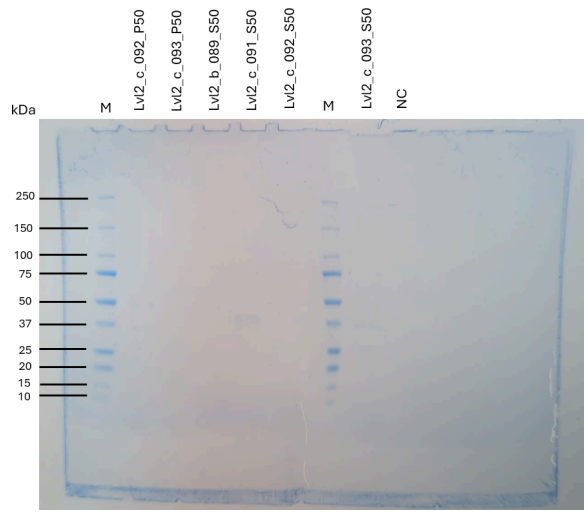
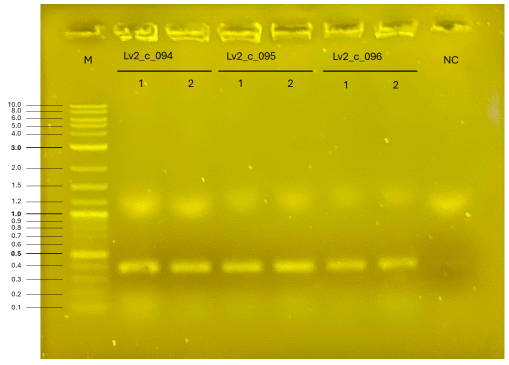
SDS-PAGE: P50: Lvl2_c_092-093, S50: Lvl2_b_089 & Lvl2_c_091-093



- [x] E. coli Trafo Alex John Thottumkal
  - 1.1_NCS
  - 1.3_NCS
- [x] S. cerevisiae cPCR of: cPCR S. cerevisiae Taq Alex John Thottumkal
  - Lvl2_c_094
  - Lvl2_c_095
  - Lvl2_c_096
    - only 2 colonies of each construct were picked to save on Taq MM
    - fw Primer: 118_cPCR_SC_fw
    - rv Primer: LP034
    - Annealing Temperature at 53 °C (Calculated with NEB Tm Calculator)
- [x] Gel electrophoresis Alex John Thottumkal
  - Lvl2_c_094
  - Lvl2_c_095
  - Lvl2_c_096

Gel Results cPCR S. cerevisiae Level 2 modules: 94 - 96



expected amplicon: 392 bp

DONNERSTAG, 10.9.2026

- [x] cPCR Alex John Thottumkal
  1.1_NCS
  1.3_NCS
- [x] gel electrophroesis Alex John Thottumkal
  1.1_NCS
  1.3_NCS

DIENSTAG, 15.9.2026

- [ ] set up precultures for:
    Lvl2_c_094
    Lvl2_c_095
    Lvl2_c_096
  canceled due to no positive result from SDS-PAGE of Encapsulin constructs. Paused until optimal incubation parameters are identified. #

DONNERSTAG, 17.9.2026

- [x] Level 2 Golden gaten: with 1.2Gal1/10 (036) according to Golden Gate Assembly Alex John Thottumkal
    construct 101 2: NCS (070) + GAL (036) + MVP (086)
    construct 102 2: INT,PABPfull (083) +GAL (036) +NCS (071)
    construct 103 2: TpQt,PABPfull (080) +GAL (036) +NCS (071)

FREITAG, 18.9.2026

- [x] Trafo of construct 101, 102 and 103 E. coli Transformation Alex John Thottumkal

DIENSTAG, 22.9.2026

- [ ] cPCR of construct 101, 102, and 103 Alex John Thottumkal Taq colony PCR

MONTAG, 28.9.2026

- [ ] 8:00 AM set up 15 mL preculture of:
    Lvl2_c_091
    Lvl2_c_092
    Lvl2_c_093
    Lvl2_c_094
    Lvl2_c_095
    Lvl2_c_096
  (induced + not induced + backbone, 15ml each)
- [ ] prepare induction medium

