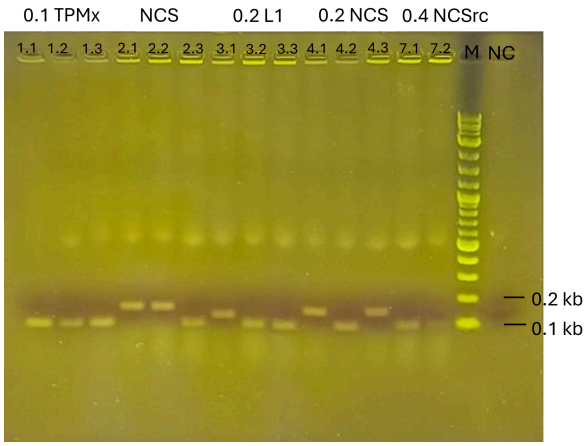
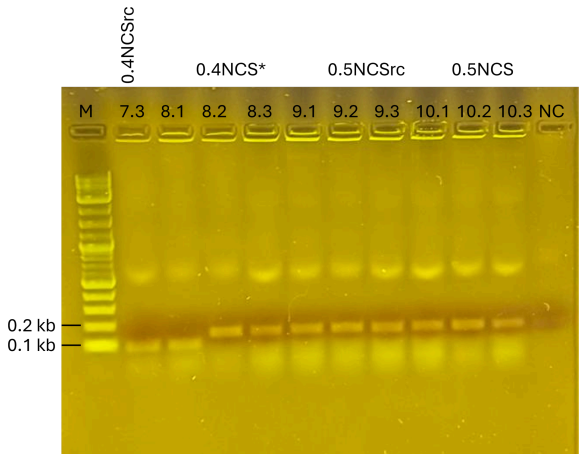
first prepare master mix distribute into PCR tubes add colony and pipet up and down.
redo colony PCR for these Inserts (same colonies as 07.05.2026)
R1: 0.1 TPMx* 150bp
R2: 0.1 NCS 160bp
R3: 0.2 L1 140bp
R4: 0.2 NCS 160bp
R7: 0.4 NCSrc 160bp
R8: 0.4 NCS* 160bp
R9: 0.5 NCSrc 160bp
R10: 0.5 NCS 160bp
2% Agarose 1kb plus ladder
loading:
gel one: 1.1 - 7.2 Ladder NC
Gel two: Ladder 7.3 - 10.3 NC

Level_0_2.png



Level_0_2.png



MONTAG, 18.5.2026

Domestication level 2 backbone:

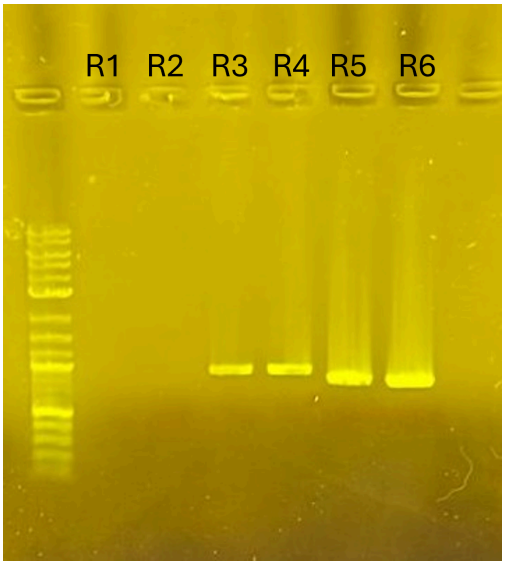
- [x] [Gibson] Backbone pESC Domestication
  Tm: 66°C, 45s
  pESC 15_2uORI_Dom_fw, 18_HIS3_Dom_rv
  expected length: 1400bp

Proof of concept level 2:

- [x] [Gibson] Level 2 Validation
  Tm: 59°C, 30s
  57_Mx_MCS2_fw, 58_Mx_MCS2_rv
  expected length: 900bp
  63_yeGFP_MCS1_fw, 64_yeGFP_MCS1_rv
  expected length: 800bp

duplicates for each reaction
R1: pESC Dom 1
R2: pESC Dom 2
R3: Mx MCS2 1
R4: Mx MCS2 2
R5: yeGFP MCS1 1
R6: yeGFP MCS1 2
=> Domestication did not yield a band
=> nested PCR with 55_pESC_bb_fw, 56_pESC_bb_rv

Gibson.png



DIENSTAG, 19.5.2026

- [x] [Gibson] PCR Clean up
  nested pESC ev conc.: 47.2
  nested pESC MCS2 conc: 48.3
  Dom 17-20 conc.: 65

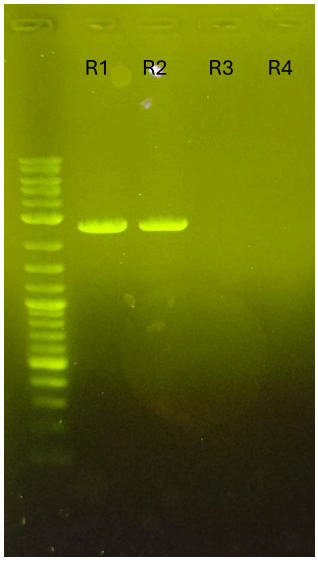
Dom 19-16 conc.: 31.5
Mx MCS 2 1 conc.: 56.1
Mx MCS2 2 conc.: 47.8
yeGFP MCS1 1 conc.: 93.6
yeGFP MCS1 2 conc.: 90.3

