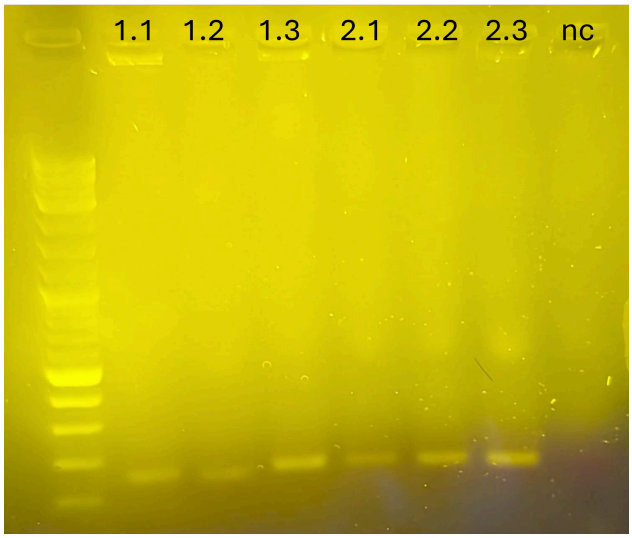
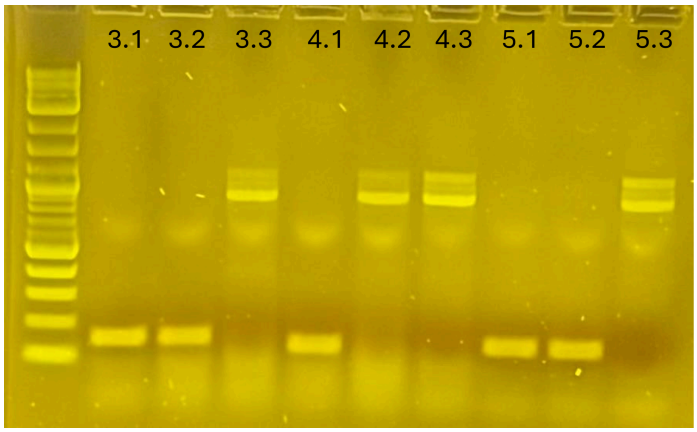
Level 0 exp length

	A	B	C
1	1	0.1TPQtStop	155bp
2	2	0.1TPTmStop	152bp
3	3	0.2NCSStop	160bp
4	4	0.4 L1	140bp
5	5	0.5TPMxStart	150bp
6	6	0.5TPQtStart	155bp
7	7	0.5TPTmStart	150bp
8	8	Enc_Mx	1000bp

cPCR_Lvl0.png



cPCR_Lvl0_2.png



pick 1.3, 2.3 3.1, 4.1 and 5.1

- [x] overnight from correct colonies and redo mini for 0.2 L1 with 3.1

1.3 0.1TPQtStop
2.3 0.1TPTmStop
3.2 0.2NCSStop
4.1 0.4 L1
5.2 0.5TPMxStart
and 3.1 0.2 L1 (13.05)

FREITAG, 22.5.2026

- [x] Level 0 Mini Prep

DIENSTAG, 26.5.2026

Proof of concept Level 2:

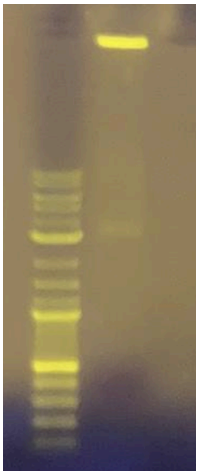
- [x] [Gibson] repeat
  https://nebuildercalculator.neb.com/
  R1: pESC MCS2 linearized, Mx MCS2
  pESC MCS2 4.8ul (0.05pmol)
  Mx MCS2 1.2 ul (0.1pmol)
  H20 2.05ul
  HiFi DNA Assembly MM 5ul
  total: 10ul

Domestication Level2 backbone:

R2: Domestication
19-16 1.7ul (0.025pmol)
17-20 0.9ul (0.05pmol)
15-18 1.5ul (0.05pmol)
H20 2.95ul
HiFi DNA Assembly MM 5ul
total: 10ul
1h at 50min

- [x] [Gibson] Trafo R1, R2
- [x] [Gibson] cPEC
  Q5 protocol without Primers, Tm depends on fragments (max 72°C) high concentration of fragment needed because there is no amplification! 100ng per fragment
  fragment 1: 15-18 30,5 ng/µl -> 3.28ul
  fragment 2:19-16 31,5 ng/µl -> 3.17ul
  fragment 3: 17-20 65 ng/µl -> 1.54ul
  8ul fragments in total
  12.5ul Q5 MM
  4.5ul H20
  Tm: 71°
  Extension: 3:45min

image.png



Level 0 cloning:

