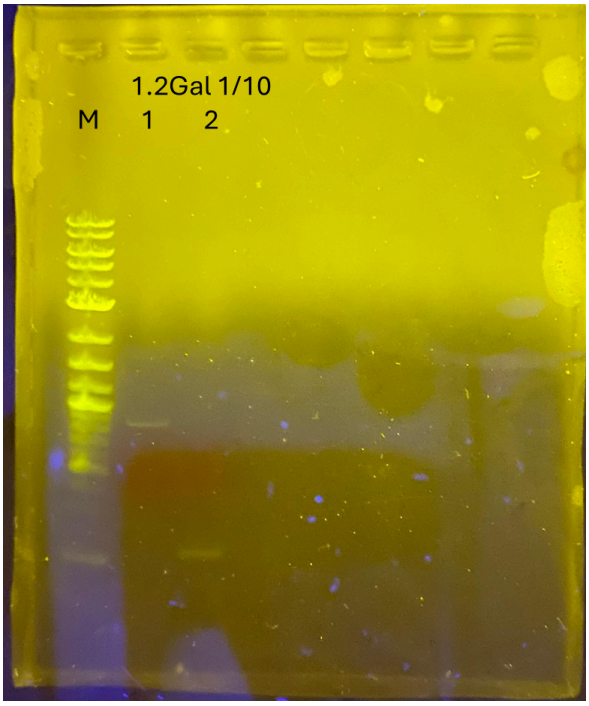
cPCR 1.2 GAL1/10



0.3E-Qt 966bp --> overnights from 0.3 E-Qt 2,3,9

0.3E-Mx 986bp --> only one colonie showes a fragment but not with the expected size to find out what it is it will be send to sequencing

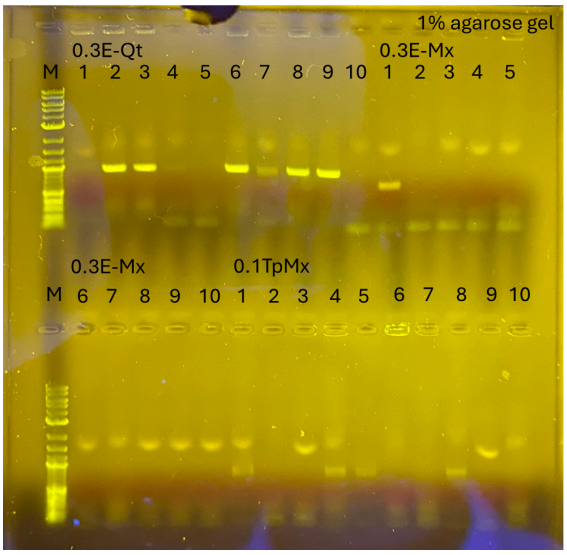
0.1 TPMx* 153bp

Tm:54°C

Elongation: 1min

65_Lvl0_bb_fw + 66_Lvl0_bb_rv

cPCR Level0



- [x] overnight from correct seq results for glycerol stock in 5 ml LB 21 0.4NCS and 23 0.5NCS

Proof of concept:

- [x] cPEC MCS2 Mx for sequencing Anica Nissen
  + 3ul LP034
  12µl of plasmid eluated containing 100 ng/µl
  3 µl of 20uM Primer
  previosly the calculation not correct insted of 40-100 ng/µl in 12 µl only 100ng in 12µl were send. somehow the sequencing did work but in the future we will prepare it like recomended by microsynth

Prepare for sequencing 15.06

	A	B	C	D	E	F
1	Seq Number		plasmid	concentration	amount for seq of plasmid in µl	amount of water in µl
2	98902287	1	cPEC 6 pESC MCS2 Mx	25.3	3.95256917	8.04743083
3	98902288	2	cPEC 7 pESC MCS2 Mx	442.1	0.226193169	11.773806831

