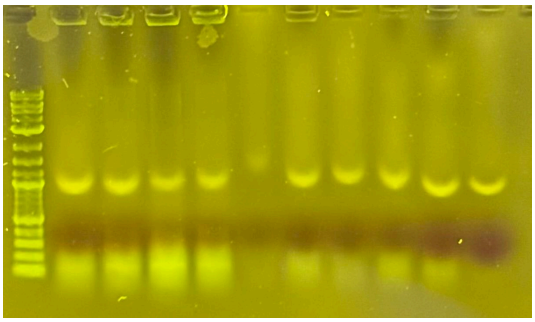
- [ ] [Gibson] cPCR repeat pESC MCS2, Domestication Taq colony PCR

  pESC MCS2 Mx M1-M4
  primer: LP022, LP034
  Domestication: D1-D5
  55_pESC_bb_fw; 56_pESC_bb_rv
  44°C, 2:45min

  **cPCR Gibson**

- [ ] overnight cultures Level 0 2.6, 4.5, 7.1; Dom 2(28.05)

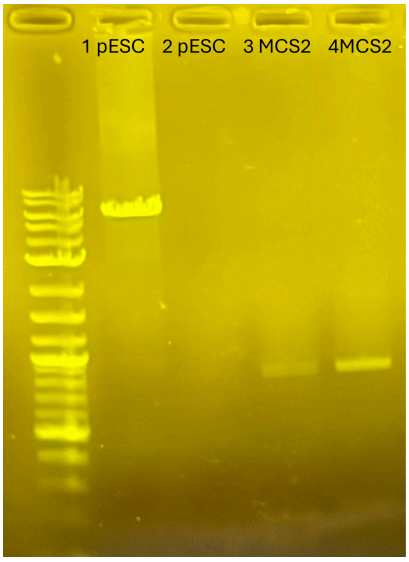
**DIENSTAG, 2.6.2026**

- [ ] [Gibson] repeat PCR for overhangs Mx MCS2, pESC linearization amplification Q5 PCR

  7_bb_MSC2_fw, 8_bb_MSC2_rv
  Tm: 61°C
  6640bp
  template: 1 pESC digested 20ng/ul -> 1ng
  2 pESC digested 4.3 ng/ul ->1ng

  57_Mx_MCS2_fw, 58_Mx_MCS2_rv
  Tm: 60°C
  904bp
  template: 3 MCS2 Mx 1ng/ul
  4 MCS2 Mx 47.8ng/ul -> 1ng

Gibson_2.png




- [x] Miniprep 2.6, 4.5, 7.1, Dom2(28.05) Alex John Thottumkal
- [x] prepare for sequencing of all level 0
  12µl of plasmid eluated containing 100 ng/µl
  add 3 µl of 20uM 69_level0_seq_fw
  sequencing will be performed by microsynth
  Full plasmid seq of domestication (Dom2)

Prepare for Sequencing

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | 98902261 | 1 | 0.1TPMx* | 258.2 | 4.64756003 | 7.35243997 | |
| 2 | 98902262 | 2 | 0.1NCS | 298.1 | 4.0254948 | 7.9745052 | |
| 3 | 98902263 | 3 | 0.2L1 [1] | 305.2 | 3.93184797 | 8.06815203 | |
| 4 | 98902264 | 4 | 0.2NCS | 314 | 3.82165605 | 8.17834395 | |
| 5 | 98902265 | 5 | 0.3E-Mx | 150.8 | 7.95755968 | 4.04244032 | |
| 6 | 98902266 | 6 | 0.4NCS | 244.3 | 4.91199345 | 7.08800655 | |
| 7 | 98902267 | 7 | 0.4NCS* | 277 | 4.33212996 | 7.66787004 | |
| 8 | 98902268 | 8 | 0.5NCS | 299.9 | 4.00133378 | 7.99866622 | |
| 9 | 98902269 | 9 | 0.5TPMxStart | 309.4 | 3.87847447 | 8.12152553 | |
| 10 | 98902270 | 10 | 0.4L1 | 257.1 | 4.66744457 | 7.33255543 | |
| 11 | 98902271 | 11 | 0.2NCS* | 308.8 | 3.88601036 | 8.11398964 | |
| 12 | 98902272 | 12 | 0.1TPTm* | 314.7 | 3.81315539 | 8.18684461 | |
| 13 | 98902273 | 13 | 0.1TPQt* | 220 | 5.45454545 | 6.54545455 | |
| 14 | 98902274 | 14 | 0.2L1 [2] | 257.4 | 4.66200466 | 7.33799534 | |
| 15 | 98902275 | 15 | 0.3E-Qt | 324 | 3.7037037 | 8.2962963 | |
| 16 | 98902276 | 16 | 0.3E-Mxpe | 272.1 | 4.41014333 | 7.58985667 | |
| 17 | 98902277 | 17 | 0.5TPQtStart | 250.2 | 4.79616307 | 7.20383693 | |
| 18 | 98902278 | 18 | 0.3 PABP | 338 | 3.55029586 | 8.44970414 | |
| 19 | 98902279 | 19 | 0.3 E-Tm | 334.2 | 3.59066427 | 8.40933573 | |
| 20 | 98902280 | 20 | 0.5 TPTmStart | 290.3 | 4.13365484 | 7.86634516 | |
| 21 | | | | | | | |
| 22 | 30372991 | 1 | Domestication | 441.2 | | | |

