toolKIT Lab Book 2.0

MONTAG, 13.7.2026

- [x] overnight for glycerol of Lvl1.1_39 c2 09.07
- [x] starting culture for competent E.coli

DIENSTAG, 14.7.2026

- [x] Level 2 backbone tspurple Drop-out
  cPEC
  Tm: 71°C
  Extension: 3.5min
  100ng Backbone 2.23ul Dom X/P 45ng/ul
  30ng Insert 1ul tspurple 30ng/ul
  9.27ul H20
  12.5ul Q5

  Gibson
  50ng vector:
  1:7 1.33ul Dom E/B 2 2.09ul tspurple 2 (53.3ng) -> H20 1.58ul
  1:10 0.85ul Dom X/P 2 2.54ul tspurple 1 (76.2ng) -> H20 1.61ul
  - [x] Trafo

- [x] overnight Lvl1.1-37 (37°C c2) and Lvl1.1-38 (37°C-c1) with Kan for glycerols

- [x] golden gate for 0.3 EncMx and 0.5NCS according to Golden Gate Assembly with oligos and use 4 µl backbone instead of 1µl (1.5 µl water for 15nm stock and 3 µl of oligo)

Following the Creating competent E.coli cells protocol the 1L Volume was injected with 10 mL of starting culture at 09:10.

competent cells

	time	OD600
1	09:10	-
2	10:10	0,028
3	11:10	0,050
4	12:10	0,159
5	12:55	0,316
6	13:05	0,364
7	13:10	0,382

55 eppis each containing 50µl of competent cells were frozen at -80 degrees for further usage.

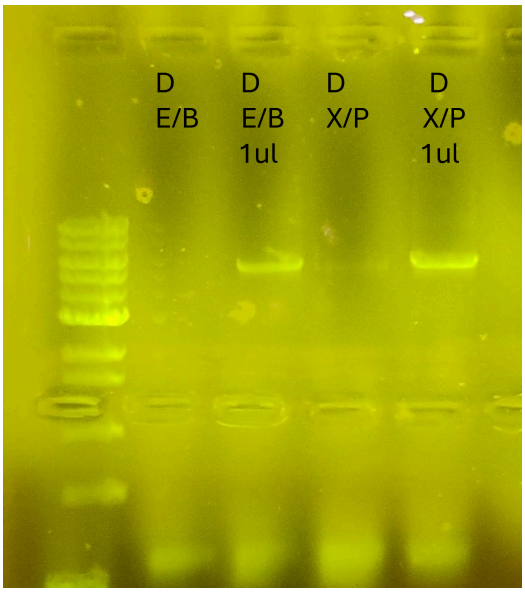
MITTWOCH, 15.7.2026

- [x] Trafo of Golden Gate according to E. coli Transformation
- [x] cPCR cPEC Level 2 tspurple
  no band

DONNERSTAG, 16.7.2026

- [x] cPCR cPEC Level 2 tspurple
  overnight culture + new colonies -> no bands
- [x] Q5 double digestion domesticated pESC for Gibson overhangs
  primer 87, 88

Gibson_overhang_backbone.png



- [x] cPCR 0.5NCS, 0.3Mx
  primer 65,66

MONTAG, 20.7.2026

- [x] domesticated pESC for Gibson overhangs: PCR cleanup of D E/B 1ul and D X/P 1ul
- [x] cPEC Level 2 tspurple
  Tm: 71°C
  Extension: 3.5min
  R1:
  D E/B 62ng/ul -> 1.61ul
  tspurple 30ng/ul -> 3.33ul
  Q5: 12.5ul
  H2O: 9.56ul

  D X/P 65ng/ul -> 1.54ul
  tspurple 25ng/ul -> 4ul
  Q5: 12.5
  H2O: 6.96ul

DIENSTAG, 21.7.2026

- [x] Trafo cPEC

5ul cPEC, 200ul SOC

- [x] Golden Gate Assembly according to Golden Gate Assembly Level 1 protocol

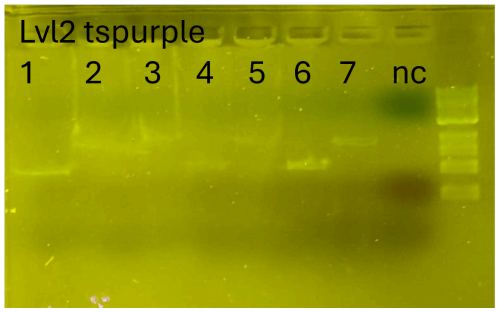
Lvl1_a_43
Lvl1_a_44
Lvl1_a_45
Lvl1_a_46

MITTWOCH, 22.7.2026

- [x] Trafo Golden Gate 43, 44, 45, 46
- [x] cPCR cPEC according to Taq colony PCR