DIENSTAG, 16.6.2026

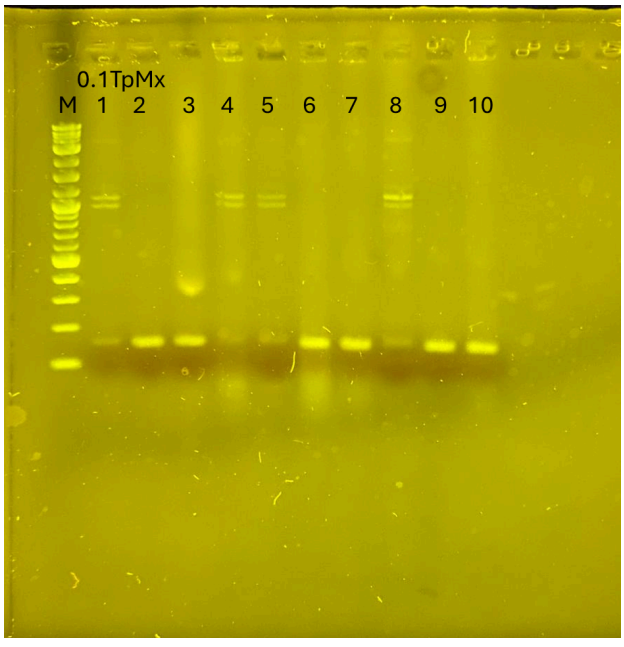
Domestication Level 2 Backbone

- [x] Miniprep Lvl2 tspurple G 10:1

Level 0 cloning

- [x] cPCR repeat TPMx* with 2% gel load 15 µl (rest from previous day)

0.1 TP Mx* cPCR 2% gel



- [x] Glycerol stocks 0.4NCS (21), 0.5NCSrc (23)

write down number / fill out construct sheet

- [x] Mini Prep for 0.1 NCS (2), 0.3 E-Qt, 0.3 E-Mx, 0.1 TPMx*

Miniprep results

	A	B	C	D	E
1	Seq number	name	concentration / ng/µL	A260/A280	A260/A230
2		level 2 tspurple	6,0	1,74	1,32
3		0.1 NCS 2	328,7	1,85	2,25
4		0.1 NCS 3	303,8	1,85	2,29
5		0.3 EQt 2	439,0	1,86	2,26
6		0.3 EQt 3	439,3	1,86	2,29
7		0.3 EQt 9	433,6	1,83	2,23
8		0.3 EMx 1	356,6	1,85	2,25
9		Gal 1/10	461,0	1,86	2,55
10		(21) 0.4 NCS 6	352,9	1,85	2,28
11		(23) 0.5 NCS 5	394,8	1,86	2,29

- [x] Prepare for sequencing
- [x] redo send to sequencing from yesterday: same as yesterday, we were not sure about the Primer dilution and it is likely that there was not 3 ml 20µM of LP34 in the tubes
- [x] 0.3Enc Mx send to sequencing --> could be lacZ

12 µl of insert 50-100 ng/µl and add 3 µl of 20uM Primer

Miniprep results1

	A	B	C	D	E	F	G
1	Seq number	name	concentration / ng/µL	amount to add of insert	amount to add of water	Primer	sequencing
2	98902289	level 2 tspurple	6	5	7	LP034	no result repeat
3	98902290	level 2 tspurple reverse	6	5	7	LP022	no result repeat
4	98902291	0.3 EQt 2	439.0	2	10	69	correct
5	98902292	0.3 EQt 3	439.3	2	10	69	correct
6	98902293	0.3 EQt 9	433.6	2	10	69	correct
7	98902294	0.3 EMx 1	356.6	2	10	69	worng but not lacZ
8	98902295	Gal 1/10	461	2	10	oSN17	correct
9	98902296	cPEC 6 pESC MCS2 Mx	25.3	3.9	8.1	LP034	wrong
10	98902296	cPEC 7 pESC MCS2 Mx	442.1	0.22	11.78	LP034	wrong

