toolKIT Lab Book 1.0

DONNERSTAG, 26.3.2026

Miniprep of pESC-HIS empty with NEB monach Spin Plasmid Kit using culture provided by supervisor

FREITAG, 27.3.2026

concentration: 108 ng/µl, 48 ng/µl

DONNERSTAG, 9.4.2026

preperation of competent cells --> 40 transformations
with NEB competent cells DH5alpha *E. coli*
as described in Creating competent E.coli cells

DONNERSTAG, 16.4.2026

Miniprep of pESC-HIS empty with NEB Kit directly from plate
-> 58 ng/µl

MONTAG, 20.4.2026

- [x] overnight pAGM9121 from MoClo Kit (further referred to as Level 0 Destination)
- [x] overnight pAGM1311 from MoClo Kit (further referred to as Level 1 Destination)

5 ml LB in reaction tubes were inoculated with DH5alpha E.coli containing the desired plasmid (glycerol stock) and incubated over night at 37°C shaking at 180rpm

DIENSTAG, 21.4.2026

- [x] preperation of competent cells (DH5alpha): 40 transformations (see 09.04 for protocol)
- [x] Miniprep of Level 0 Destination according to the NEB kit
  concentration: 327.8 ng/ul
- [x] Miniprep of Level 1 Destination according to the NEB kit
  concentration: 285 ng/ul

Proof of concept Level 2:

- [x] [Gibson] pESC Backbone linearization

Q5 PCR was prepared and run according to protocol Q5 PCR

(R1) Reaction1: pESC-HIS MSC1
  primer:
  1_bb_MSC1_fw
  2_bb_MSC1_rv
  template pESC
  Tm 57°C
  expected length: **6632bp**

(R2) Reaction2: pESC-HIS MSC2
  primer:
  7_bb_MCS2_fw
  8_bb_MC2_rv
  template pESC
  Tm 68°C
  expected length: **6640bp**

Enc-Mx and TPMx-yeGFP PCR amplification and to add overhangs

Q5 PCR was prepared and run according to protocol Q5 PCR

(R3) Reaction3: MCS1 C-term TP Mx yeGFP

primer:
3_Mx_MCS1_fw
6_Mx_Qt_Tm_MCS1_rv
template MCS1
Tm 61°C
expected length: **874bp**
concentration: 459.3
-> there was a band, not visible in the picture propably because the PCR did not yield a lot of product

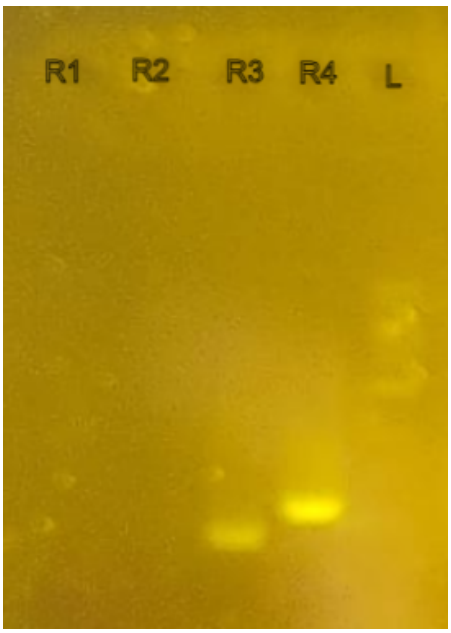
(R4) Reaction4: MCS2 Mx

primer:
9_Mx_Tm_Qt_MCS2_fw
10_Mx_Tm_Qt_MCS2_rv
template MCS2
Tm 70°C
expected length: **1055bp**
concentration: 428.4 ng/ul
-> there was a band, not visible in the picture

important: the annealing temperature has to match the primer Tm:
Reaction 1&3 annealing at 59°C
Reaction 2&4 annealing at 69°C

To analyse 6 µl of reaction was loaded onto a 1% Agarose gel and run for 25 min at 120 V. the 1kb + NEB ladder was used

pESC MCS1, MCS2



we will try a double restriction digest prior to the PCR for the MCS1 and MCS2

MITTWOCH, 22.4.2026

Proof of concept Level 2: pESC digest and insert preperation

[Gibson] Double Restriction Digest of pESC-HIS

digest was prepared and perfored according to protocol double restriction digest

for MCS1: EcoRI, PacI

for MCS2: XhoI, BamHI

pESC Backbone linearization

Q5 PCR was performed according to protocol Q5 PCR

(R1) Reaction1: pESC-HIS (digested by EcoRI, PacI) MSC1

1_bb_MSC1_fw

2_bb_MSC1_rv

template pESC-HIS (digested by EcoRI, PacI)