Domestication Level 2 backbone:

[Gibson] Domestication repeat, nested PCR

Duplicates with 2 different templates: pESC empty vector and pESC MCS2 digested with BamHI/XhoI, linearized (22.04.)
R1: 55_pESC_bb_fw, 56_pESC_bb_rv; pESC ev
R2: 55_pESC_bb_fw, 56_pESC_bb_rv, pESC MCS2 linearized
Tm: 58°C, 1:45min
expected length: 2600bp
R3: 15_2uORI_Dom_fw, 18_HIS3_Dom_rv; pESC ev
R4: 15_2uORI_Dom_fw, 18_HIS3_Dom_rv; pESC MCS2 linearized
Tm: 66°C, 45s
expected length: 1400bp

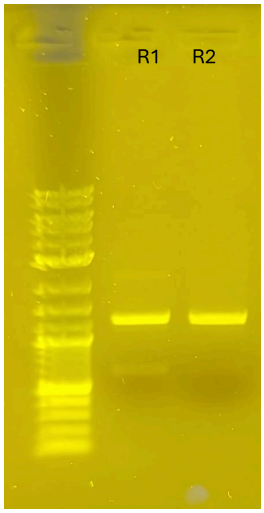
Domestication.png



[Gibson] nested PCR on R1 and R2

Template:
R1: nested pESC ev conc.: 47.2
R2: nested pESC MCS2 linearized conc: 48.3
15_2uORI_Dom_fw, 18_HIS3_Dom_rv
=> take R2 for Gibson Assembly, less background bands

Domestication2.png



Level 0 cloning:

- [x] oligo annealing according to Oligo Annealing

18 µl water each and 1µl forward 1 µl reverse (100µM)

Level 0 cloning overview

	A	B	C	D	E	F
1	number	name	Primer	Nanodrop ng/µl	length bp	used for GG µl
2	1	0.1TPQtStop	27+28	172,7	81	2,5
3	2	0.1TPTmStop	29+30	154,0	78	2,5
4	3	0.2NCSrcStop	33+34	156,5	84	2,5
5	4	0.4 L1	37+38	235,9	62	2,5
6	5	0.5TPMxStart	43+44	179,5	75	2,5
7	6	0.5TPQtStart	45+46	237,4	81	2,5
8	7	0.5TPTmStart	47+48	172,4	76	2,5
9	8	Enc_Mx	use from last week		898	1 from 15mM stock

- [x] golden gate cloning Charlotte Lenné

prepare the 15mM dilution for backbone and Enc_Mx

use the Golden Gate Assembly table to calculate and prepare the reactions

backbone: 2659bp

MITTWOCH, 20.5.2026

Proof of concept Level 2:

- [ ] [Gibson] Assembly Mx MCS2 in pESC digested MCS2 according to Gibson Assembly

  Backbone/ Insert: 0.02pmol

(0.02pmol*6640bp*650Da)/1000=86.32ng
pESC MCS2 conc.: 43 ng/ul -> 2ul
Using NEBioCalculator: Insert 33.70 ng (3:1)
Mx MCS2 2 conc.: 47.8ng/ul -> 0.7ul

Domestication Level 2 backbone:

- [x] [Gibson] Assembly Domestication according to Gibson Assembly

PCR Cleanup Dom 15-18
Dom 15-18 conc.: 25.7ng/ul
Dom 17-20 conc.: 65ng/ul
Dom 19-16 conc.: 31.5ng/ul
Backbone/ Insert: 0.02pmol
Backbone=longest insert -> Dom 19-16 -> 1.5ul
(0.02pmol*3633bp*650Da)/1000=47.23ng
Using NEBioCalculator:
Dom 15-18 (3:1) 50.19 ng -> 1.95ul
Dom 17-20 (3:1) 72.15 ng -> 1.11ul

- [x] [Gibson] Trafo

Level 0 cloning:

- [x] prepare specR plates with X Gal

take the LB Agar from the cold room (400ml) and heat it up in the microwave till the agar is completly disolved. Add Spec (stock concentration 60 mg/ml) to end up with 60 µg/ml Spec in the medium.

- [x] Trafo 10:00

before plating 40 µl of the XGals (DMSO) stock 20mg/ml has to be added to the plate and distributed evenly. (for the lacZ selection)

-> all plates showed white colonies

according to E. coli Transformation

- [x] plate ca. 13:30

DONNERSTAG, 21.5.2026

Level 0 cloning:

- [x] colony PCR Level 0 modules

no colonies on the Enc_Mx plate and no white colonies for construct 6 and 7
according to Taq colony PCR protocol
65_Lvl0_bb_fw
66_Lvl0_bb_rv