--> we found out that the 0.1 NCS from the previous sequencing is correct so we do not need to sequence the new ones (discard new MiniPrep 0.1 NCS 2 and 3 !)

prepare new LP034 20µM from 100µM stock for level 2
prepare new 69 20µM from 100 µM stock for level 0
prepare new oSN17 20 µM from 100 µMstock for level 1
prepare new oSN18 20 µM from 100 µMstock for level 1

- [x] Trafo Qt sample that matches E-Mxpe after sequencing to get colonies with 0.3 E Mxpe and Dom2 according to E. coli Transformation

30 µl were plated

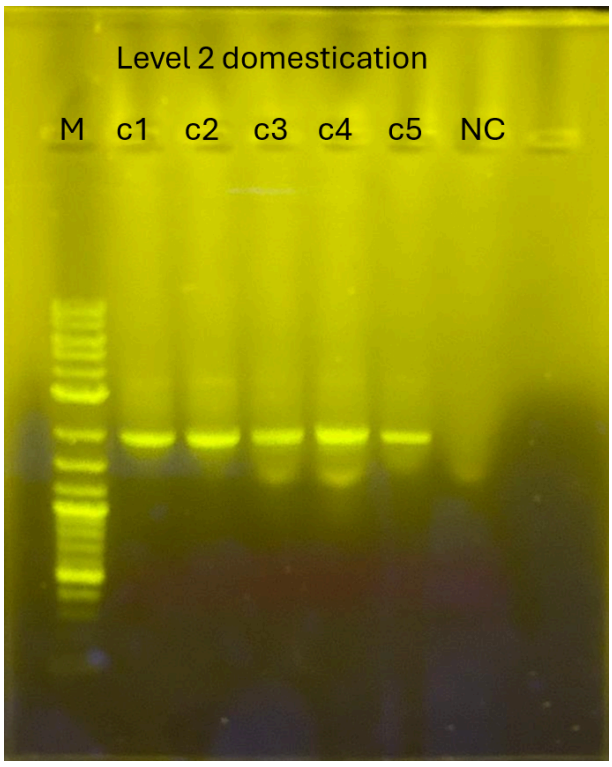
MITTWOCH, 17.6.2026

Domestication Level 2

- [x] cPCR for Dom2 (Primer LP22 and LP34) Taq colony PCR

100 V 35 min 1% agaros

cPCR Domestication for glycerol stock



--> c1 is used for the glycerol stock

Level 0 Golden Gate cloning

- [x] 0.3Mxpe did not show any colonies so TRAFo is to be repeated. we used 5 µl of 324 ng/l plasmid. could be to much so i try again with 1:3 dilution (2µl stock to 4µl water) two Trafos first with 1 µl and second with 2 µl
- [x] annealing of 0.1 NCSrc (Primer 23 and 24)and 0.5 NCS (Primer 61 and 62) Oligo Annealing
- [x] golden gate for missing level 0 0.1 NCSrc and 0.5 NCS Golden Gate Assembly

BbsI and 2,5 µl annealed oligo is used
reaction 1 0.1NCSrc

reaction 2 0.5 NCS

- [x] overnight for positiv 0.1 TPMx* 2,7,9 (15.06)
- [x] overnight for glycerol stock 0.1 NCS 1 (08.06) Spec added and dom2 1 (16.06.26) Amp added