Tm 57°C

concentration: 398 ng/ul

expected fragment: **6632bp**

(R2) Reaction2: pESC-HIS (digested by XhoI, BamHI) MSC2

7_bb_MCS2_fw

8_bb_MCS2_rv

template pESC-HIS (digested by XhoI, BamHI)

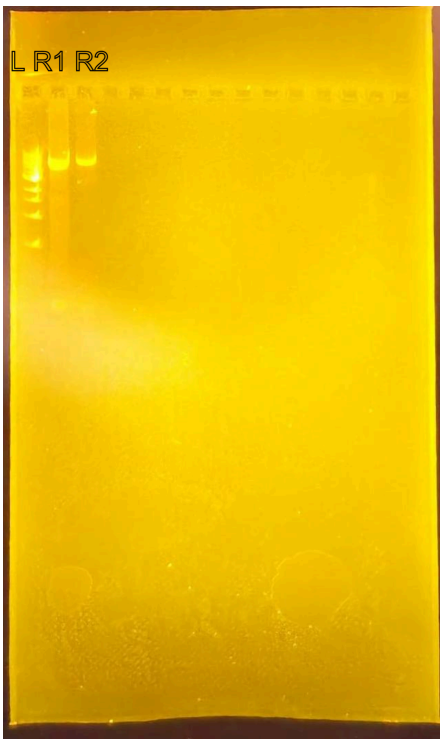
Tm 68°C

concentration: 430.6

expected length: **6640bp**

analysis: the reaction was checked using 1% agarose gel and the 1kb + ladder from NEB. The gel ran for 20 min at 120 V

BB Linearization



R2 was used for Gibson Assembly of Enc-Mx in pESC MCS2

DONNERSTAG, 23.4.2026

Proof of concept Level 2: cloning MCS2

- [x] [Gibson] Assembly of MCS2 Mx in pESC-HIS (digested by XhoI, BamHI) MSC2

Reaction 2 pESC-His MCS2 (22.04.26) and Reaction 4 Enc-Mx (21.04.26) according to protocol from NEB

Gibson Assembly

Backbone/ Insert: 0,02pmol

(0.02pmol*6640bp*650Da)/1000=86,32ng

Using NEBioCalculator:

Insert 41.15ng (3:1)

BB MCS2: 430.6ng/ul ->0.2 ul -> 1:10 dilution 2ul

MCS2 Mx: 428.4ng/ul -> 0.09ul -> 1:10 dilution 0.9ul

in total 2,9 µl were used for the reaction

incubation at 50°C for 1h

- [x] [Gibson] transformation Level 2 Enc-Mx according to E. coli Transformation

FREITAG, 24.4.2026

Proof of concept Level 2: cPCR

- [x] [Gibson] colony PCR Level 2 Enc-Mx

only one colony was picked

primer: LP022, LP034

expected length: ~3kb

the colony PCR was performed according to the protocol Taq colony PCR

analysis: with a 1% agarosegel run for 25 min at 120V Ladder: 1 kb plus

cPCR pECS Enc-Mx



-> weak band was observed therefore Miniprep was performed

MONTAG, 27.4.2026

- [x] [Gibson] overnight culture Level 2 Enc-Mx in 5 ml LB
- [x] overnight culture empty pESC-HIS (2x) in 5ml LB
- [x] overnight cultures preparation: reaction tubes were filled with 5 ml steril LB and stored in the cold room @Marina

DIENSTAG, 28.4.2026

Proof of concept Level 2: MiniPrep

- [x] [Gibson] Miniprep Level 2 Enc-Mx, Miniprep empty pESC-HIS (2x)

the plasmid isolation was peformed following the instructions of the NEB Miniprep Kit. the concentration and purety was mesured using NanoDrop:
pESC empty vector 1: 331,2 ng/µl A260/A280: 1,91 A260/A230: 2,27
pESC empty vector 2: 336,4 ng/µl A260/A280: 1,90 A260/A230: 2,31
pESC Mx: 108,9 ng/µl A260/A280: 1,81 A260/A230: 1,27 --> not as pure as desired so we will repeat the isolation tomorrow.
Overnight culture from master plate was inoculated (5ml LB).