**MITTWOCH, 3.6.2026**

[Gibson] Assembly pESC MCS2 Gibson Assembly

calculation https://punnettsquare.org/gibson-assembly-calculator/
1h at 50°C

1 Reaction: (like 26.05) https://nebuildercalculator.neb.com/
R1: pESC MCS2 linearized, Mx MCS2
pESC MCS2 1.1ul (0.1pmol)
Mx MCS2 1.2ul (1:10) (0.1pmol)
H2O: 2.8ul
Hifi MM: 5ul

2 Reaction: insert: backbone -> 7:1 ratio
50ng backbone 1 pESC digested conc.: (1:10) 37.3ng/ul -> 1.3ul
47.7ng insert 4 MCS2 Mx conc.: (1:10) 47.4ng/ul -> 1ul
H2O: 2.7ul

3 Reaction: insert:backbone -> 10:1 ratio
50ng backbone -> 1.3ul (1:10)

68.1ng insert -> 1.44ul (1:10)
H2O: 2.26ul
Hifi MM: 5ul

4 Reaction: cPEC
Q5 protocol without Primers, Tm depends on extensions (max 72°C) high concentration of fragment needed because there is no amplification! 100ng per fragment
Backbone: 373ng/ul -> (1:10)
Mx: 474ng/ul
12.5ul Q5 MM
7.71ul H20
Tm: 66°C
Extension: 4min 7500bp

- [x] Trafo

**FREITAG, 5.6.2026**

The whole plasmid seq for the domestication was correct. the four point mutations were inclued. the plasmid can now be used for insertig the ts purple drop out.

## Level 0 cloning:

to prepare for sequencing 100 ng were added to water and send for sequencing.

**Sequencing results 02.06**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | seq number | | construct | ng/ml | plasmid amount | water amount | result | |
| 2 | 98902261 | 1 | 0.1TPMx* | 258.2 | 4.64756003 | 7.35243997 | wrong! | do again |
| 3 | 98902262 | 2 | 0.1NCS | 298.1 | 4.0254948 | 7.9745052 | partly wrong! | do again |
| 4 | 98902263 | 3 | 0.2L1 [1] | 305.2 | 3.93184797 | 8.06815203 | wrong but we have 2 | |
| 5 | 98902264 | 4 | 0.2NCS | 314 | 3.82165605 | 8.17834395 | correct | |
| 6 | 98902265 | 5 | 0.3E-Mx | 150.8 | 7.95755968 | 4.04244032 | wrong | do again |
| 7 | 98902266 | 6 | 0.4NCS | 244.3 | 4.91199345 | 7.08800655 | wrong | do again |
| 8 | 98902267 | 7 | 0.4NCS* | 277 | 4.33212996 | 7.66787004 | partly correct | do again |
| 9 | 98902268 | 8 | 0.5NCS | 299.9 | 4.00133378 | 7.99866622 | wrong | do again |
| 10 | 98902269 | 9 | 0.5TPMxStart | 309.4 | 3.87847447 | 8.12152553 | correct | |
| 11 | 98902270 | 10 | 0.4L1 | 257.1 | 4.66744457 | 7.33255543 | correct | |
| 12 | 98902271 | 11 | 0.2NCS* | 308.8 | 3.88601036 | 8.11398964 | correct | |
| 13 | 98902272 | 12 | 0.1TPTm* | 314.7 | 3.81315539 | 8.18684461 | correct | |
| 14 | 98902273 | 13 | 0.1TPQt* | 220 | 5.45454545 | 6.54545455 | correct | |
| 15 | 98902274 | 14 | 0.2L1 [2] | 257.4 | 4.66200466 | 7.33799534 | correct | |
| 16 | 98902275 | 15 | 0.3E-Qt | 324 | 3.7037037 | 8.2962963 | **Mxpe correct somehow? do again?** | use as Mxpe |
| 17 | 98902276 | 16 | 0.3E-Mxpe | 272.1 | 4.41014333 | 7.58985667 | wrong | |
| 18 | 98902277 | 17 | 0.5TPQtStart | 250.2 | 4.79616307 | 7.20383693 | correct | |
| 19 | 98902278 | 18 | 0.3 PABP | 338 | 3.55029586 | 8.44970414 | correct | |
| 20 | 98902279 | 19 | 0.3 E-Tm | 334.2 | 3.59066427 | 8.40933573 | correct | |
| 21 | 98902280 | 20 | 0.5 TPTmStart | 290.3 | 4.13365484 | 7.86634516 | correct | |
| 22 | | | | | | | | |
| 23 | 30372991 | 1 | Domestication | 441.2 | | | | |