DONNERSTAG, 18.6.2026

Level 0 Golden Gate brick preperation

- [x] Glycerol 0.1NCS (and Mini Prep) and Dom2
- [x] Mini Prep 0.1TPMx* 2,7,9

for colony 2 the 230/260 was very low so it will not be send to seq

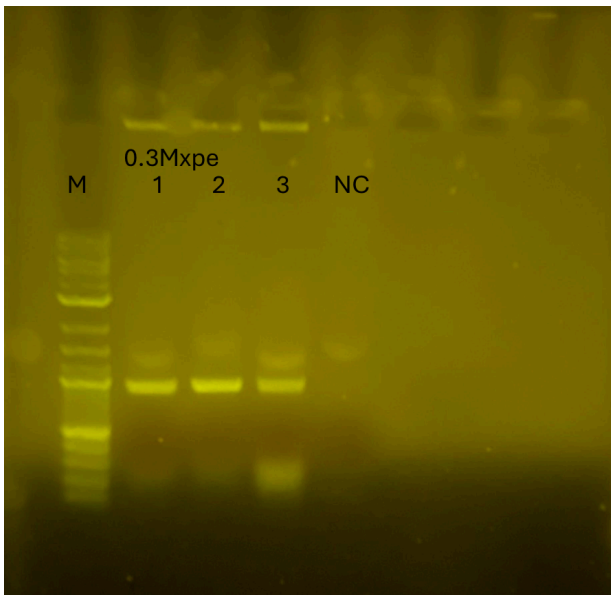
- [x] prepare for sequencing

Sequencing

	A	B	C	D	E	F	G
1	Seq number	name	concentration / ng/µL	amount to add of insert	amount to add of water	Primer	sequencing
2	98902298	0.1TPMx* 7	327.1			69	one basepair missing
3	98902299	0.1TPMx* 9	317.1			69	overhang correct but also point mutation

- [x] cPCR for 0.3Mxpe (1060bp) pick three colonies test with level 0 bb primer 65 and 66 Taq colony PCR

cPCR 0.3Mxpe



- [x] Trafo 0.1NCSrc and 0.5 NCS

plate on Spec with xGal 40ul

- [x] O/N tspurple for new mini and then full plasmid sequencing
- [x] O/N 0.3E-Qt 9 and Gal 1/10 for glycerol
- [x] O/N 0.3Mxpe colony 1 for glycerol

FREITAG, 19.6.2026

Golden Gate Brick preperation:

- [x] glycerol stock for 0.3E-Qt and 1.2 Gal1/10 and 0.3Mxpe --> sadly the culture did not grow ... so we have to repeat it next week
- [x] Miniprep for 0.3E-Qt and Gal1/10 and tspurple

Miniprep results 16.06

	A	B	C	D
1	name	concentration / ng/µL	A260/A280	A260/A230
2	level 2 tspurple	9.7	1.48	0.63
3	0.3 EQt	537.1	1.9	2.16
4	1.2 Gal 1/10	392.2	1.88	2.2

the bacteria with tspurple plasmid likely discared it because there was no antibiotic pressure. we need to repeat the overnight from the original colony using ampicilin.

MONTAG, 22.6.2026

- [x] cPCR for 0.1NCSrc (6 colonies)and 0.5 NCS (8 colonies) Ahmed Haj Kacem like described: Taq colony PCR
- [x] test the one not tested colonies from the plates of 0.1TPMx*

for all:
Tm:54°C
Elongation: 1min
Primer: 65_Lvl0_bb_fw + 66_Lvl0_bb_rv
expected fragment: TPMx* 150bp, 0.1NCSrc 150 bp and 0.5NCS 150bp