LP22, LP34
Tm: 50°C
Extension: 2min, expected length: 1850bp

cPCR_Lvl2_tspurple.png

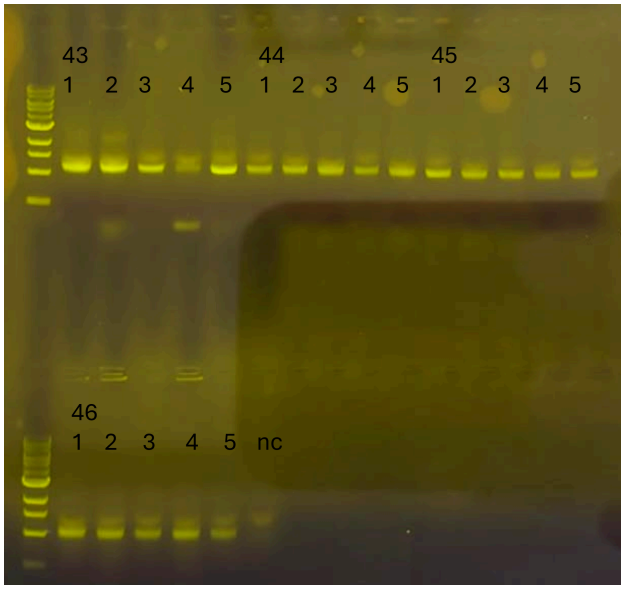


pick c3 and c7, sequencing via E.coli NightSeq from microsynth

DONNERSTAG, 23.7.2026

- [x] cPCR Lvl1.3 43, 44, 45, 46
  expected length: 1200bp

cPCR_1.3.png



Lvl1.3_a_43 Mx: c3

Lvl1.3_a_044 Mxpe: c1

Lvl1.3_a_045 Qt: c1

1.3_a_046 Tm: c1

sequencing preperation 24.07.

	A	B
1	Construct	concentration / ng/µL
2	ts purple 3	47.2
3	ts purple 7	45.6
4	Lvl1.3_a_43 Mx	95.9
5	Lvl1.3_a_044 Mxpe	193.5
6	Lvl1.3_a_045 Qt	160
7	1.3_a_046 Tm	141.7
8		

MONTAG, 27.7.2026

Level 2 Destination Backbone tspurple correct

c7 overnight for glycerol

DIENSTAG, 28.7.2026

S. cerevisiae transformation with Level2 Destination tspurple
5ul of 115ng/ul
10ul of 115ng/ul
20min heatshock
all level 1.3 sequencing results were positiv

MITTWOCH, 29.7.2026

- [x] Mini Prep 37 1.1CTPMx GFP and 38 1.1CTPQtGFP for level 2 golden gate
- [x] Glycerol of 1.3 culture (43,44,45,46)

Miniprep results 29.07

	A	B	C	D	E
1	number	name	concentration / ng/µL	A260/A280	A260/A230
2	1	37	198.7	1.86	2.05
3	2	38	143.1	1.86	2.01
4	3	43	37.8	1.73	1.14
5	4	44	140	1.84	1.87
6	5	45	89.7	1.86	2.0
7	6	46	159.5	1.84	1.77

- [x] level 2 Golden gate

47: 2 C-term TP Mx yeGFP - Mx --> 37+36+43
48: 2 C-term TP Mx yeGFP - Mxpe --> 37+36+44
49: 2 C-term TP Qt yeGFP - Qt --> 37+36+45
add 1.1 and 1.3 module after distributing the MM.

Table2

	A	B	C	D	E
1		reaction PCR amplified or ordered (15nM stock)	MM 4.2		
2			4.2		
3	1.1	1			
4	1.2	1	4.2		
5	1.3	1			
6	10x T4 Ligase Buffer	1	4.2		
7	T4 Ligase	0.5	2.1		
8	BbsI	0.2	0.84		
9	Backbone levl2 (1 µl of 15nM stock)	5.3	22.26		
10					

DONNERSTAG, 30.7.2026

- [x] Trafo 47, 48, 49 recovery in 200 µl SOC plate everything

FREITAG, 31.7.2026

- [x] cPCR of level 2 using LP34 and LP22 Primer (annealing temp 50 °C), the colonies were still small so there was no purple colore visible yet. we rendomly selected 5 colonies per plate to test. Taq colony PCR elongation time 2min 15 sec

47: 2 C-term TP Mx yeGFP - Mx : probe 1.1 -1.5 expected size: 3769bp -> c5

48: 2 C-term TP Mx yeGFP - Mxpe probe 2.1-2.5 expected size: 3750 bp -> c4

49: 2 C-term TP Qt yeGFP - Qt probe 31-3.5 expected size: 3765 bp -> c1

89: probe 4.1-4.5 expected size: 3500 bp (Nanoreactor) -> c5