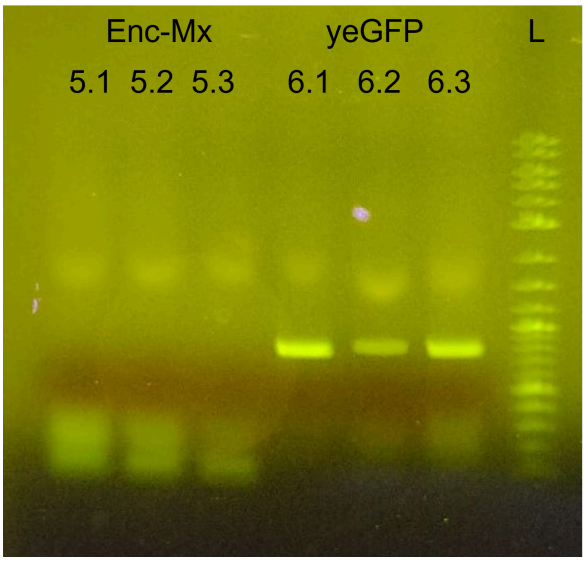
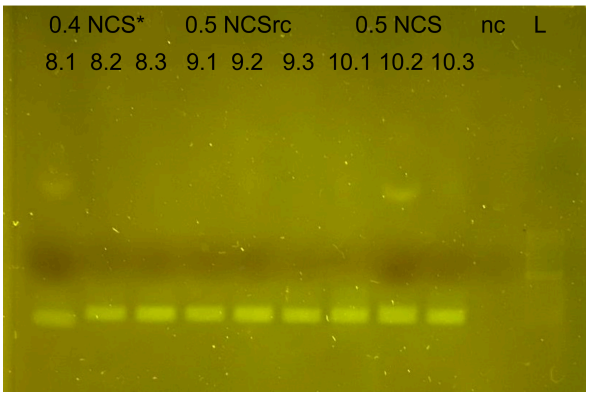
cPCR_R5_R6.png



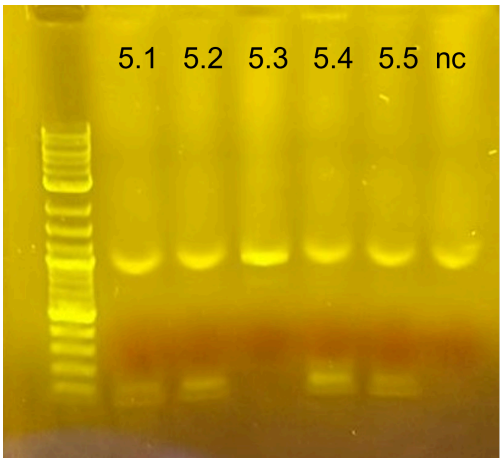
cPCR_R8_R9_R10.png



-> repeat of colonyPCR for R5 with 5 more colonies, same conditions

-> pick 5.3 for miniprep

cPCR_R5_repeat



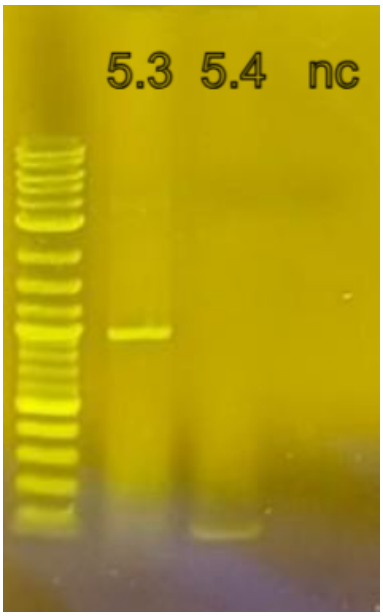
Unexpected "fragments" were observed on all gels that are probebly caused by the used Taq MasterMix so the redo of Mx will be performed using 1Taq MM.