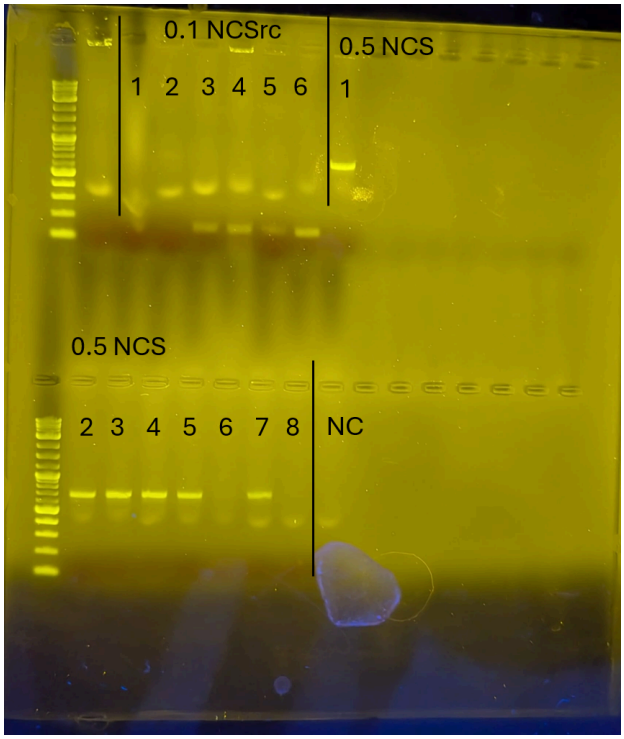
prepare a 2% gel

cPCR

	A	B	C
1		**25ul Reaction**	16.5 x MM
2	2x Taq MM	12.5	206.25
3	10uM P fw	0.5	8.25
4	10um P rv	0.5	8.25
5	Template	colony	colony
6	H20	11.5	189.75

run gel for 25 min with 120 V

cPCR Level 0



--> 0.1NCSrc 3,4,6 for overnight

--> 0.5NCS no correct colonies possibly lacZredo Goldengate reaction

- [x] overnight for positiv cPCR 0.1 NCSrc 3,4,6 in LB and redo cryo 0.3Mxpe c3 use Spec in LB, redo tspurple from colony directly with Amp in LB. Also prepare overnights for 0.1TPMx* from the masterplate colony 6 and 10
  Ahmed Haj Kacem Anica Nissen

DIENSTAG, 23.6.2026

- [x] cryo for 0.3 Mxpe (16) and Mini and send to seqencing
- [x] tspurple cryo (37)
- [x] Mini for Tspurple and full plasmid seq 20 µl of 20ng/ml plasmid

--> so 5,41 µl of plasmid and 10,59 µl water

- [x] Mini Prep 0.1TPMx* c6 and 10 prepare for seq
- [x] Mini Prep for correct 0.1NCSrc

Miniprep results 23.06

	A	B	C	D	E
1	number	name	concentration / ng/µL	A260/A280	A260/A230
2	1	0.1 NCSrc 3	398.1	1.87	2.31
3	2	0.1 NCSrc 4	391.5	1.86	2.31
4	3	0.1 NCSrc 6	393.4	1.85	2.55
5	4	0.1 TpMx* 6	303.7	1.85	2.43
6	5	0.1 TpMx* 10	386.8	1.86	2.43
7	6	0.3 Mxpe C3	369.5	1.8	2.42
8	7	tspurple original C	59.1	1.86	3.39

- [x] send to seq:

use 12 µl with 40-100 ng/µl of plasmid add 3 µl of 20µM Primer

sequencing preperation

	A	B	C	D	E	F	G
1	sequencing number	name	concentration / ng/µL	amount to add of insert	amount to add of water	Primer 3µl (20µM)	result
2	98902300	0.1 NCSrc 3	398.1	2	10	69	correct
3	98902301	0.1 NCSrc 4	391.5	2	10	69	correct
4	98902302	0.1 NCSrc 6	393.4	2	10	69	correct
5	98902303	0.1 TpMx* 6	303.7	2	10	69	correct
6	98902304	0.1 TpMx* 10	386.8	2	10	69	correct
7	98902305	0.3 Mxpe C3	369.5	2	10	69	correct
8	30372992	tspurple original C	59.1	5.41	10.59	non because full plasmid seq	

- [x] 0.5NCS run the colonie sample 6 and 8 again with 15 µl loaded --> no fragment so the golden gate will be repeated

