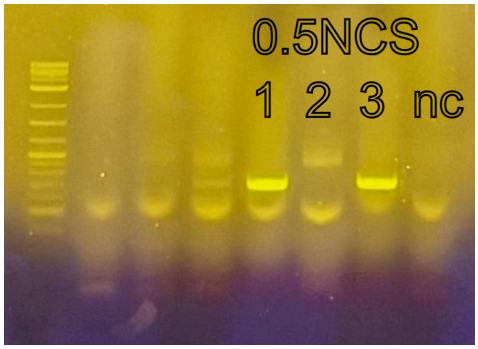
expected fragment size: 1 kb

- [x] Level 0 cPCR

Primer: 65,66 Tm 54°C

expected fragment size: 150 bp

cpcr_0.5NCS.jpeg



- [x] Trafo again for LvL_1.1_38 and 39 with 1 µl as well as 37 for the first time 1 µl and 5 µl plate all cells
- [x] Trafo again of 0.5NCS use rest and plate 50 µl

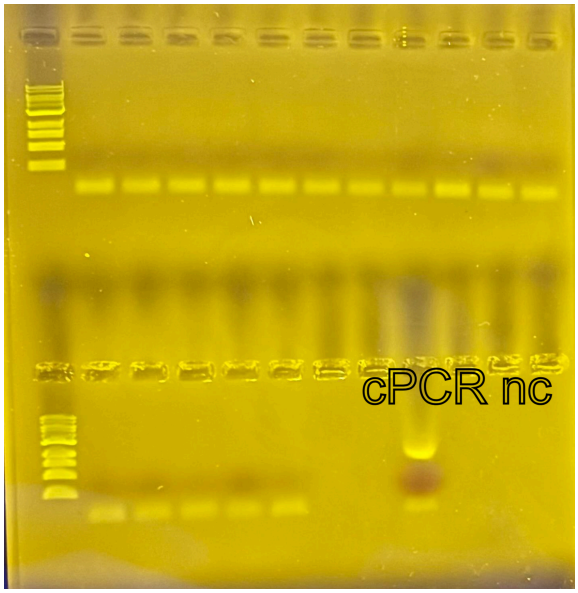
FREITAG, 3.7.2026

- [x] [Gibson] Gradient PCR for Backbone Linearization Q5 PCR

  87/88

  55°C-62°C with digested Domestication and undigested Domestication to find optimal Tm

