ciVCPO + TP_Mx pESC-Ura
4. Qt pESC-His and ciVCPO + TP_Qt pESC-Ura
5. Mx pESC-His
6. Qt pESC-His

in new yeast strain BY4742

DIENSTAG, 6.10.2026

Cultivation and induction according to Purification of Encapsulin (S.cerevisiae) :

1. 2_b_089 LGB_L3rc_ST + SC_L3_Qt and NLucP pESC-Ura
2. SpyCatcher + Enc_Qt pESC-His and NLucP pESC-Ura

till OD600: 1

MITTWOCH, 7.10.2026

Cultivation and induction according to Purification of Encapsulin (S.cerevisiae) :

1. mxpe vcpo induced
2. mxpe vcpo not induced
3. mxpe dummy induced
4. mxpe dummy not induced
5. qt vcpo induced
6. qt vcpo Not induced
7. qt Dummy induced
8. qt Dummy not induced

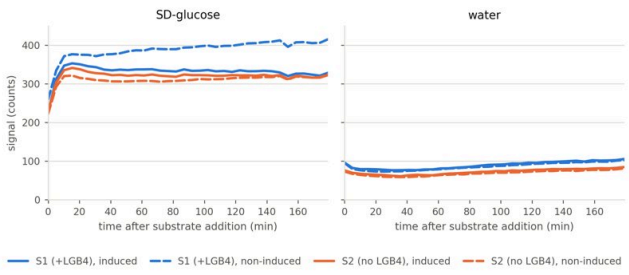
till OD600: 1

FREITAG, 9.10.2026

nanoluc live cell assay:

results aren't usable, because the measurement was taken in flourescence mode:

Figure3_NLucP_measurement.png



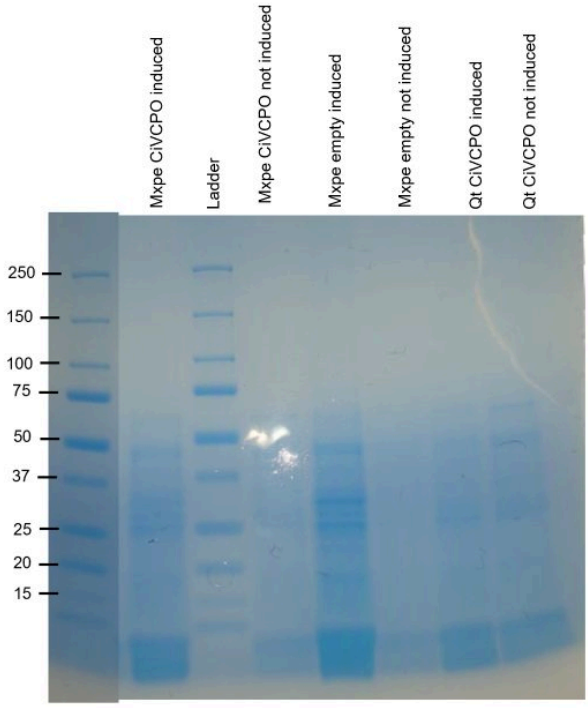
MONTAG, 12.10.2026

lysis according to Purification of Encapsulin (S.cerevisiae) but used tris 1M instead of pbs because it inhibits VCPOs

