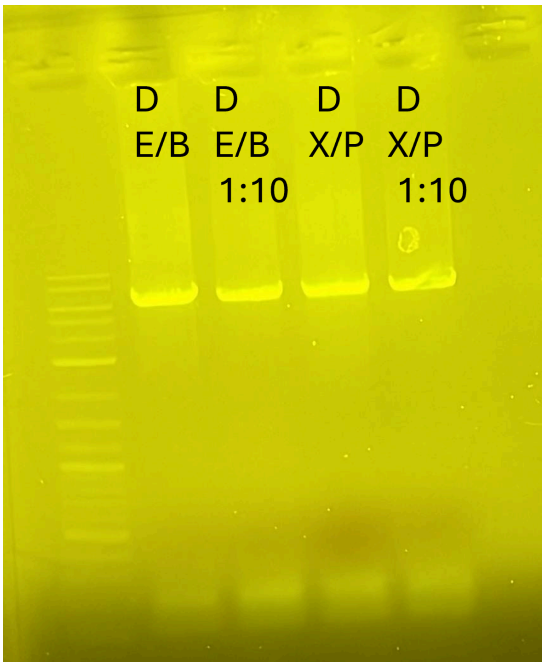
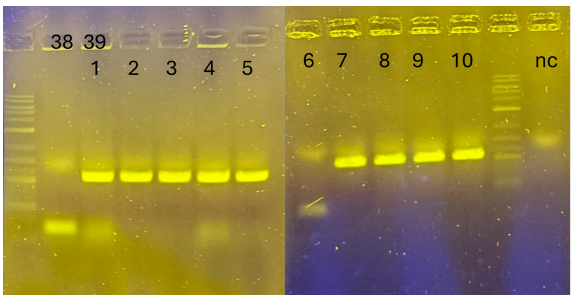
0.4ul of 1:10
1ul of 10.1ng/ul PacI/XhoI
1ul of 1:10
Tm: 58°C
expected length: 5804bp -> 3min elongation

Gibson_overhang_Dom.png



- [x] cPCR Lvl1.1 38, 39 Taq colony PCR

cPCR_Lvl1.png



- [x] cPCR Lvl0 0.5NCS 65,66
11x
colony 21 from 06.07.

MITTWOCH, 8.7.2026

- [x] test different Trafo conditions

--> use 250 µl SOC for recovery
--> use 5 µl gg reaction and 2 µl

--> plate all cells

- [x] Trafo of Golden Gate (37°C level 1 37, 38, 39)

add 5 µl of GG reaction to competent cells follow the protocol E. coli Transformation but plate all cells instead of 30 µl!
dont forget to add the 40 µl X Gal

- [x] overnight Lvl1 39-2, 39-3

- [x] Gibson/cPEC Level 2 backbone tspurple
  calculated with https://punnettsquare.org/gibson-assembly-calculator/
  50ng vector:
  1:3 0.85ul Dom E/B 0.76ul tspurple (22.8ng) -> H20 3.39ul
  1:7 0.85ul Dom E/B 0.93ul tspurple (53.3ng) -> H20 3.37ul
  1:10 0.93ul Dom X/P 2.54ul tspurple (76.2ng) -> H20 1.53ul
  cPEC
  2ul Dom E/B
  2.9ul tspurple
  12.5 Q5
  6.6 H20