DIENSTAG, 29.9.2026

- [ ] 8:00 AM measure OD of preculture
- [ ] animpfen im kolben (Glucose)
- [ ] nach 5 h OD messen um abzuschätzen, wann induziert werden soll
  - [ ] 1:10 verdünnen bei der Messung, wenn OD über 0,4. Dann aber auch Blank 1:10 verdünnen
  - [ ] 800 µL passen auch in den Küvetten, statt 1 mL
- [ ] bei OD = 1 ins Induktionsmedium wechseln (OD = 0,8 wäre auch ok).
  - [ ] auch notieren, zu welchem Zeitpunkt OD=1 erreicht wurde bzw. wann induziert wurde fürs Induktionsprotokoll
  - [ ] beim wechseln ins Induktionsmedium steril aus Kolben in Falcon
  - [ ] Falkon abzentrifugieren in der großen Zentrifuge im S2 Labor 10 min 4700 rpm
    - [ ] steriles Arbeiten
    - [ ] zweimal waschen, überall die gleiche Menge an MilliQ Wasser hinzugeben wegen dem tarieren der Zentrifuge
    - [ ] nach dem zweiten Waschschritt das Zellpellet resuspendieren in 30 ml Induktionsmedium, etwas schütteln, Kolben bei 30 °C für min. 22 h induzieren (die Zellen stecken in der exponentiellen Phase)

MITTWOCH, 30.9.2026

- [ ] Golden Gate of 1.1 casette (INT+L1+GFP+NCS+NCS)
  Golden Gate Assembly

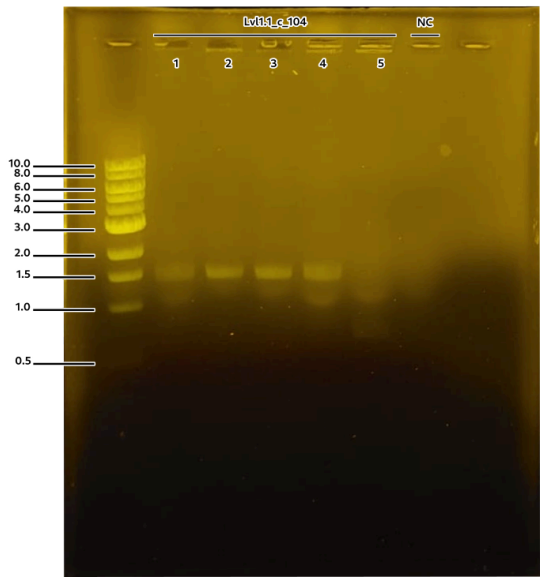
  Trafo of 1.1 casette Tim

DONNERSTAG, 1.10.2026

- [ ] Induktion stoppen
- [x] cPCR of 1.1 casette Taq colony PCR

FREITAG, 2.10.2026

lvl1.1_c_104



MONTAG, 5.10.2026

- [x] set up prepre-culture. due to not having any SD-His plates left for a streaking plate, the pre-precultures were set up in 2 mL microcentrifuge tubes. Therefore 500 µL SD-His Glucose medium was put there. following constructs were set up at 06:00 PM in a shaking incubator (180 rpm at 30 °C).
    - Lvl2_c_091
    - Lvl2_c_092
    - Lvl2_c_093
    - Lvl2_c_094
    - Lvl2_c_095
    - Lvl2_c_096

DIENSTAG, 6.10.2026

- [x] 9:30 AM set up preculture of:

precultures from 06.10.2026

	Volume of medium [mL]	type of medium	Construct name	Going to be induced?	notes
1	15	SD-His Glucose	Lv2_c_92	yes	
2	15	SD-His Glucose	Lv2_c_94.1	no	
3	20	SD-His Glucose	Lv2_c_94.2	yes	additional 5 mL for TEM
4	15	SD-His Glucose	Lv2_c_95	yes	
5	15	SD-His Glucose	Lv2_c_96	yes	

- [x] overnight culture of E.coli from the glycerol stock. Construct no. 55: Level 2 destination vector Ahmed Haj Kacem