- [x] level 0 0.5NCS new golden gate with 2.5µl annealed oligos and BbsI
- [x] level 0 0.1TPMx* golden gate again with 2.5 µl annealed oligos
- [x] level 1 construct C terminal GFP cargo: with BsaI level 1 backbone Golden Gate Assembly
  - 0.1 TPTm* / 0.1 TPQt*
  - 0.2 L1
  - 0.3 yeGFP
  - 0.4 NCSrc
  - 0.5 NCSrc

tube 1: Lvl0.5_a_030 (0.5NCS)
tube 2: Lvl0.1_a_007 (0.1 TPMx*)
tube 3: Lvl1_1_a_038 (C terminal GFP with TPQt*)
tube 4: Lvl1_1_a_039(C terminal GFP with TPTm*)

MITTWOCH, 24.6.2026

Level 0 and 1 cloning

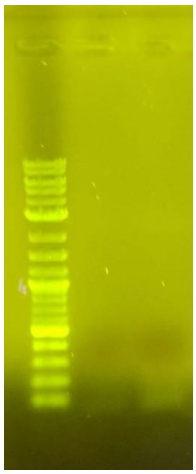
- [x] Trafo of:

tube 1: Lvl0.5_a_030 (0.5NCS)
tube 2: Lvl0.1_a_007 (0.1 TPMx*)
tube 3: Lvl1_1_a_038 (C terminal GFP with TPQt*)
tube 4: Lvl1_1_a_039(C terminal GFP with TPTm*)
according to E. coli Transformation
plate on selection plate with X-Gal Spec plate for Level 0 and Kan Plate for level 1. we plated 30 µl

- [x] Q5 PCR with new primers for 0.3Enc-Mx Q5 PCR

level 0 primer 83,84; fragment size: 898bp
MCS2 Gibson 85,86; fragment size: 904 bp

WhatsApp_Image_2026-06-24_at_17



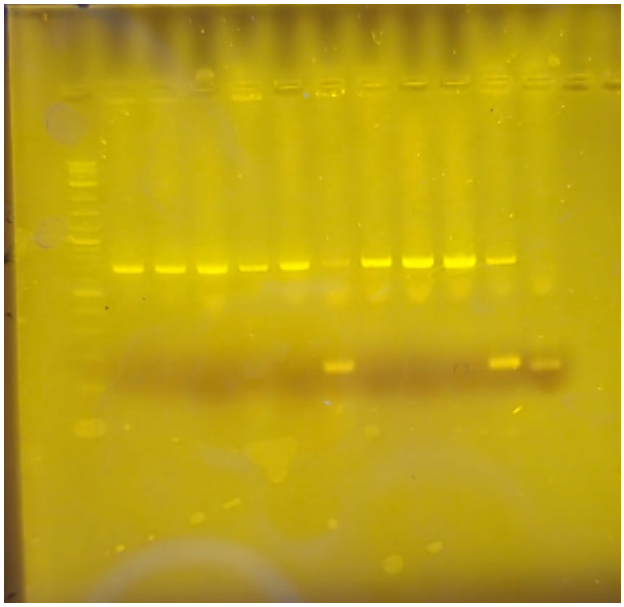
- [x] Inoculation of yeast preculture in YPD medium and incubation at 30°C overnight

DONNERSTAG, 25.6.2026

- [x] cPCR of: Taq colony PCR

tube 1: Lvl0.5_a_030 (0.5NCS)
tube 2: Lvl0.1_a_007 (0.1 TPMx*)
with Primer for Level 0 backbone 65,66 Tm 54°C