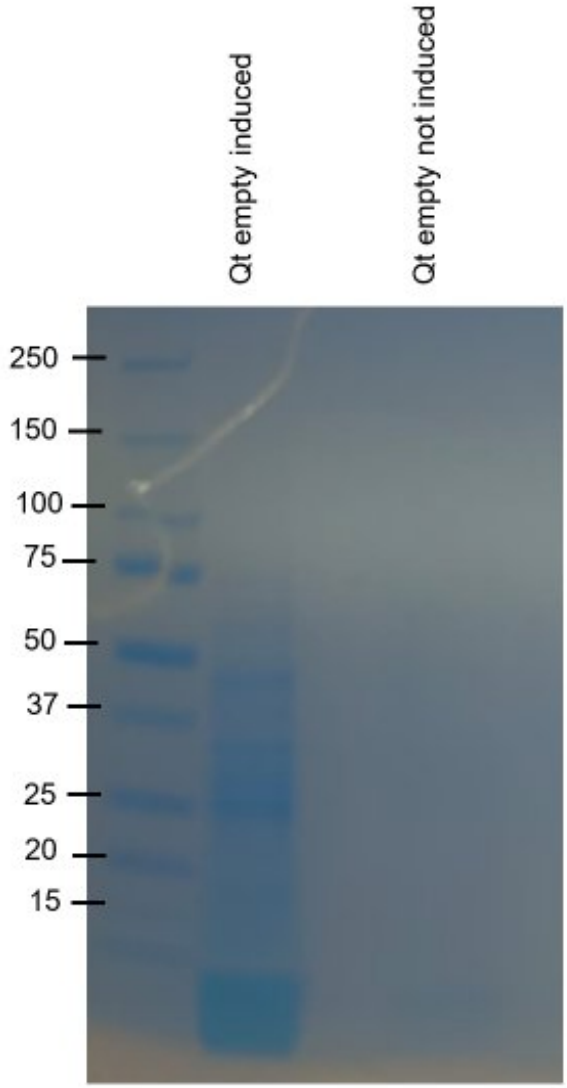
DONNERSTAG, 15.10.2026

SDS VCPO

SDS lysate 1

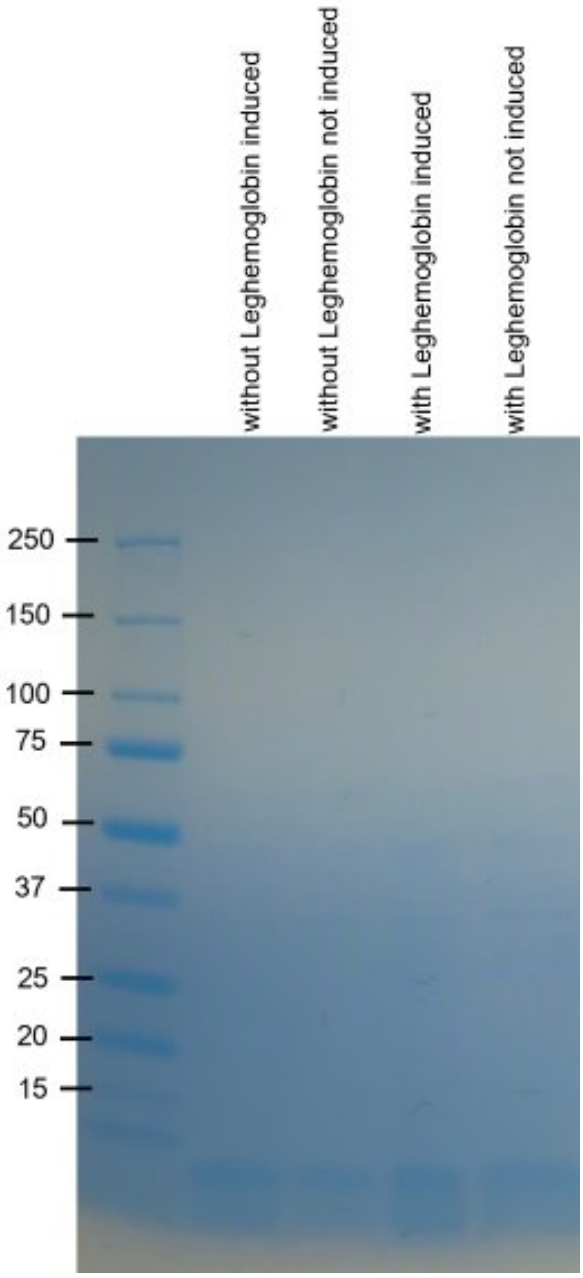

Mxpe CiVCPO induced
Ladder
Mxpe CiVCPO not induced
Mxpe empty induced
Mxpe empty not induced
Qt CiVCPO induced
Qt CiVCPO not induced
250
150
100
75
50
37
25
20
15

SDS lysate 2



phenol red assay: 80 µL 0.5 mM phenol red, 20 µL 50 mM KBr, 50 µL 50 mM H_2O_2, 50 µL enzyme crude extract, and 1 M citrate buffer pH 5.0 with 1 mM Na_3VO_4 to a total volume of 250 µL, absorbance measured for 30 minutes at 592 nm
-> no increase of OD measured

Screenshot_2026-10-17_20415333.png



Golden Gate Assembly

Introduction

We will follow the Goldan gate insert to backbone ratio calculation from our supervisor. Insert and backbone are diluted to 15mM and 1µl each is added to the reaction

For oligos: 2,5 µl of the annealing reaction is directly used.