MITTWOCH, 7.10.2026

- [x] 9:30 AM measuring of the OD:

precultures from 06.10.1

	Volume of medium [mL]	type of medium	Construct name	Going to be induced?	OD	notes
1	15	SD-His Glucose	Lv2_c_92	yes	0.367	
2	15	SD-His Glucose	Lv2_c_94.1	no	0.363	
3	20	SD-His Glucose	Lv2_c_94.2	yes	0.361	additional 5 mL for TEM
4	15	SD-His Glucose	Lv2_c_95	yes	0.357	
5	15	SD-His Glucose	Lv2_c_96	yes	0.364	

- [x] 12:30 set up main-culture of:

maincultures from 06.10.2

	Volume of medium [mL]	type of medium	Construct name	Going to be induced?
1	180	SD-His Glucose	Lv2_c_92	yes
2	180	SD-His Glucose	Lv2_c_94.1	no
3	180	SD-His Glucose	Lv2_c_94.2	yes
4	180	SD-His Glucose	Lv2_c_95	yes
5	180	SD-His Glucose	Lv2_c_96	yes
6	60	SD-His Glucose	Lv2_c_94.2_TEM	yes

- [x] 14:45 measure OD600 of:

maincultures from 06.10.1

	Volume of medium [mL]	type of medium	Construct name	Going to be induced?	OD600
1	180	SD-His Glucose	Lv2_c_92	yes	0.089
2	180	SD-His Glucose	Lv2_c_94.1	no	0.098
3	180	SD-His Glucose	Lv2_c_94.2	yes	0.109
4	180	SD-His Glucose	Lv2_c_95	yes	0.111
5	180	SD-His Glucose	Lv2_c_96	yes	0.107
6	60	SD-His Glucose	Lv2_c_94.2_TEM	yes	0.110

- [x] 17:15 measure OD600 of:

maincultures from 06.10.3

	Volume of medium [mL]	type of medium	Construct name	Going to be induced?	OD600
1	180	SD-His Glucose	Lv2_c_92	yes	0.174
2	180	SD-His Glucose	Lv2_c_94.1	no	0.216
3	180	SD-His Glucose	Lv2_c_94.2	yes	0.226
4	180	SD-His Glucose	Lv2_c_95	yes	0.226
5	180	SD-His Glucose	Lv2_c_96	yes	0.232
6	60	SD-His Glucose	Lv2_c_94.2_TEM	yes	0.202

After measurement place the cultures into fridge

- [x] Miniprep of the E.coli culture carrying construct no. 55 (level 2 destination vector) Ahmed Haj Kacem
- [ ] golden gate cloning of the level 2 carrying the level 1 modules:
  Lvl1.3_c_086MVP

Lvl1.2_a_036 GAL

Lvl1.1_c_104 INT + GFP

(the plasmid concentration is around 10 nM. 15 nM are needed for GG cloning)

- [x] Overnight culture of E. coli from the glycerol stock, carrying the level two destination vector (construct no. 55) Ahmed Haj Kacem

DONNERSTAG, 8.10.2026

- [x] overnight culture of E. coli carrying GFP with INT (construct no. 104). Picking colony number 2 Ahmed Haj Kacem
- [x] golden gate cloning of the level 2 carrying the level 1 modules: Tim

  Lvl1.3_c_086MVP

  Lvl1.2_a_036 GAL

  Lvl1.1_c_104 INT + GFP
- [x] Main-cultures from 06.10 were placed into the incubator, after 2h incubation measure the OD600 of at 11:45

maincultures from 06.10.4

	Volume of medium [mL]	type of medium	Construct name	Going to be induced?	OD600
1	180	SD-His Glucose	Lv2_c_92	yes	0.64
2	180	SD-His Glucose	Lv2_c_94.1	no	0.89
3	180	SD-His Glucose	Lv2_c_94.2	yes	0.89
4	180	SD-His Glucose	Lv2_c_95	yes	0.79
5	180	SD-His Glucose	Lv2_c_96	yes	1.01
6	60	SD-His Glucose	Lv2_c_94.2_TEM	yes	0.87

