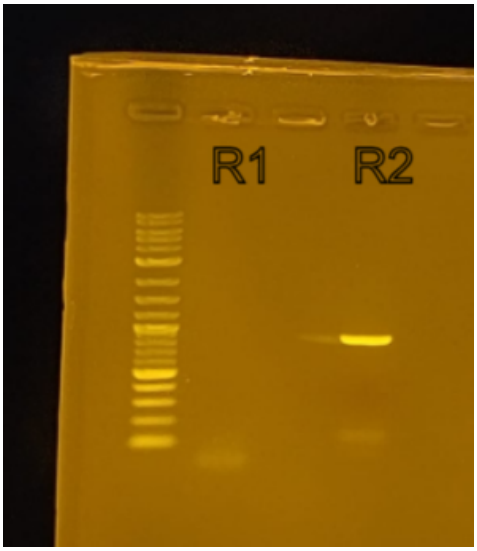
Dom, Level 2 Destination



MONTAG, 4.5.2026

Level 0 cloning

- [ ] transformation Level 0 moduls 1-10 in DH5alpha Ziyue Zhou , Charlotte, Ahmed acording to E. coli Transformation

  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

Proof of concept Level 2:

- [ ] [Gibson] transformation pESC MCS2 Enc-Mx with MCS1 TP-Mx yeGFP

  -> no colony, repeat of pESC MCS2 backbone linearization und TP-Mx yeGFP PCR for overhangs

Domestication Level 2 backbone:

- [ ] [Gibson] Domestication pESC Repeat

  Reaction1:

  15_2uORI_Dom_fw, 18_HIS3_Dom_rv

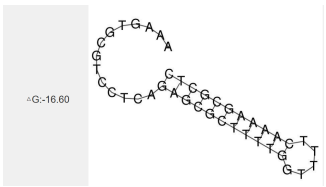
  Tm: 62 °C

  length: 1300bp

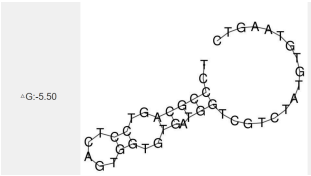
  Elongation: 1min

  -> repeat with touchdown PCR, primers probably have secondary structures (predicted with VectorBuilder)

hairpin 15



hairpin 18



DIENSTAG, 5.5.2026

Domestication Level 2 backbone:

- [x] [Gibson] Domestication Touchdown PCR Tim
  Design touchdown PCR: 4 cycles at 72 °C (Tm+10°C), 29 cycles 72°C -0.5°C per cycle to 58°C (Tm-4°C)
  Elongation: 45s

Proof of concept Level 2:

- [x] overnight pESC Enc-Mx in 5 ml LB 37°C

Level 0 module 1-10 Trafo plates were checked and all showed white and blue colonies in the center. the white coloniers can be screend with cPCR--> white color suggestes that the xgal cassat was substetued with the insert.

MITTWOCH, 6.5.2026

Domestication Level 2 backbone:

- [x] [Gibson] Repeat Domestication nested PCR
  template: newer Miniprep pESC-HIS empty
  R1:
  used primers: 15_2uORI_Dom_fw, 20_GAL1,10_Dom_rv
  Tm: 63°C
  expected length: ~3kb
  R2: as control
  used primers: 17_HIS3_Dom_fw, 20_GAL1,10_Dom_rv
  Tm: 65°C
  expected length: 1800bp
  -> no bands
  -> design of new primers (18bp, better Tm)
  -> Repeat PCR on linearized backbone (pESC-HIS (digested by XhoI, BamHI) MSC2)
  second PCR on fragment with 15_2uORI_Dom_fw, 18_HIS3_Dom_rv
  Gel cleanup

Proof of concept Level 2:

- [x] [Gibson] Miniprep pESC Enc-Mx + already isolated pESC Enc-Mx from 29.04
  1 pESC Mx: 62,5 ng/µl A260/A280: 1,91 A260/A230: 2,44
  2 pESC Mx: 90,7 ng/µl A260/A280: 1,91 A260/A230: 2,37
  3 (cleaned up again) pESC Mx: 28,0 ng/µl A260/A280: 1,97 A260/A230: 2,61

-> sequencing

12µl of plasmid eluated containing 50-100 ng/µl

1 pESC::His Mx 62,5 ng/µl 06.05

2 pESC::His Mx 181 ng/µl 29.04

3 pESC ::His Mx 90,7 ng/µl 06.05

add 3 µl of 20mM Primer, in this case Primer LP034 stock was diluted

sequencing will be performed by microsynth

DONNERSTAG, 7.5.2026

Level 0 cloning:

- [x] colony PCR Golden Gate Level 0 Moduls according to Taq colony PCR

  Tm: 54°C

  Elongation: 1min

  3x colonies per plate

  R1: 0.1 TPMx* 150bp

  R2: 0.1 NCS 160bp

  R3: 0.2 L1 140bp

  R4: 0.2 NCS 160bp

  R5: 0.3 Enc-Mx 1kb

  R6: 0.3 yeGFP 800bp

  R7: 0.4 NCSrc 160bp

  R8: 0.4 NCS* 160bp

  R9: 0.5 NCSrc 160bp

  R10: 0.5 NCS 160bp

cPCR_R1_R2_R3_R4_R7.png