Materials

- Insert
- Backbone
- Ligase buffer
- Ligase
- Restriction enzyme (BbsI (level0 and 2) or BsaI(level1))

Procedure

Protocol

1. If not already done dilute bigger inserts to 15nM using the table to calculate. make sure to mix propably before continuing

calculation 15nM stock preperation

	A	B	C	D	E	F	G	H	I
1	**Fragment**	**Size [bp]**	**Mass. Conc. [ng/µl]**	**Mol. Conc. [nM]**	**Target Mol. [nM]**	**Target Vol. [µl]**	**Vol. DNA [µl]**	**Vol. H2OMQ [µl]**	
2	calculation:			(C2*10^-9)/((B2*617.96)+36.04)*10^15			E2/D2*F2	F2-G2	
3	Level 0 backbone	2659	327.8	199.4898124119	15	50	3.76	46.24	
4	0.3 Enc_Mx	898	394.6	711.0369585695	15	50	1.05	48.95	
5	0.3 Enc_Mxpe	877	10	18.4506284063	15	25	20.32	4.68	
6	0.3 Enc_Tm	832	10	19.4484890819	15	25	19.28	5.72	
7	0.3 Enc_Qt	883	10	18.3252643529	15	25	20.46	4.54	
8	PABP	548	10	29.5265604994	15	25	12.7	12.3	
9	1.2 GAL1/10	705	95.6	219.4181178406	15	25	1.71	23.29	
10	level 1bb	2621	285	175.9575113854	15	25	2.13	22.87	
11	0.1TPTm* plasmid	2109	314.7	241.4614415771	15	25	1.55	23.45	
12	0.1TPQt* plasmid	2112	220	168.5607325392	15	25	2.22	22.78	
13	0.2 L1 plasmid	2093	257.4	199.0063106185	15	25	1.88	23.12	
14	0.3 GFP plasmid	2792	329.7	191.0883000894	15	25	1.96	23.04	
15	0.4 NCSrc plasmid	2114	244.3	187.0019513412	15	25	2.01	22.99	
16	0.5 NCSrc plasmid	2118	299.9	229.1280119779	15	25	1.64	23.36	

2. Set up the following reaction mix on ice:

1µl DNA 15nM Insert PCR reactions
if oligos 2,5 µl direct from annealing reaction
1 µl 15nM Backbone
1 µl 10x T4 DNA Ligase Buffer (extremely heat sensitive, will not survive multiple heat/thaw cycles)
0.5 µl T4 DNA Ligase (extremely heat sensitive)
0.5 µl BsaI-HFv2 (relatively heat sensitive)
Make **up to 10 µl** of dH2O

mix preperation

	A	B	C	D	E	F	G	H	I	J	K
1		reaction oligo	reaction PCR amplified or ordered (15nM stock)	MM 7.2 oligo reactions	MM 5.2 ordered PCR reactions	MM 2.1 reaction oligo	MM 2.1	MM 6.2 reaction oligo			
2	DNA Insert	2.5	1								
3	Backbone (1 µl of 15nM stock)	1	1	7.2	5.2	2.1	2.1	6.2			
4	10x T4 Ligase Buffer	1	1	7.2	5.2	2.1	2.1	6.2			
5	T4 Ligase	0.5	0.5	3.1	2.6	1.05	1.05	3.1			
6	BbsI or BsaI	0.5	0.5	3.1	2.6	1.05	1.05	3.1			
7	H2O	4.5	6	32.4	31.2	9.45	12.6	27.9			
8				add 7.5 µl each	add 9 µl each	add 7.5 µl each	add9 µl each				

3. Mix gently by pipetting up and down. Tap on the bench or briefly centrifuge (1 sec) to ensure no bubbles in the reaction mixture.

4. Transfer the reaction tube(s) to a thermal cycler and run the following program:

    1. 5 min 37°C
    2. 5 min 16°C)

    Step 1&2 x 50 cycles

    5 min 50°C

    5min 80°C

    If you’re running overnight, add a Hold at 15°C.