0.5 NCS cPCR

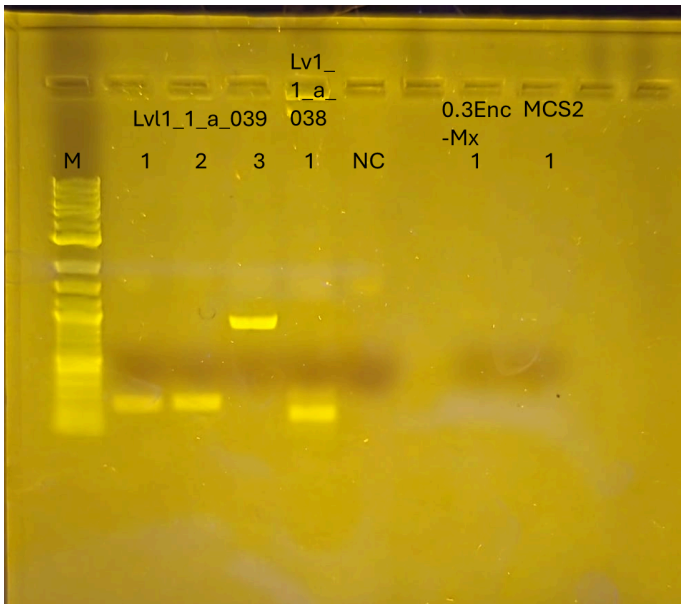


tube 3: Lvl1_1_a_038 (C terminal GFP with TPQt*)
tube 4: Lvl1_1_a_039(C terminal GFP with TPTm*)
with primer for Level 1 backbone oSN 17 oSN 18 Tm 57 °C

- [ ] repeat Q5 PCR with new primers for 0.3Enc-Mx Q5 PCR

level 0 primer 83,84; fragment size: 898bp
MCS2 Gibson 85,86; fragment size: 904 bp

cPCR Level 1 and Q5 for 0.3Enx-Mx

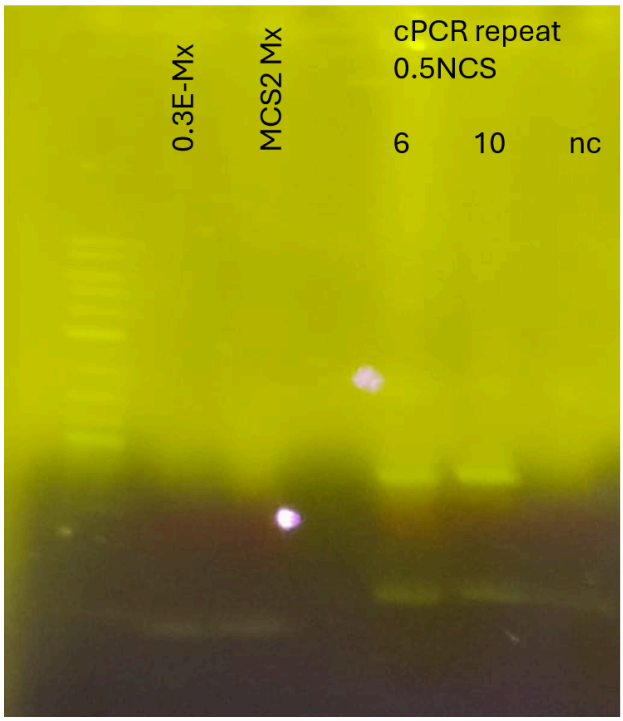


- [ ] overnight culture for 0.1NCSrc colony 3, 0.1TPMx* colony 10 use 3ml LB with 3µl Spec for cryo
- [ ] overnight for Miniprep of Lvl1_1_a_039(C terminal GFP with TPTm*) colony 3 in 3 ml LB
- [ ] overnight for Miniprep of 0.5NCS colonie 6 and 10

- [x] touchdown PCR 0.3Mx + MCS2 Mx

81°C to 67°C

cPCR 0.5NCS and Q5 for 0.3E-Mx



FREITAG, 26.6.2026

- [x] glycerol stock for 0.1 NCSrc (029) 0.1TPMx* (007)
- [x] Minprep from all overnights

Miniprep results 26.06

	A	B	C	D	E
1	number	name	concentration / ng/µL	A260/A280	A260/A230
2	1	0.1NCSrc	219,7	1,83	2,55
3	2	0.1TPMx*	122,9	1,84	2,11
4	3	Lvl1.1_39	101,7	1,87	2,29
5					
6					
7					
8					