the sequencing results arrived

**Level 0 construct overview 05.06**

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | level 0 construct | length | Primer | cPCR size | ng/µl | seq result | |
| 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 | correct | |
| 5 | 0.1 TPTm* | 29 | 29,30 | 152 | 314.7 | correct | |
| 6 | 0.2 L1 | 18 | 31,32 | 140 | 257.4 | correct | |
| 7 | 0.2 NCS | 41 | oligo | 160 | 314 | correct | |
| 8 | 0.2NCS* | 42 | 33,34 | 160 | 308.8 | correct | |
| 9 | 0.3 yeGFP | 751 | 35,36 | 800 | 329.7 | correct | |
| 10 | 0.3 PABP | >1700 | twist | | 338 | correct | |
| 11 | 0.3 PABP-tr | | 67,68 | | | | |
| 12 | 0.3 E-Tm | 832 | twist | 917 | 334.2 | correct | |
| 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 | 0.3 Qt labeled matches somehow use this | |
| 16 | 0.4 L1 | 18 | 37,38 | 140 | 257.1 | correct | |
| 17 | 0.4 NCS | 41 | 39,40 | 160 | 244.3 | | |
| 18 | 0.4 NCS* | 41 | 59,60 | 160 | 277 | correct | |
| 19 | 0.5 NCS | 41 | 61,62 | 160 | 299.9 | | |
| 20 | 0.5 TP-MxSt | 24 | 43,44 | 150 | 309.4 | correct | |
| 21 | 0.5 TP-QtSt | 30 | 45,46 | 155 | 250.2 | correct | |
| 22 | 0.5 TP-TmSt | 25 | 47,48 | 150 | 290.3 | correct | |

# Proof of concept Level 2:

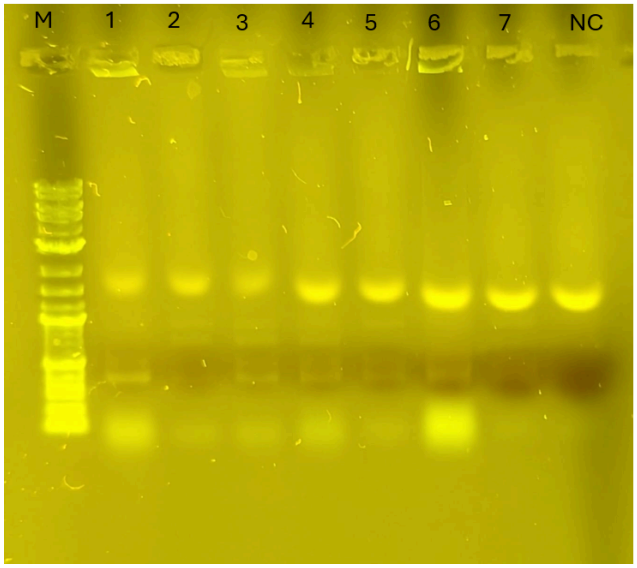
proof of concept level 2 cPCR for Enc-Mx according to Taq colony PCR from 4 plates 7 colonies were picked (5 frome one and one each from two plates. one plate had no colonies)

