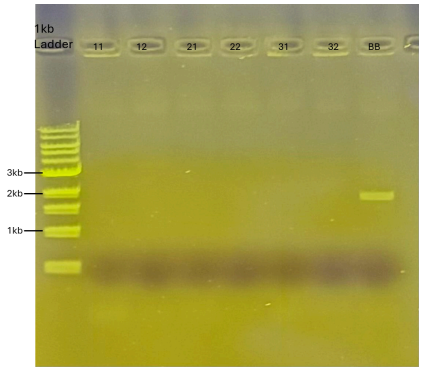
cPCR yeast




the fragment in the bb control has the expected size (1.8 kb tspurple) so the **cell opening mechanism worked.** the problem probably lies with the fragment size (3.7 kp) so a new primer amplyfing a 460 bp fragment has been ordered.

- [x] transfer colonies on selection media (short streak) 5 each (47,48,48, BB)

## MONTAG, 17.8.2026

- [x] Add recipes for all buffers for purification to the recipe list.
  - 10x Raffinose stock: 200 g/L in 500 ml sterile Charlotte Lenné
  - Induction medium with 2% Raffinose and 1% Galactose
  - Lysis buffer, sterile Ahmed Haj Kacem --> we use PBS pH 7.4
  - PBS pH 7.4, sterile and outgassed Ahmed Haj Kacem
  - Running buffer for SDS Anica Nissen
  - Ethanol, outgassed Charlotte Lenné
  - Milli-Q, sterile and outgassed Charlotte Lenné

- [x] Prepare sterile flasks for the buffer: a total of seven, as well as centrifuge tubes and shaking flasks (two 1 L and one 250 ml for the preculture). Autoclave.
- [x] Prepare the buffer, including sterile filtration. and outgassed (ultrasound bath 30 min)

PBS buffer: less concentrated (2L water was given instead of filling to 2L).

- [x] Start the preculture of Lvl2_a_047 (Mx_GFP) in dropout medium including glucose. (10.08. c2) 10:40 a clock (2x 5ml SD His Glucose with chloramphenicol) --> tube 1 and 2

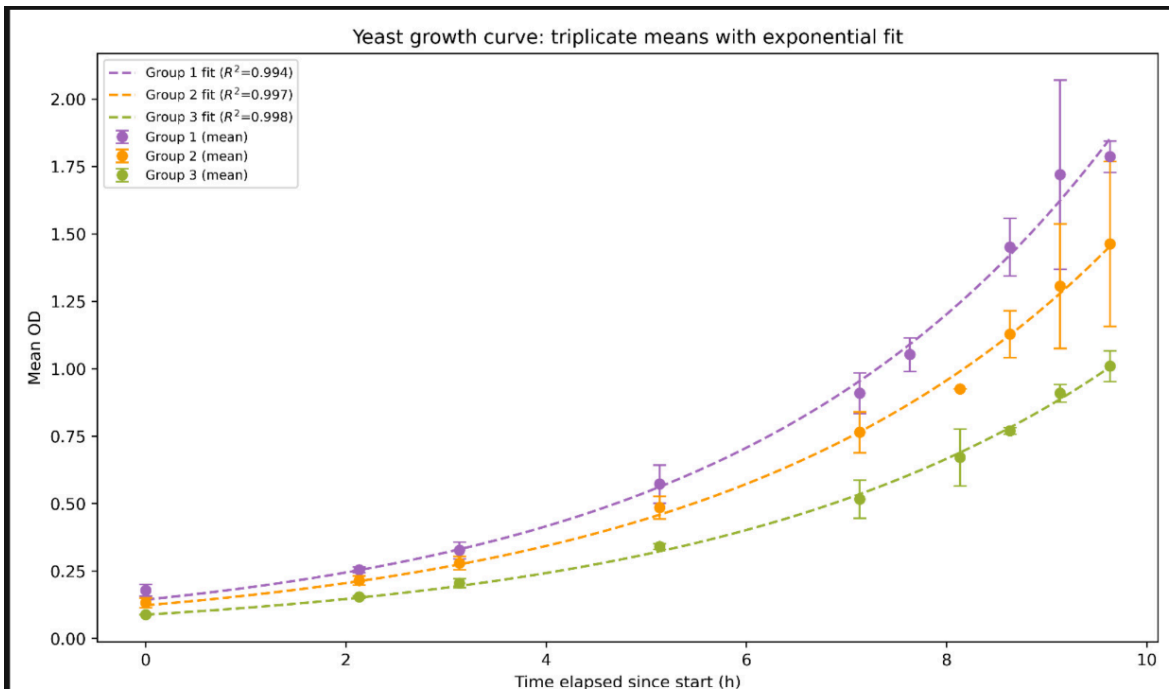
because there was no visible growth (density) in case a third overnight was inoculated using a colony for 47 (not tested) with more cellmass 16:00 --> backup