Domestication Level 2 backbone:

- [x] [Gibson] Domestication pESC-HIS

for the domestication Q5 PCR was performed according to Q5 PCR

(R1) Reaction1:
used Primers:
15_2uORI_Dom_fw, 18_HIS3_Dom_rv
Tm: 62°C
expected fragment: **1300bp**
-> no band observed -> repeat

(R2) Reaction2:
used Primers:
17_HIS3_Dom_fw, 20_GAL1,10_Dom_rv
Tm: 65°C
expected fragment: **1800bp**

(R3) Reaction3:
used Primers:
19_GAL1,10_Dom_fw, 16_2uORI_Dom_rv
Tm: 65°C
expected fragment: **3600bp**

Annealing temperature at 64°C

- [x] DpnI Digest (1 µl per reaction) of all reactions to degrade the rest of the plasmid (clean up), 1h at 37°C

Level 0 cloning: Insert preperation

- [x] Oligo Annealing according to protocol Oligo Annealing

used Primers:
25_0.1TPMx*_fw, 26_0.1TPMx*_rv conc:
31_0.2L1_fw, 32_0.2L1_rv conc: 250,5 ng/µL

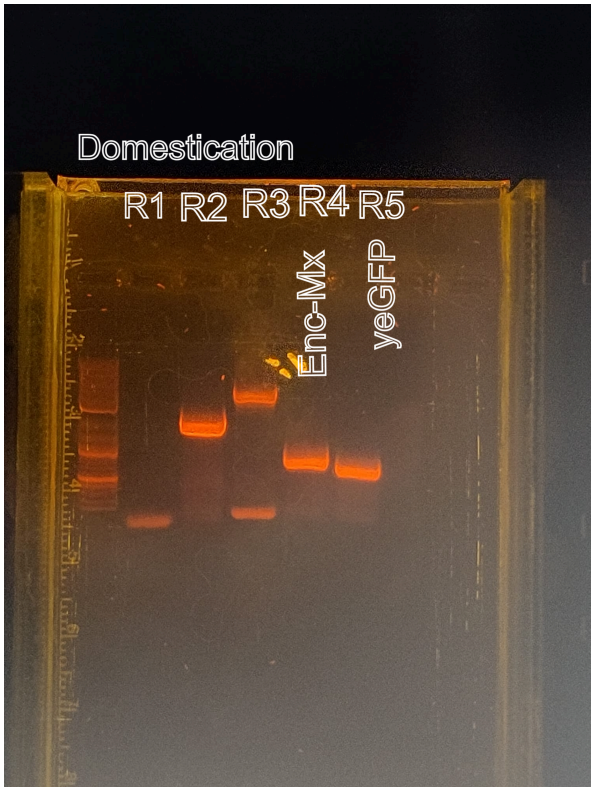
39_0.4NCSrc_fw, 40_0.4NCSrc_rv conc: 353,3 ng/µL
41_0.5NCSrc_fw, 42_0.5NCSrc_rv conc: 775,7 ng/µL
49_0.1NCS_fw, 50_0.1NCS_rv conc: 107,14 ng/µL
51_0.2NCS_fw, 52_0.2NCS_rv conc: 646,1 ng/µL
59_0.4NCS*_fw, 60_0.4NCS*_rv conc: 643,9 ng/µL
61_0.5NCS_fw, 62_0.5NCS_rv conc: 658,3 ng/µL

- [x] Q5 PCR yeGFP, Enc-Mx
  according to Q5 PCR

(R4) Reaction4:
used Primers:
53_0.3EncMx_fw, 54_0.3EncMx_rv
Tm: 64°C
expected fragment: **898bp**

(R5) Reaction5:
used Primers:
35_0.3yeGFP_fw, 36_0.3yeGFP_rv
Tm: 54°C
expected fragment: **751bp**

Dom, MCS1, MCS2



MITTWOCH, 29.4.2026

- [x] make LB Agar @Ziyue Tim

prepare LB Medium 2L
transfer 400ml each into a new flask and add 20 g/L of Agar
prepare for autoclave

- [x] make plates Spec @Ziyue

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.