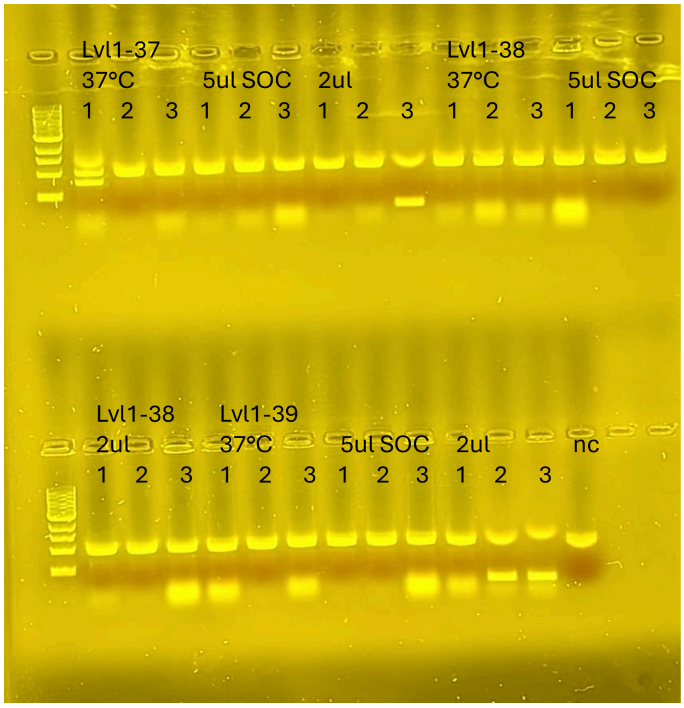
- [x] Golden Gate Assembly 0.3E-Mx

DONNERSTAG, 9.7.2026

- [x] cPCR level 0 --> not possible because there are no colonies on the plate (adding more insert is not the call!)
- [x] cPCR level 1

three colonies for each condition --> total 27, first three from 37°C reaction, next three 5µl SOC and last three from 2 µl in Trafo
1.1_37 1-9
1.1_38 10-18
1.1_39 19-27
one nc
Taq colony PCR
Primer N17 and N18 Tm: 57 °C
expected fragment size: 1 kb

cPCR_Lvl1-373839.png



overnight from 1.1_37 37°C c2, 1.1_37 SOC c2, 1.1_38 37°C c1, 1.1_38 SOC c2

- [x] miniprep Lvl1 39-2, 39-3

Miniprep results 09.07

	A	B	C	D	E
1	number	name	concentration / ng/µL	A260/A280	A260/A230
2	1	1.1_39 c2	368	1.87	2.33
3	2	1.1_39 c3	185.7	1.85	2.34

- [x] prepare for seq Send to Sequencing (Mycrosynth)

sequencing preperation 09.07

	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	98902316	Lvl1.1_39 c2	368	2	10	N17	correct
3	98902317	Lvl1.1_39 c2	368	2	10	N18	
4	98902318	Lvl1.1_39 c3	185.7	3	9	N17	correct
5	98902319	Lvl1.1_39 c3	185.7	3	9	N18	
6							
7							
8							

- [ ] Trafo 0.3E-Mx 5 µl into 50 µl cells and plate 30 µl after spinning down and rest onto second plate

- [ ] cPCR Level 2 tspurple
  LP22/ LP34
  Tm: 64°C
  expected length: 1860bp

cPEC_tspurple.png

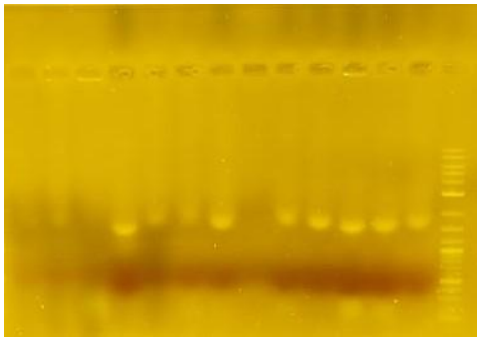


repeat Gibson and cPEC

FREITAG, 10.7.2026

- [ ] cPCR 0.5NCS 0.3E-Mx Taq colony PCR

cPCR



no expected fragments. but for the clonig 5 U enzymes were used istead of 10 U!

- [x] Miniprep of the 1.1_37 37°C c2, 1.1_37 SOC c2, 1.1_38 37°C c1, 1.1_38 SOC c2

Miniprep results 10.07

	A	B	C	D	E
1	number	name	concentration / ng/µL	A260/A280	A260/A230
2	1	1.1_37 37°C c2	197.3	1.87	2.25
3	2	1.1_37 SOC c2	227.7	1.86	2.24
4	3	1.1_38 37°C c1	223.6	1.86	2.3
5	4	1.1_38 SOC c2	153.6	1.86	2.12

- [x] prepare for seq Send to Sequencing (Mycrosynth)

sequencing preperation 10.07

	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	98902320	1.1_37 37°C c2	197.3	3	9	N17	
3	98902321	1.1_37 SOC c2	227.7	3	9	N17	
4	98902322	1.1_38 37°C c1	223.6	3	9	N17	
5	98902323	1.1_38 SOC c2	153.6	4	8	N17	
6							
7							
8							