- [x] send to sequencing of Lvl1.1_39 with Primer N17 and N18

sequencing preperation1

	A	B	C	D	E	F	G
1	sequencing number	name	concentration / ng/µL	amount to add of insert	amount to add of water	Primer 3µl (20µM)	result
2	98902306	Lvl1.1_39	101,7	6	6	N17	
3	98902307	Lvl1.1_39	101,7	6	6	N18	
4							
5							
6							
7							
8							

MONTAG, 29.6.2026

- [x] cPCR of single colonies for 0.5NCS at least 8 each Primer for level 0 backbone Taq colony PCR
  use a 2% gel

- [x] redo trafo for tube 3: Lvl1_1_a_038 (C terminal GFP with TPQt*) 24.06.26 but with **2 µl** of the golden gate sample, and plate 100 µl of the culture before spinning down on one plate and then spin down the culture discard supernatant except for 50 µl and plate the rest on a second plate! the drop is X-Gal plate 40 µl of it on the plate before plating the cells and level1 needs Kanamycin as an antibiotic.

DIENSTAG, 30.6.2026

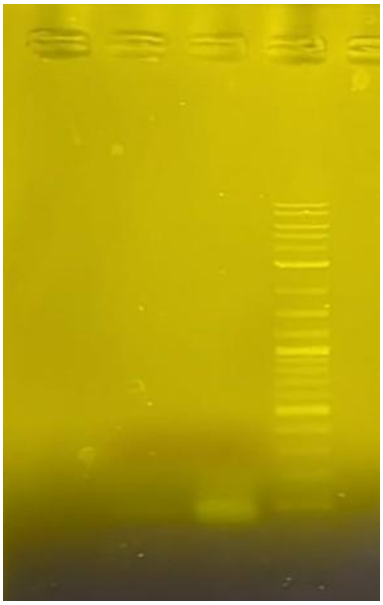
- [x] golden gate repeat

tube 1: Lvl1_1_a_038 (C terminal GFP with TPQt*)
tube 2: Lvl1_1_a_039(C terminal GFP with TPTm*)

again oligo annealing of 0.5 NCS and then repeat the golden gate! Oligo Annealing

- [x] repeat Q5 PCR of Enc-Mx and use 3% DMSO so 0.75 µl
  level 0 primer 83,84; fragment size: 898bp
  MCS2 Gibson 85,86; fragment size: 904 bp
  Tm: 71°C

Q5 Mx



MITTWOCH, 1.7.2026

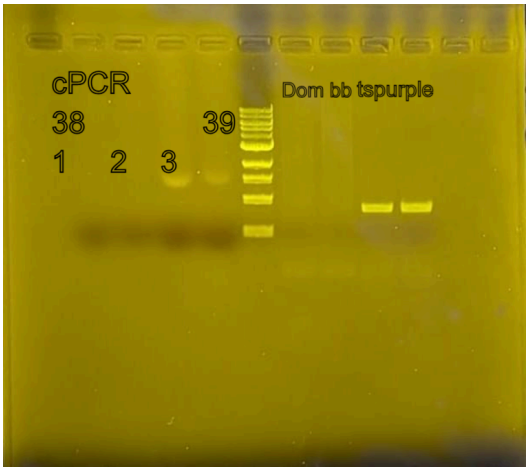
- [x] trafo Golden Gate

tube 1: Lvl1_1_a_038 (C terminal GFP with TPQt*)
tube 2: Lvl1_1_a_039(C terminal GFP with TPTm*)
tube 3: 0.5NCS --> 6

DONNERSTAG, 2.7.2026

- [x] [Gibson] tspurple Level 2 Q5 PCR
  2 replicates each with 1ng/ul and 4ng/ul
  87/88 58°C -> expected length: 5800bp 3min
  89/90 67°C -> expected length: 884bp 30s
  DpnI digest, 1h at 37°C

cPCR level 1 and tspurple



- [x] Level 1 cPCR according to Taq colony PCR

Primer N17 and N18 Tm: 57 °C