Level 0 cloning

- [x] Golden Gate Assembly Level 0 moduls according to protocol Golden Gate Assembly

  T4 Buffer Aliquots 20ul
  3:1 insert, 100ng Level 0 Destination, 32.70ng/ul -> 3.05ul
  calculations with NEBioCalculator

  R1: 0.1 TPMx* 7.334 ng 289ng/ul -> 2.53ul of 1:100
  R2: 0.1 NCS 8.8ng 1074.4ng/ul -> 0.82ul of 1:100
  R3: 0.2 L1 5.867ng 250.5ng/ul -> 2.3ul of 1:100
  R4: 0.2 NCS 8.236ng 646.1ng/ul -> 1.2ul of 1:100
  R5: 0.3 Enc-Mx 97.48ng 394.6ng/ul -> 2.47ul of 1:10
  R6: 0.3 yeGFP 80.90ng 408.5ng/ul -> 1.98ul of 1:10
  R7: 0.4 NCSrc 8.236ng 353.3ng/ul -> 2.3ul of 1:10
  R8: 0.4 NCS* 8.236ng 643.9ng/ul -> 1.3ul of 1:10
  R9: 0.5 NCSrc 8.687ng 775.7ng/ul -> 1.1ul of 1:10
  R10: 0.5 NCS 8.687ng 658.3ng/ul -> 1.3ul of 1:10

Golden Gate Reaction

	A	B	C	D	E	F	G	H	I	J	K	L	M
1		10ul Reaction	MM 10.2x	0.1TPMx	0.1NCS	0.2L1	0.2NCS	0.3Enc-Mx	0.3yeGFP	0.4NCSrc	0.4NCS*	0.5NCSrc	0.5NCS
2	DNA Insert			2.53	0.82	2.3	1.2	2.47	1.98	2.3	1.3	1.1	1.3
3	Backbone (100ng)	3.05	31.11	3.05	3.05	3.05	3.05	3.05	3.05	3.05	3.05	3.05	3.05
4	10x T4 Ligase Buffer	1	10.2	1	1	1	1	1	1	1	1	1	1
5	T4 Ligase	1	10.2	1	1	1	1	1	1	1	1	1	1
6	BbsI	1	10.2	1	1	1	1	1	1	1	1	1	1
7	H2O			1.42	3.13	1.65	2.75	1.48	1.97	1.65	2.65	2.85	2.65

Proof of concept Level 2

- [x] [Gibson] Miniprep Level 2 Enc-Mx

the plasmid isolation was performed after distributing the overnight culture into two 2 ml eppis to reduce the amount of bacteria per isolation.
pESC Mx: 184.4 ng/µl A260/A280: 1,86 A260/A230: 2,20
pESC Mx: 184,9 ng/µl A260/A280: 1,87 A260/A230: 2.27

- [x] [Gibson] Level 2 Enc-Mx Double Digest MCS1 @Marina

  for MCS1: EcoRI, PacI
  see Double Restriction Digest
  20min at 37°C
- [x] [Gibson] Backbone Linearization Level 2 Enc-Mx @Marina

  according to Q5 PCR
  pESC-HIS (digested by EcoRI, PacI) MSC1
  1_bb_MSC1_fw, 2_bb_MSC1_rv
  Tm: 57°C
  Reaction L
  concentration: 470.7ng/ul
  bad A260/230: 0.7

pESC MCS2 Linearization



DONNERSTAG, 30.4.2026

Proof of concept Level 2:

- [Gibson] Assembly pESC MCS2 Enc-Mx with MCS1 TP-Mx yeGFP @Marina

  assembly was performed according to protocol Gibson Assembly

  Backbone/ Insert: 0,02pmol

  (0.02pmol*7595bp*650Da)/1000=98.74ng

  Using NEBioCalculator:

  Insert 34.09 ng (3:1)

  conc. pESC MCS2 digested EcoRI, PacI: 47ng/ul -> 2.1ul

  conc. MCS1 TP-Mx yeGFP: 45.9ng/ul -> 0.74ul

  incubation 1h at 50°C

Domestication Level 2 backbone:

- [Gibson] Domestication pESC Repeat

  according to Q5 PCR

  R1: used primers: 15_2uORI_Dom_fw, 18_HIS3_Dom_rv

  Tm: 62°C

  length: **1300bp**

  Elongation: 30s -> Repeat 2 increase to 1min

- [Gibson] Level 2 Destination Cloning: Q5 PCR tsPurple

  according to Q5 PCR

  R2: used primers: 13_Level2purple_fw, 14_Level2purple_rv

  Tm: 67°C

  length: **800bp**

  analysis: with a 1% agarose gel run for 25 min 120 V