Primer: LP022 and LP034

expected fragment: 2.5 kb

1% agarose gel 135V 20min

cPCR proof of concept Enc-Mx pESC




**MONTAG, 8.6.2026**

# Level 0 cloning:

- [x] Overnight culture for Glycerol stocks Golden Gate Level 0 Ahmed Haj Kacem

**Level 0 construct overview 05.1**

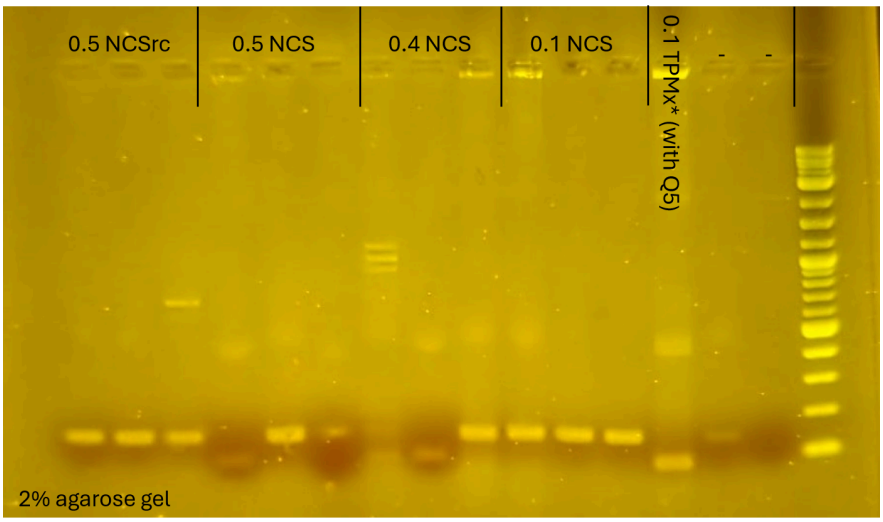
| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | level 0 construct | length | Primer | cPCR size | ng/µl | seq result | lable so far |
| 2 | 0.1 TPQt* | 34 | 27,28 | 155 | 220 | correct | 1.3 21.05 |
| 3 | 0.1 TPTm* | 29 | 29,30 | 152 | 314.7 | correct | 2.3 21.05 |
| 4 | 0.2 L1 | 18 | 31,32 | 140 | 257.4 | correct | 3.1 07.05 |
| 5 | 0.2 NCS | 41 | oligo | 160 | 314 | correct | 4.3 07.05 |
| 6 | 0.2NCS* | 42 | 33,34 | 160 | 308.8 | correct | 3.2 21.05 |
| 7 | 0.3 yeGFP | 751 | 35,36 | 800 | 329.7 | correct | 6.1 07.05 |
| 8 | 0.3 PABP | >1700 | twist | | 338 | correct | 4.5 29.05 |
| 9 | 0.3 E-Tm | 832 | twist | 917 | 334.2 | correct | 2.6 29.05 |
| 10 | 0.4 L1 | 18 | 37,38 | 140 | 257.1 | correct | 4.1 21.05 |
| 11 | 0.4 NCS* | 41 | 59,60 | 160 | 277 | correct | 8.3 07.05 |
| 12 | 0.5 TP-MxSt | 24 | 43,44 | 150 | 309.4 | correct | 5.2 21.05 |
| 13 | 0.5 TP-QtSt | 30 | 45,46 | 155 | 250.2 | correct | 6.2 28.05 |
| 14 | 0.5 TP-TmSt | 25 | 47,48 | 150 | 290.3 | correct | 7.1 28.05 |

- [x] cPCR Level0 from original cloning plates
  except for 0.1TPMx*, all cPCRs were performed with taq MM