- [x] prepare Induction medium with 2% Raffinose (100 ml 10x stock) and 1% Galactose (50 ml 10x stock): first SD His (1.92g) in MiliQ to autoclave (750ml) then add YNB (100ml 10x stock) and sugar

## DIENSTAG, 18.8.2026

- [x] prepare centrifuge tube (autoclave)
- [x] prepare SD His induction media by mixing compounds SD His Medium (selection Medium)
- [x] Use part of the preculture to prepare a glycerol stock of Lvl2_a_047

image.png




- [x] Start the main culture in SD glucose (200 ml). Calculate how much needs to be inoculated to reach the desired OD in 6–7 hours. What is the desired OD for induction?

inoculation to reach OD600: 1 --> 18:30
then main culture inoculate with OD600= 0.15 followed by ca. 10 hour growth shaking
calculation:
preculture
tube 1: 1/10: 0.313 so OD 3.13
tube 2 1/10: 0.711 so OD 7.11
mix both together and measure OD again 1/20: 0.687 so OD: 13.74
0.15*200ml/13.74 = **2.183 ml inoculated at 8:30 --> measurement was wrong culture did not grow**

Important: the growth curve was tested in YPD we dont know how much slower the growth is on SD His medum (selection pressure)!

Change to the induction medium by centrifuging the cells down. 18:30 --> not possible only OD of 0.071
solution let it grow at RT shaking --> change Medium tomorrow morning

**MITTWOCH, 19.8.2026**

- [x] Change to the induction medium by centrifuging the cells down. 9:00 a clock. measure OD600

OD600: 1.4 at 9:10 a clock
induced at 11:00 a clock

**DONNERSTAG, 20.8.2026**

## Purification until SEC for Validation of Lysis/Precipitation:

Purification of Encapsulin (S.cerevisiae)

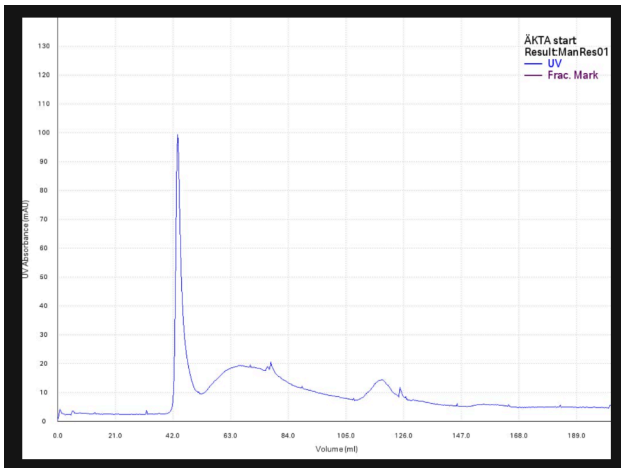
- [x] harvest start 7:00 a clock Alex John Thottumkal Charlotte Lenné ;

total of 0.48 g cell dry weight

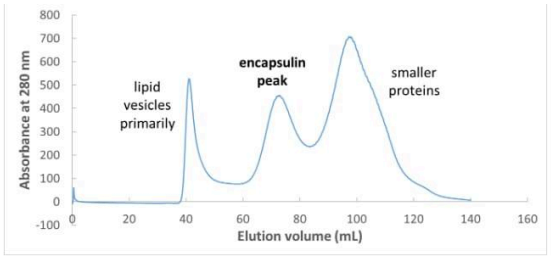
- [x] lysis ca. start 8:00 a clock Alex John Thottumkal Felix Cuntz ;
- [x] precipitation with ammonium sulphate start 9:00 a clock Alex John Thottumkal Felix Cuntz ;
- [x] SEC start 9:45 a clock Alex John Thottumkal ;

learnings:
remember to take samples for SDS (add to protocol) note down the sample taking on the SEC with time!
we underestimated the time that includes encapsulin peak