- [x] 12:10 Measure OD600, cell harvest, washing with miliQ

  Transferred into induction-medium at 16:42

inductioncultures from 06.10.5

	Volume of medium [mL]	type of medium	Construct name	Going to be induced?	OD600
1	180	SD-His Glc+Raf	Lv2_c_92	yes	1.015
2	180	SD-His Glc+Raf	Lv2_c_94.2	yes	1.335
3	180	SD-His Glc+Raf	Lv2_c_95	yes	1.195
4	180	SD-His Glc+Raf	Lv2_c_96	yes	1.41
5	60	SD-His Glc+Raf	Lv2_c_94.2_TEM	yes	1.265

FREITAG, 9.10.2026

- [x] Measure OD600 of:

inductioncultures from 06.10.1

	Volume of medium [mL]	type of medium	Construct name	Going to be induced?	OD600
1	180	SD-His Glc+Raf	Lv2_c_94.2	yes	5.12
2	60	SD-His Glc+Raf	Lv2_c_94.2_TEM	yes	4.85

- [x] set up backup-prepre-culture of:
  - Lvl2_c_091
  - Lvl2_c_092
  - Lvl2_c_093
  - Lvl2_c_094
  - Lvl2_c_095
  - Lvl2_c_096
- [x] Bacterial transformation in E. coli. The inserted construct no. 105 MVP + GAL + INT + GFP Ahmed Haj Kacem E. coli Transformation

Table1

	Cell pellet	weight empty falcon / g	weight falcon with sample / g	net weight of wet cell pellet / g	E
1	92' stress	13.3219	13.9495	0.6276	
2	92'' stress	13.5999	14.1310	0.5311	
3	92' no stress	13.5039	14.1048	0.6009	
4	92'' no stress	13.6037	13.9869	0.3832	
5	94' stress	13.4970	14.0980	0.601	
6	94'' stress	13.3581	13.9359	0.5778	
7	94'' no stress	13.4836	14.0641	0.5805	
8	94'' no stress	13.8253	14.4137	0.5884	
9	95' stress	13.5566	14.1663	0.6097	
10	95'' stress	13.3242	13.9618	0.6376	
11	95' no stress	13.4551	14.0714	0.6163	
12	95'' no stress	13.2305	13.8035	0.573	
13	96' stress	13.5285	13.9863	0.4578	
14	96'' stress	13.8382	14.3172	0.479	
15	96' no stress	13.4439	14.0500	0.6061	
16	96'' no stress	13.6276	14.2351	0.6075	
17	95' TEM	13.4846	13.8550	0.3704	
18	95'' TEM	13.4892	13.9042	0.415	
19	92''' stress	13.3742	13.4760	0.1018	for SDS
20	92''' no stress	13.3275	13.4236	0.0961	for SDS
21	94''' stress	13.3856	13.4949	0.1093	for SDS
22	94''' no stress	13.6188	13.7620	0.1432	for SDS
23	95''' stress	13.4838	13.6052	0.1214	for SDS
24	95''' no stress	13.5196	13.6276	0.108	for SDS
25	96''' stress	13.4857	13.6103	0.1246	for SDS
26	96''' no stress	13.3424	13.4782	0.1358	for SDS
27	94''' TEM	13.6547	13.7689	0.1142	for SDS

wurden für die TEM proben proben gezogen aus 94 oder 95??? AUS 94.

SAMSTAG, 10.10.2026

- [x] colony PCR to validate the transformation of 09.10.2026 Ahmed Haj Kacem Taq colony PCR
  six colonies were picked
  a master plate was prepared

Preparation of a master mix for colony PCR

	A	B	C
1		**25ul Reaction**	x 6.2
2			
3	2x Taq MM	12.5	77.5
4	10uM P fw	0.5	3.1
5	10um P rv	0.5	3.1
6	Template	colony	colony
7	H20	11.5	71.3
8			

Size of the amplicon: ca. 400 bp
Elongation: 24 s
Annealing temperature of the primers: 53 degrees
the primers:

- fw Primer: 118_cPCR_SC_fw
- rv Primer: LP034

--> false amplicon: it should be used solely for the validation of the transformation of *S. cerevisiae*
--> cPCR should be conducted again