**cPCR colonies**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | | number on plate | cPCR tube | shows the expected fragment (x if yes) |
| 2 | 0.3 E-Mx | 7 | 2 | |
| 3 | 0.3 E-Mx | 8 | 3 | |
| 4 | 0.3 E-Mx | 9 | 4 | |
| 5 | 0.3 E-Qt | 6 | 5 | |
| 6 | 0.3 E-Qt | 7 | 6 | |
| 7 | 0.3 E-Qt | 8 | 7 | |
| 8 | 0.5NCSrc | 4 | 8 | x |
| 9 | 0.5NCSrc | 5 | 9 | x |
| 10 | 0.5NCSrc | 6 | 10 | |
| 11 | 0.5NCS | 4 | 11 | |
| 12 | 0.5NCS | 5 | 12 | x |
| 13 | 0.5NCS | 6 | 13 | |
| 14 | 0.4NCS | 4 | 14 | |
| 15 | 0.4NCS | 5 | 15 | |
| 16 | 0.4NCS | 6 | 16 | x |
| 17 | 0.1NCS | 1 | 17 | x |
| 18 | 0.1NCS | 2 | 18 | x |
| 19 | 0.1NCS | 3 | 19 | x |
| 20 | 0.1TPMx* (with Q5) | 1.3 (Masterplate) | 1 | |

cPCR Level 0




for overnicht: 0.5NCSrc 5, 0.4NCS 6, 0.1NCS 1

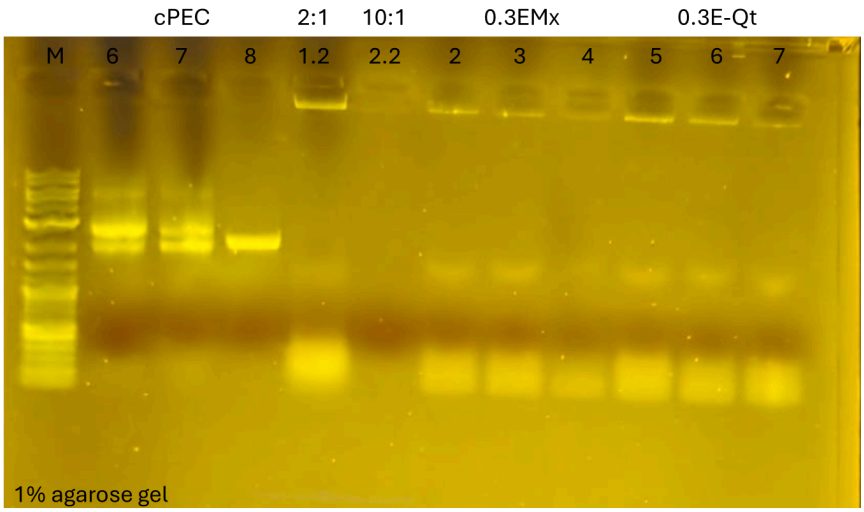
[Gibson] cPCR with Q5 MM

**cPCR Level 2 domestication**

| | A | B | C |
|---|---|---|---|
| 1 | | number on plate | cPCR tube |
| 2 | cPEC | 6 | 6 |
| 3 | cPEC | 7 | 7 |
| 4 | cPEC | 8 | 8 |
| 5 | 2:1 Gibson | 1.1 | 1.2 |
| 6 | 10:1 Gibson | 2.1 | 2.2 |

125V for 33 min:

cPCR




expected band at 2800bp
cPEC 6,7 has one thicker band right below 3kb

## Domestication Level 0 backbone:

- [x] domestication overnight culture Dom2

**DIENSTAG, 9.6.2026**

## Level 0 cloning:

- [x] glycerol stocks, Mini Prep
- [x] overnight for constructs that showed positiv cPCR results in 3 ml LB

0.5NCSrc 5, 0.4NCS 6, 0.1NCS 1

- [x] Level 1.2 cloning: GAL1/10 Promoter overhangs on domesticated promoter (Dom2) Q5 PCR

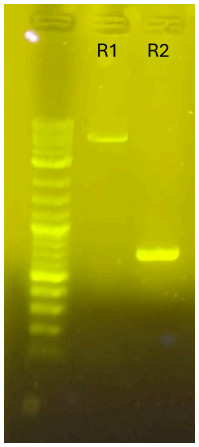
  R2: 21_GAL1,10_fw, 22_GAL1,10_rv
  62°C
  ~700bp