- [ ] hydrate twist sequences --> spin down 400 xg 1000 ng each so dilute in 100 µl to have a total concetration of **10ng/µl**
- [ ] Golden Gate Assembly Ziyue Zhou Golden Gate Assembly
  Enc-Mxpe, Enc-Qt, Enc-Tm, PABP in Level 0 --> use twist seq directly as insert
  repeat 0.5TPQtStart (6) and 0.5TPTmStart (7) --> oligos
  repeat Enc-Mx

1. 0.3 Enc-Mxpe
2. 0.3 Enc-Tm
3. 0.3 Enc-Qt
4. 0.3 PABP
5. 0.3 Enc-Mx
6. 0.5 TPQtStart
7. 0.5TPTmStart

Level 0 construct overview

	A	B	C	D	E	F
1	level 0 construct	length	Primer	cPCR size	ng/µl	seq dilution
2	0.1 NCS	78	23,24	160	298.1	
3	0.1 TPMx*	65	25,26	150	258.2	
4	0.1 TPQt*	81	27,28	155	220	
5	0.1 TPTm*	78	29,30	152	314.7	
6	0.2 L1	52	31,32	140	257.4	
7	0.2 NCS	73	oligo	160	314	
8	0.2NCS*	84	33,34	160	308.8	
9	0.3 yeGFP	751	35,36	800	329.7	
10	0.3 PABP	548	twist			
11	0.3 PABP-tr		67,68			
12	0.3 E-Tm	832	twist	917		
13	0.3 E-Qt	883	twist	967		
14	0.3 E-Mx	898	53,54	980	153.8	
15	0.3 E-Mxpe	877	twist	961		
16	0.4 L1	62	37,38	140	257.1	
17	0.4 NCS	75	39,40	160	244.3	
18	0.4 NCS*	73	59,60	160	277	
19	0.5 NCS	77	61,62	160	299.9	
20	0.5 TP-MxSt	75	43,44	150	309.4	
21	0.5 TP-QtSt	81	45,46	155		
22	0.5 TP-TmSt	76	47,48	150		

MITTWOCH, 27.5.2026

Level 0 cloning: Trafo

- [x] Trafo Golden Gate level 0 Charlotte Lenné E. coli Transformation
- [x] add X Gal to the Spec Plates 40 µl

plate 30 µl

Proof of concept Level 2:

- [x] [Gibson] cPCR Taq colony PCR

  Note: for Gibson plate 200ul

  pESC MCS2:

  primer: LP022, LP034

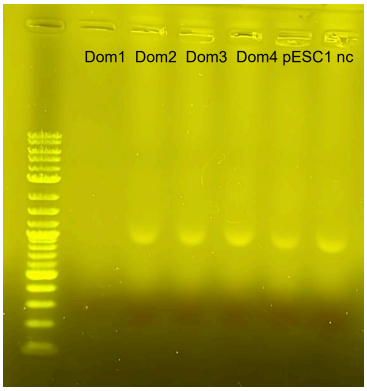
  expected length: 2800bp

Domestication Level 2 backbone:

Domestication:

55_pESC_bb_fw; 56_pESC_bb_rv
expected length: 2700bp
Tm: 64°C
Extension: 2:45min

cPCR_Dom.png



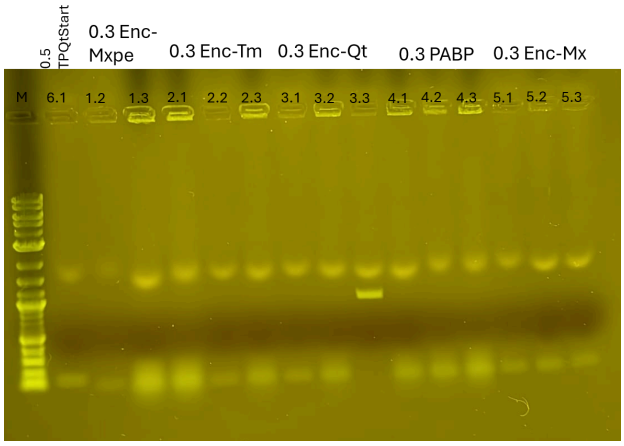
DONNERSTAG, 28.5.2026

Level 0 cloning:

- [ ] cPCR from golden gate level0 + gel Taq colony PCR Alex John Thottumkal

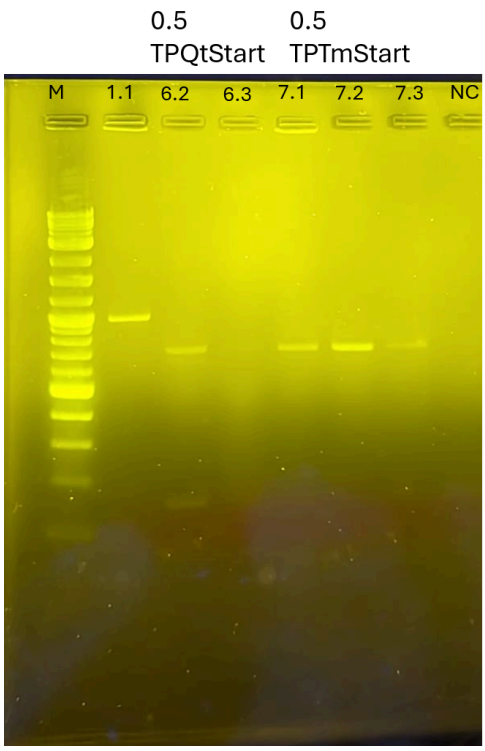
Primer dilution 65 and 66 stock 100µM to 10 µM
Tm 54°C and 1 min elongation
1% gel

cPCR level 0



2% gel

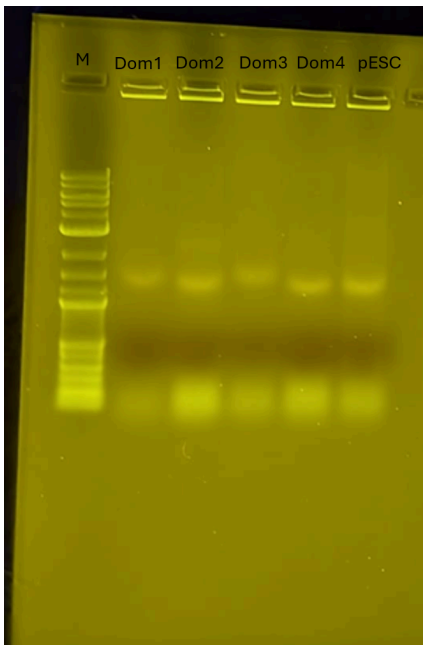
cPCR level 0



- [x] Repeat cPCR with Annealing Temp: 44°C

ladder Dom1 Dom2 Dom 3 Dom4 pESC

domestication cPCR



-> Dom2 weak band

FREITAG, 29.5.2026

- [x] Mini Prep Alex John Thottumkal

Level 0 construct overview 29.05.26

	A	B	C	D	E	F	G
1	level 0 construct	length	Primer	cPCR size	concentration ng/µl		
2	0.1 NCS	78	23,24	160	298.1		
3	0.1 TPMx*	27	25,26	150	258.2		
4	0.1 TPQt*	34	27,28	155	220		
5	0.1 TPTm*	29	29,30	152	314.7		
6	0.2 L1	18	31,32	140	257.4		
7	0.2 NCS	41	oligo	160	314		
8	0.2NCS*	42	33,34	160	308.8		
9	0.3 yeGFP	751	35,36	800	329.7		
10	0.3 PABP	>1700	twist				
11	0.3 PABP-tr		67,68				
12	0.3 E-Tm	832	twist	917			
13	0.3 E-Qt	883	twist	967	324		
14	0.3 E-Mx	898	53,54	980	153.8		
15	0.3 E-Mxpe	877	twist	961	272.1		
16	0.4 L1	18	37,38	140	257.1		
17	0.4 NCS	41	39,40	160	244.3		
18	0.4 NCS*	41	59,60	160	277		
19	0.5 NCS	41	61,62	160	299.9		
20	0.5 TP-MxSt	24	43,44	150	309.4		
21	0.5 TP-QtSt	30	45,46	155	250.2		
22	0.5 TP-TmSt	25	47,48	150			

- [x] cPCR repeat Golden Gate 6 colonies each Taq colony PCR

2 0.3 Enc-Tm 2.1 -2.6
4 0.3 PABP 4.1 -4.6
5 0.3 Enc-Mx 5.1-5-6
7 0.5TPTmStart 7.1-7.6
primer 65, 66 Tm 54°C and 1 min elongation