PCR cycle

	A	B	C
1	STEP	TEMP	TIME
2	Initial Denaturation	95°C	30 seconds
3	25-33 Cycles	95°C 45-68°C* 68°C	15-30 seconds 15-60 seconds 24 seconds
4	Final Extension	68°C	5 minutes
5	Hold	4-10°C	

MONTAG, 12.10.2026

- [ ] colony PCR to validate the transformation of 09.10.2026 Ahmed Haj Kacem Taq colony PCR
  5 colonies were picked --> the sixth colony showed some purple cells

Preparation of a master mix for colony PCR 1

	A	B	C
1		**25ul Reaction**	x 6.2
2			
3	2x Taq MM	12.5	77.5
4	10uM P fw	0.5	3.1
5	10um P rv	0.5	3.1
6	Template	colony	colony
7	H20	11.5	71.3
8			

Size of amplicon: 5897 bp --> elongation time: 6min

Primers: LP22, LP34

PCR cycle 1

	A	B	C
1	STEP	TEMP	TIME
2	Initial Denaturation	95°C	30 seconds
3	25-33 Cycles	95°C 45-68°C* 68°C	15-30 seconds 15-60 seconds 6min
4	Final Extension	68°C	5 minutes
5	Hold	4-10°C	

- [x] set up pre-culture with backup-prepre-culture of:
  - Lvl2_c_092
  - Lvl2_c_094
  - Lvl2_c_095
  - Lvl2_c_096
- [x] Measure the OD600 at the end of the pre-culture of:

preculture of prepreculture 10.10

	Volume of medium [mL]	type of medium	Construct name	OD600
1	15	SD-His Glc	Lvl2_c_092	3.44
2	15	SD-His Glc	Lvl2_c_094	3.25
3	15	SD-His Glc	Lvl2_c_095	3.45
4	15	SD-His Glc	Lvl2_c_096	3.39

DIENSTAG, 13.10.2026

- [x] 9:05 set up mainculture of

mainculture of prepreculture 10.10

	Volume of medium [mL]	type of medium	Construct name
1	180	SD-His Glc	Lvl2_c_092
2	180	SD-His Glc	Lvl2_c_094
3	180	SD-His Glc	Lvl2_c_095
4	180	SD-His Glc	Lvl2_c_096

- [x] 17:30 Measure OD600 and stop incubation of

mainculture of prepreculture 10.10.1

	Volume of medium [mL]	type of medium	Construct name	OD600
1	180	SD-His Glc	Lvl2_c_092	0.61
2	180	SD-His Glc	Lvl2_c_094	0.72
3	180	SD-His Glc	Lvl2_c_095	0.57
4	180	SD-His Glc	Lvl2_c_096	0.64

- [x] 22:30 set up a new group of main-culture of

mainculture with raffinose

	Volume of medium [mL]	type of medium	Construct name	added culture medium
1	180	SD-His Raf	Lvl2_c_092	pre-culture 12.10, 6ml
2	180	SD-His Raf	Lvl2_c_094	pre-culture 7.10, 6ml

- [ ] Gel electrophoresis of monday's cPCR

MITTWOCH, 14.10.2026

- [ ] 09:30 AM measure OD600 of

mainculture with raffinose1

	Volume of medium [mL]	type of medium	Construct name	OD600
1	180	SD-His Raf	Lvl2_c_092	0.11
2	180	SD-His Raf	Lvl2_c_094	0.10

- [ ] Repeat cPCR

  5 colonies were picked --> the sixth colony showed some purple cells

Preparation of a master mix for colony PCR 2

	A	B	C
1		**25ul Reaction**	x 6.2
2			
3	2x Taq MM	12.5	77.5
4	10uM P fw	0.5	3.1
5	10um P rv	0.5	3.1
6	Template	colony	colony
7	H20	11.5	71.3
8			

Size of amplicon: 5897 bp --> elongation time: 6min

Primers: LP22, LP34

PCR cycle 2

	A	B	C
1	STEP	TEMP	TIME
2	Initial Denaturation	95°C	30 seconds
3	25-33 Cycles	95°C 45-68°C* 68°C	15-30 seconds 15-60 seconds 6min
4	Final Extension	68°C	5 minutes
5	Hold	4-10°C	