**chromatogram**



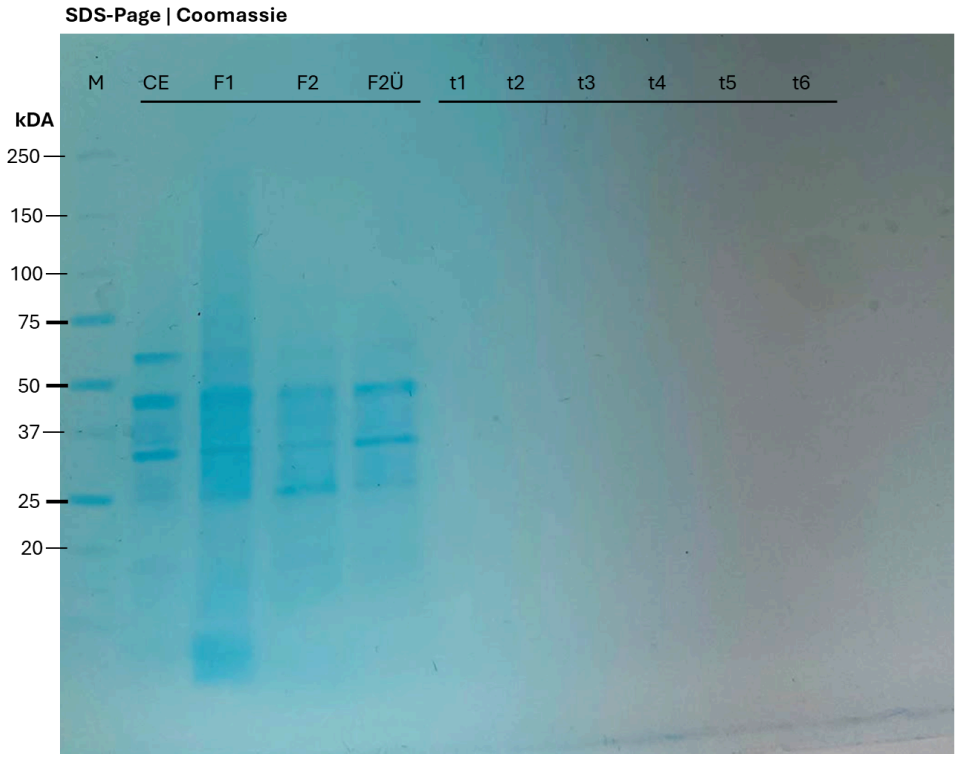

**Paper reference**




**…plementary Figure 13. Example FPLC chromatograph of size-exclusion chromatography purification of encapsulins.** The encapsulin peak elutes at ~65-85 mL. Size-exclusion …natography is the first column-based step in the purification process, leading to reasonably pure but not completely homogeneous sample.

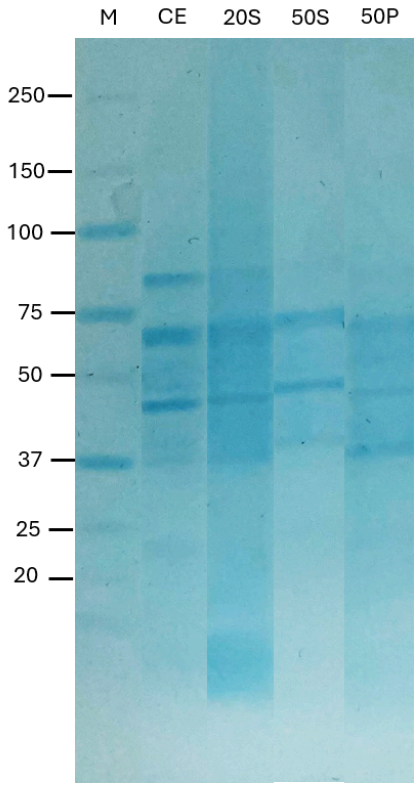
- [x] concentrate -->store in freezer start ca. 14:00 a clock Ahmed Haj Kacem --> only store in freezer
- [x] SDS 1.5 h Ahmed Haj Kacem Alex John Thottumkal