## Domestication Level 2 Backbone

- [x] Domestication backbone linearization Q5 PCR

  R1: 11_Level2bb_fw, 12_Level2bb_rv
  58°C
  ~5700bp

Domestication_lin_GAL11




- [x] overnight pECS MCS2 Mx cPEC 6,7

**MITTWOCH, 10.6.2026**

- [x] Miniprep Level 0 0.5TP-TmSt, 0.5NCS, 0.4NCS, 0.1NCS

## Level 0 cloning:

golden gate cloning Golden Gate Assembly first prepare 15mM stock for Level 1 BB and Gal1/10

**Level 0 construct for golden gate 10.06**

| | A | B | C |
|---|---|---|---|
| 1 | level 0 construct | length | concentration |
| 2 | 0.1 TPMx* | 27 | |
| 3 | 0.3 E-Qt | 883 | |
| 4 | 0.3 E-Mx | 898 | |
| 5 | 1.2 GAL1/10 | 705 | 95.6 |
| 6 | | | |

- [x] Clean up GAL1/10
- [x] level 1 Gal1/10 golden gate BsaI and Level1 BB

## Proof of Concept

- [x] Miniprep pESC MCS2 Mx cPEC 6,7
- [x] Prep for Sequencing

- [x] [Gibson] Level 2 Backbone
  - [x] Clean up

conc.: Dom linearization 39.7ng/ul

conc.: tsPurple overhangs 56.7ng/ul

3:1

Dom lin: 1.26
tspurple: 0.41
Hifi: 5
H20: 3.33

10:1
Dom lin: 1.26
tspurple: 1.36
Hifi: 5
H20:2.38

- [x] cPEC Level 2 Backbone

Dom lin: 2.52
tspurple: 1.76
Q5: 12.5
H2O: 8,22
Annealing: 72°C

- [x] Trafo

**DONNERSTAG, 11.6.2026**

# Level 0 cloning

- [x] Trafo Golden Gate
- [x] make Kan plates

- [x] Prep for Sequencing Level 0 0.5TP-TmSt, 0.5NCS, 0.4NCS, 0.1NCS
  12µl of plasmid eluated containing 100 ng/µl
  3 µl of 20uM 69_level0_seq_fw

# proof of concept

- [x] Prep for Sequencing pESC MCS2 Mx cPEC 6,7
  12µl of plasmid eluated containing 100 ng
  3 µl of 20uM LP022

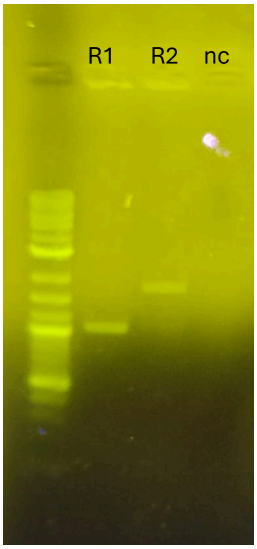
**Sequencing results**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | seqencing number | | construct | ng/µl | plasmid added | water added | result | |
| 2 | 98902281 | 1 | 0.5TP-TmSt | 125.2 | 0.7987220447 | 11.2012779553 | correct | |
| 3 | 98902282 | 2 | cPEC 6 pESC MCS2 Mx | 25.3 | 3.95256917 | 8.04743083 | too short | send again with LP034 |
| 4 | 98902283 | 3 | 0.1 NCS | 345 | 0.2898550725 | 11.7101449275 | correct | |
| 5 | 98902284 | 4 | 0.4 NCS | 337.3 | 0.2964719834 | 11.7035280166 | partly incorrect but correct overhangs | |
| 6 | 98902285 | 5 | 0.5 NCSrc | 283.3 | 0.3529827038 | 11.6470172962 | correct | |
| 7 | 98902286 | 6 | cPEC 7 pESC MCS2 Mx | 442.1 | 0.226193169 | 11.773806831 | too short | send again with LP034 |

## Domestication Level 2:

- [x] cPCR Lvl2 tspurple with Q5
  ~1900bp
  Tm:64°C
  R1: cPEC
  R2: G 10:1 -> correct, send for sequencing

Lvl2_tspurple.png




**MONTAG, 15.6.2026**

## Domestication Level 2 Backbone

- [x] overnight Lvl2 tspurple G 10:1

## Level 0 cloning:

- [x] cPCR Golden Gate Taq colony PCR Ahmed Haj Kacem Alex John Thottumkal
  1.2 GAL1/10 890bp
  Tm: 57°C
  Elongation: 1min
  oSN17 + oSN18