E. coli Transformation

Introduction

Standard E. coli Transformation with heatshock

Materials

›

- 50ul competent cells (stored in -80°C)
- 2 Agar plates with coresponding Antibiotics according to transformation plasmid resistance (plate praperation: Amp Stock 50mg/ml → final concetration 1:1000 50ug/ml)
- 1ml LB Medium

Procedure

1. Take competent cells out of -80°C and thaw on ice (approximately 20-30 mins).
2. Remove agar plates (containing the appropriate antibiotic) from storage at 4°C and let warm up to room temperature and then (optional) incubate in 37°C incubator.
3. Mix 1 - **5 µ**l of DNA (usually 10 pg - 100 ng) into 20-**50 µL** of competent cells in a microcentrifuge or falcon tube. GENTLY mix by flicking the bottom of the tube with your finger a few times.
4. Incubate the competent cell/DNA mixture on ice for 20-30 mins.
5. Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 30-60 secs (45 secs is usually ideal, but this varies depending on the competent cells you are using).
6. Put the tubes back on ice for 2 min.
7. Add 250-**1,000 µl LB** or SOC media (without antibiotic) to the bacteria and grow in 37°C shaking incubator for 45 min.
8. Plate some or all of the transformation onto a 10 cm LB agar plate containing the appropriate antibiotic.

   Herunterzentrifugieren (short taste gedrückt halten bis 14000 rpm erreicht wird und dann loslassen)und 900µL Überstand abnehmen
   plate 50 µL on one plate and the rest on a second plate. This gives the best chance of getting single colonies, while allowing you to recover all transformants. If the culture volume is too big, gently collect the cells by centrifugation and resuspend in a smaller volume of LB so that there isn't too much liquid media on the agar plates. If the agar plate doesn't dry adequately before the cells begin dividing, the bacteria diffuse through the liquid and won't grow in colonies.
   10-25 µl for level 0
   X-Gal: 40 µl

- [x] 9. Incubate plates at 37°C overnight

Oligo Annealing

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

›

Procedure

Protocol

- 1. Combine 1 µL of 100µM forward and reverse Oligo in a PCR tube with 18 µL water, for a total volume of 20 µL.
- 2. In a thermocycler, heat at 95°C for 3 min then cool to room temperature over ~30-60 min. (There should be a temperature step function on the thermocycler, e.g. cool by 0.1°C every second.)
- 3. Annealed oligo fragments can be stored at -20°C.

Taq colony PCR

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

›

Procedure

Taq colony PCR

Master Mix preparation

- 1. Thaw primers completely. Thaw Taq MM completely on ice. Prepare all reagents on ice.
- 2. Label PCR tubes.
- 3. Calculate the total number of reactions including:
  - samples
  - negative control
  - excess volume (recommended 10 - 20%)
- 4. Prepare the Master Mix in the following order:
  - H2O
  - fw Primer
  - rv Primer
  - 2x Taq Master Mix
- 5. Mix gently by pipetting up and down or gently flicking the tube.

Table1

	A	B	C	D	E	F	G	H	I	J	K	L	M
1		50ul Reaction	**25ul Reaction**	32x MM	2.2 x MM	11.2	24.2x MM	26 x MM	19.5x MM	5.2xMM	23 x MM	4.2 xMM	8,2 xMM
2	2x Taq MM	25ul	12.5	400	27,5	140	302.5	325	243.75	65	287.5	52.5	102,5
3	10uM P fw	1ul	0.5	16	1,1	5.6	12.1	13	9.75	2.6	11.5	2.1	4,1
4	10um P rv	1ul	0.5	16	1,1	5.6	12.1	13	9.75	2.6	11.5	2.1	4,1
5	Template	colony	colony	colony	colony								colony
6	H20	to 50ul	11.5	368	25,3	128.8	278.3	299	224.25	59.8	264.5	48.3	94,3
7													

Distribution of the Master Mix

- 6. Aliqot 25 µL of Master Mix into each PCR tube
- 7. Keep reactions on ice during setup

Colony Picking

- 8. Use a ssterile pipette tip
- 9. Lightly touch a single bacterial colony. Avoid transferring too much biomass as excessive cells can inhibit PCR
- 10. Streak the same colony onto a fresh agar plate for backup
- 11. Dip the same tip into the corresponding PCR tube and mix briefly by pipetting up and down
- 12. Discard the tip

Controls

- 13. use nuclease-free water (milliQ) water instead of template DNA

PCR Cycling Conditions

- 14. To reduce non-specific amplification and primer dimer formation, reactions should be kept on ice during setup
- 15. Manual hot start: Reactions should be transferred into the thermocycler only after the cycler has reached the initial denaturation temperature (95°C).