SDS Page from SEC of 20.08.2026




M: Precision Plus Protein All Blue (Biorad 1610373)

image.png




CE --> directly after glass bead lysis

F1 --> precipitation 1 Pellet --> after 20% precipitation  new definition: 20 Precipitat

F2 --> precipitation 2 Pellet --> after 50 % precipitation new definition: 50 precipitat

F2Ü --> precipitation 2 supernatant --> after 50 % precipitation: 50 supernatant

EncA Mx: 32.5kDa

- [x] store protein solution in glycerol in -80°C for IEX in week (30.08-4.9)

- [x] Repeat the cPCR of the yeast using the new primer 118 as the forward primer and 34 as the reverse primer
  Karoline Kaminski cPCR S. cerevisiae Taq

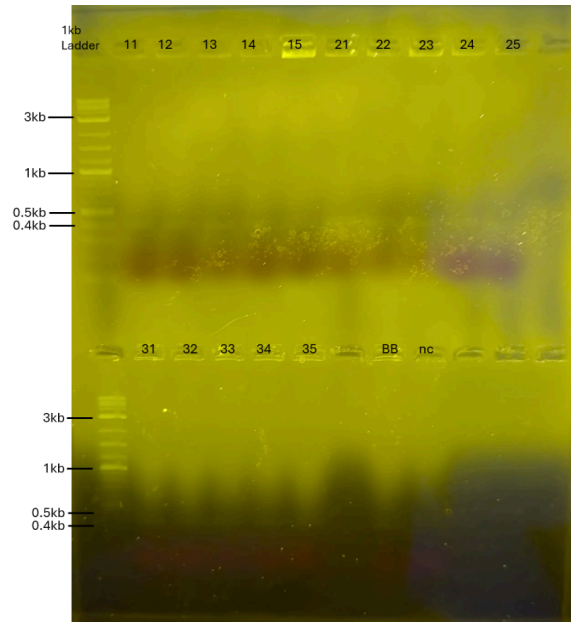
Tm: 53°C
fragment: 391bp --> 30 sec
2% gel

**colony plate**



5 colonies per construct (47.48,49) 1 colony BB and 1 nc (plate)
1.1 is the same colony used for the first encapsulin purification

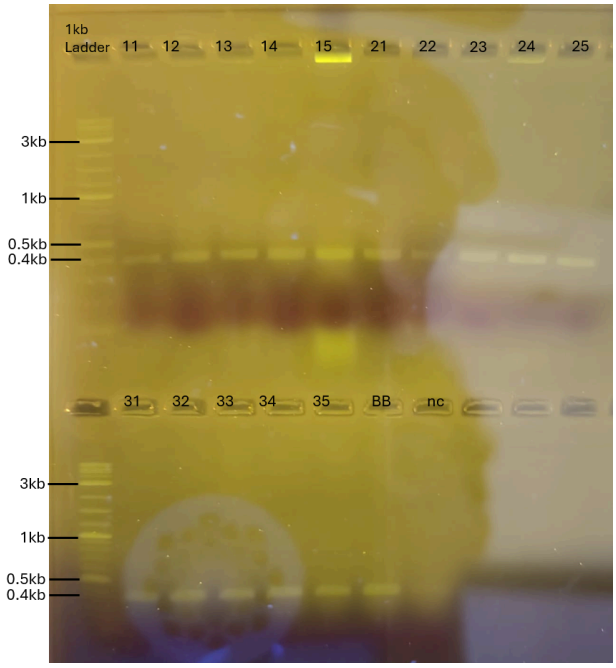
**cPCR yeast**



**FREITAG, 21.8.2026**

- [x] redo gel: 2% 17 samples 35 min 120 V  load everthing thats left (20 µl) --> samples in cold room, pcr rack labeld cPCR Sc (important let the agar cool down before adding the stain to ensure better staining)

cPCR second load




- [x] stain sds --> transfer nice sample (fraction) (tubes are in the cold room) for further use