FREITAG, 8.5.2026

Level 0 cloning:

- [x] colonyPCR repeat of Enc.Mx with onetaq Tim

CPCR_R5_onetaq



MONTAG, 11.5.2026

Level 0 cloning:

- [x] overnight culture in 5ml LB for all the Level 0 colonies with a positive result in colony PCR (total 10) (choose colonies) Charlotte Lenné

1.3 2.1 3.2 4.3 5.3 6.1 7.1 8.3 9.2 10.3

DIENSTAG, 12.5.2026

Level 0 cloning:

- [x] Mini Prep of all Level 0, measure the amout of plasmid using Nano Drop (total 10) @Ziyue(Vormittag) Ahmed Haj Kacem

level 0 1.3 :258.2 ng/µl	A260/A280: 1,91 A260/A230: 2,19
level 0 2.1 :298.1 ng/µl	A260/A280: 1,91 A260/A230: 2,91
level 0 3.2 :305.2 ng/µl	A260/A280: 1,92 A260/A230: 2,24
level 0 4.3 :314.0 ng/µl	A260/A280: 1,91 A260/A230: 2,25
level 0 5.3 :150.8 ng/µl	A260/A280: 1,89 A260/A230: 2,25
level 0 6.1 :329.7 ng/µl	A260/A280: 1.92 A260/A230: 2.32

level 0 7.1 :244.3 ng/µl A260/A280: 1.88 A260/A230: 2.26
level 0 8.3 :277.0 ng/µl A260/A280: 1.91 A260/A230: 2.20
level 0 9.2 :306.9 ng/µl A260/A280: 1.91 A260/A230: 2.21
level 0 10.3 :299.9 ng/µl A260/A280: 1.91 A260/A230: 2.25

- [x] Prepare for sequencing Ahmed Haj Kacem (ab 13 Uhr) Felix Cuntz

12µl of plasmid eluated containing 50-100 ng/µl
dilute if nessesary!
add 3 µl of 20mM Primer, in this case Primer 65_Lvl0_bb_fw stock (100mM) was diluted
sequencing will be performed by microsynth

Seq pipetting scheme

	A	B	C	D	E	F	G
1		conc (ng/µl)	end conc (ng/µl)	dilution factor (DNA : H_2O)	DNA ul	H_2O ul	end volume
2	level 0 1.3 (R1)	258.2	86.1	1 : 2	4 µl	8 µl	12 µl
3	level 0 2.1 (R2)	298.1	99.4	1 : 2	4 µl	8 µl	12 µl
4	level 0 3.2 (R3)	305.2	76.3	1 : 3	3 µl	9 µl	12 µl
5	level 0 4.3 (R4)	314.0	78.5	1 : 3	3 µl	9 µl	12 µl
6	level 0 5.3 (R5)	150.8	75.4	1 : 1	6 µl	6 µl	12 µl
7	level 0 6.1 (R6)	329.7	82.4	1 : 3	3 µl	9 µl	12 µl
8	level 0 7.1 (R7)	244.3	81.4	1 : 2	4 µl	8 µl	12 µl
9	level 0 8.3 (R8)	277.0	92.3	1 : 2	4 µl	8 µl	12 µl
10	level 0 9.2 (R9)	306.9	76.7	1 : 3	3 µl	9 µl	12 µl
11	level 0 10.3 (R10)	299.9	75.0	1 : 3	3 µl	9 µl	12 µl

Label for Sequencing:
R1: 98 902 251 -> primer too close to insert, redo sequencing with newly designed primer
R2: 98 902 252
R3: 98 902 253
R4: 98 902 254
R5: 98 902 255 -> no results, redo Lvl 0 Assembly
R6: 98 902 256
R7: 98 902 257
R8: 98 902 258
R9: 98 902 259
R10: 98 902 260

MITTWOCH, 13.5.2026

Level 0 cloning:

- [x] colony PCR 1-10 (except 5 and 6) Charlotte Lenné ab Anica Nissen

according to Taq colony PCR protocol
65_Lvl0_bb_fw
66_Lvl0_bb_rv