Gradient PCR



-> again with gel extraction after double digestion

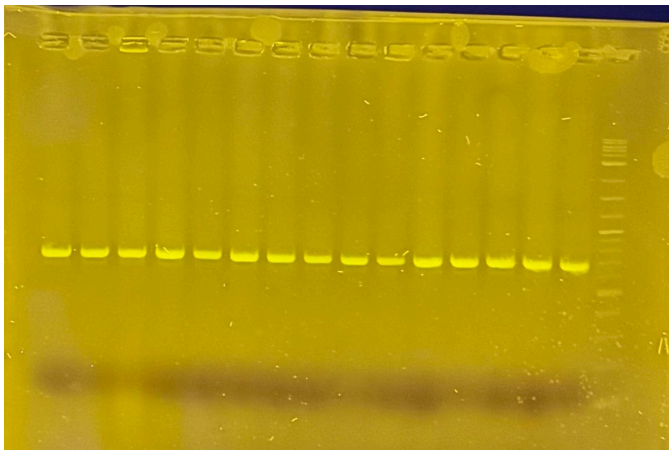
- [x] cPCR 0.5NCS

  65/66

  Tm:54°C

expected length: 150bp

cPCR 0.5NCS



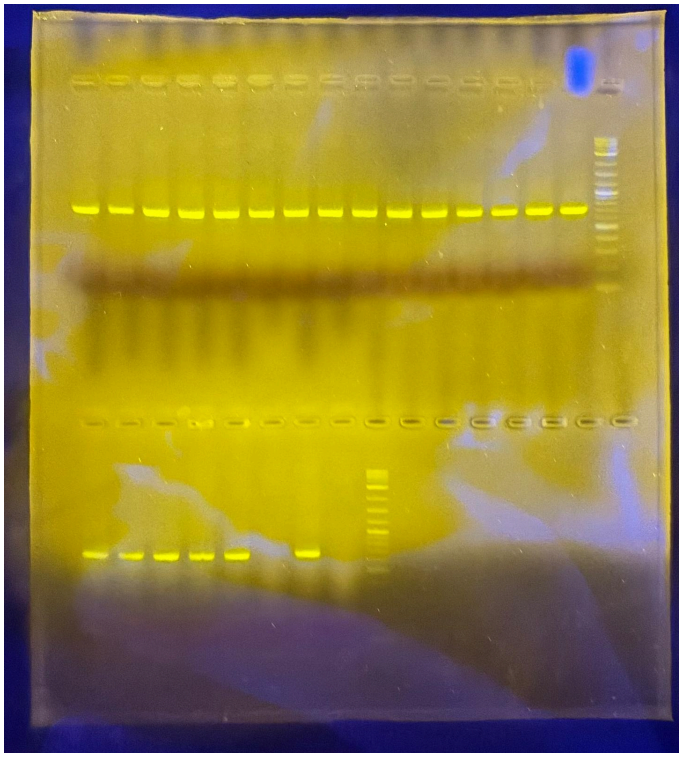
- [x] Trafo: 1.1_38 and 1.1_39 again with 2 µl and plate 100 µl liquid and the the rest of the cells after spinning down
- [x] Trafo redo of 0.5NCS with 5 µl plasmid and 30 µl plating after spinning down

MONTAG, 6.7.2026

- [x] new Spec plates Ahmed Haj Kacem
- [x] prepare overnight tubes with 3 ml LB Ahmed Haj Kacem
- [x] cPCR of 0.5 NCS even though the selection with x gal did not work. Screen 22 colonies Taq colony PCR

Tm:54°C
Elongation: 30 sec
Primer: 65_Lvl0_bb_fw + 66_Lvl0_bb_rv

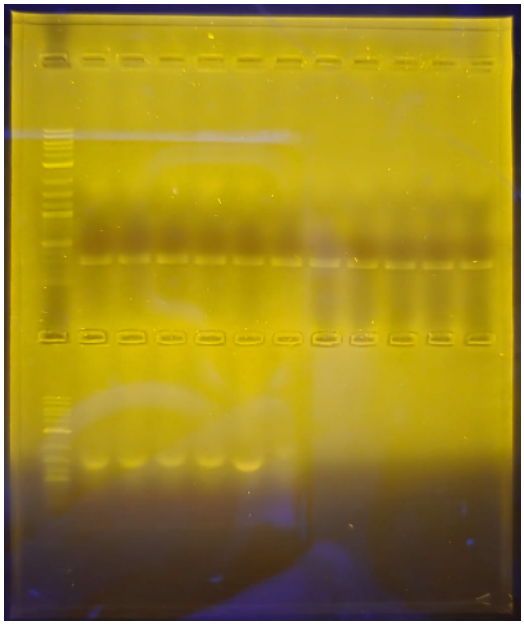
0.5NCS_06.07.2026.jpeg



- [x] do single colonies for the 0.5NCS again that showed two fragments 25.06.2026
- [x] cPCR level 1 construct 38,39 all present colonies (needed to grow a day longer) with the Primers N17 and N18

Primer N17 and N18 Tm: 57 °C
expected fragment size: 1 kb

cPCR of 39 (1-12) and 38 (13-16)



DIENSTAG, 7.7.2026

- [x] test new GG programm for level 1 (5h 37°C) for 37,38,39

- [x] redo golden gate normal

reaction:

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

3 µl backbone (instead of water)!

Golden Gate Level 1 C terminal GFP

	A	B	C	D	E
1	component	amount for one reaction (µl) normal	amount for reaction with more backbone (µl)	MM for 3 reactions (1for each TP) --> x3.1	
2	0.1 TPTm*/Qt*/Mx* (15nM stock)	1	1		
3	0.2 L1 (15nM stock)	1	1	3.1	
4	0.3 yeGFP (15nM stock)	1	1	3.1	
5	0.4 NCSrc (15nM stock)	1	1	3.1	
6	0.5 NCSrc (15nM stock)	1	1	3.1	
7	Level 1 BB (15nM stock)	1	3	9.3	
8	10x T4 Ligase buffer	1	1	3.1	
9	T4 Ligase	0.5	0.5	1.55	
10	BsaI	0.5	0.5	1.55	
11	H2O	2	0	0	
12				place 9 µl in each reaction tube and add 0.1TP	

Test one reaction for each TP with 5h 37°C and the other one with standard cycle overnight

- [x] 0.5 NCS gg new with more insert use 4 µl instead of 2.5 µl (adjust water!)

- [x] double digest Domestication with XhoI, PacI and BamHI, EcoRI double restriction digest

  37°C 1h

  Gel extraction of longer fragment, Gal promoter should appear at ~800bp

  Gibson overhangs: primer 87/88 Q5 PCR

  4 reactions

  1ul of 26.9ng/ul EcoRI/BamHI