Table2

	A	B	C
1	STEP	TEMP	TIME
2	Initial Denaturation	95°C	30 seconds
3	25-33 Cycles	95°C 45-68°C* 68°C	15-30 seconds 15-60 seconds 1 minute/kb
4	Final Extension	68°C	5 minutes
5	Hold	4-10°C	

16. Prepare gel 2% inf the fragment is shorter than 200bp (120V 30-35 min otherwise use a 1% gel. it has to run for 20-25 min at 120V.

Taq colony PCR

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

›

Procedure

Taq colony PCR

Master Mix preparation

- 1. Thaw primers completely. Thaw Taq MM completely on ice. Prepare all reagents on ice.
- 2. Label PCR tubes.
- 3. Calculate the total number of reactions including:
  - samples
  - negative control
  - excess volume (recommended 10 - 20%)
- 4. Prepare the Master Mix in the following order:
  - H2O
  - fw Primer
  - rv Primer
  - 2x Taq Master Mix
- 5. Mix gently by pipetting up and down or gently flicking the tube.

Table1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1		50ul Reaction	**25ul Reaction**	13.2x MM	2.2 x MM	17,2 MM	11.2	24.2x MM	26 x MM	19.5x MM	9.5xMM	23 x MM	4.2 xMM	8.2 xMM	28.2 xMM
2	2x Taq MM	25ul	12.5	165	27,5	215	140	302.5	325	243.75	118.75	287.5	52.5	102,5	352.5
3	10uM P fw	1ul	0.5	6.6	1,1	8.6	5.6	12.1	13	9.75	4.75	11.5	2.1	4,1	14.1
4	10um P rv	1ul	0.5	6.6	1,1	8.6	5.6	12.1	13	9.75	4.75	11.5	2.1	4,1	14.1
5	Template	colony	colony	colony	colony									colony	colony
6	H20	to 50ul	11.5	151.8	25,3	197.8	128.8	278.3	299	224.25	109.25	264.5	48.3	94,3	324.3
7															

Distribution of the Master Mix

6. Aliqot 25 µL of Master Mix into each PCR tube

7. Keep reactions on ice during setup

Colony Picking

8. Use a sterile pipette tip

9. Lightly touch a single bacterial colony. Avoid transferring too much biomass as excessive cells can inhibit PCR

10. Streak the same colony onto a fresh agar plate for backup. Backup needs to be incubated

11. Dip the same tip into the corresponding PCR tube and mix briefly by pipetting up and down

12. Discard the tip

Controls

13. use nuclease-free water (milliQ) water instead of template DNA

PCR Cycling Conditions

14. To reduce non-specific amplification and primer dimer formation, reactions should be kept on ice during setup

15. Manual hot start: Reactions should be transferred into the thermocycler only after the cycler has reached the initial denaturation temperature (95°C).

Table2

	A	B	C
1	STEP	TEMP	TIME
2	Initial Denaturation	95°C	30 seconds
3	25-33 Cycles	95°C 45-68°C* 68°C	15-30 seconds 15-60 seconds 1 minute/kb
4	Final Extension	68°C	5 minutes
5	Hold	4-10°C	

- [x] 16. Prepare gel 2% inf the fragment is shorter than 200bp (120V 30-35 min otherwise use a 1% gel. it has to run for 20-25 min at 120V.

Oligo Annealing

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

›

Procedure

Protocol

- 1. Combine 1 µL of 100µM forward and reverse Oligo in a PCR tube with 18 µL water, for a total volume of 20 µL.
- 2. In a thermocycler, heat at 95°C for 3 min then cool to room temperature over ~30-60 min. (There should be a temperature step function on the thermocycler, e.g. cool by 0.1°C every second.)
- 3. Annealed oligo fragments can be stored at -20°C.