**MONTAG, 24.8.2026**

- [x] culture for glycerol stock Lvl2_a_048 and Lvl2_a_049

**DIENSTAG, 25.8.2026**

## Lysis and Precipitation of Lvl2_a_047 (Mx-GFP) (**Valdiation of induction with negative control**)

- [x] 10:30am preculture of Lvl2_a_047 2x 5ml SD HIS Glucose
  3pm OD600: 0.00
  learning: inoculate more than 5ml
  grow for at least 15h

**MITTWOCH, 26.8.2026**

preculture did not grow -> too little cell mass from glycerol? => preparation of new glycerol for Lvl2_a_047

**MONTAG, 31.8.2026**

- [x] IEX Column buffer preperation (Tris HCl) according to IEX Buffer Preparation Charlotte Lenné Alex John Thottumkal
- [x] 9am preculture of 047, 048, 049 (5ml for microscopy, 2x 10ml of 047 for purification, 1x10ml 055)
  2 different negativ controls:

- Yeast 047 not induced (from preculture)
- Yeast 055 (tspurplebb) induced (from microscopy sample)

- [x] preparation of agar slides for microscopy (Charlotte)

## DIENSTAG, 1.9.2026

preculture did not grow sufficient

## MITTWOCH, 2.9.2026

- [x] preculture Mo 12am - Mi 8am (44h)

**OD 600 preculture 8am**

| | A | B |
|---|---|---|
| 1 | | OD600 |
| 2 | 047 1 | 1.62 |
| 3 | 047 2 | 1.92 |
| 4 | 48 | 1.66 |
| 5 | 49 | 1.5 |
| 6 | 55 | 1.5 |

2 different negative controls:

- Yeast 047 not induced (from preculture) SDS: 047nc
- Yeast 055 (tspurplebb) induced (from microscopy sample) SDS: 055nc

- [x] mainculture at 8:45am (6h) 1x 200ml 047, 3x50ml 048, 049, 055 according to 96-well yeast growth curve

start OD600: 0.15
OD600 2pm
47 200ml: 0.2
47 50ml: 0.3
48 0.4
49 0.5
55 0.5

OD600 5pm
47 200ml: 0.45 0.88 -> induction
47 50ml: 0.65 0.9 -> induction
48 0.807 -> induction
49 0.856 -> induction
55 0.922 -> induction

- [x] induction 8:30pm

only 1 washing step

## DONNERSTAG, 3.9.2026

- [x] until 9am production for microscopy (13h) 047, 048, 049
- [x] directly from induction culture for microscopy

055nc
047induc
048induc
049induc

wash 1x with MilliQ

resuspend in MilliQ

- [x] until 12am production 047 (17h)
  12am Lysis + Precipitation (Felix)
  047 induc 200ml directly from induction media

- [x] 10:30am Microscopy session of Lvl2_a_047, 048 and 049 in vivo (Alex, Charlotte, Marina)

- [x] plate Lvl2_a_049 for next week purification from glycerol

**FREITAG, 4.9.2026**

- [x] SDS Page
  whole cell SDS: according to cPCR S. cerevisiae protocol
    047nc, 055nc
    047induc 200ml, 047induc 50ml, 048induc, 049induc
    047CE (=1), 047F1(=2), 047F2(=3), 047F2Ü(=4)

**SAMSTAG, 5.9.2026**

- [x] preculture 049
- [x] preculture mRNA + Leg
- [x] SDS gel destaining

**MONTAG, 7.9.2026**

## Temperatur-dependent production -> Yield optimization

preculture 170rpm 30°C ~48h

mainculture 049 9am ~7h 2x200ml cultivation
  Start OD600: 0.13
  049 (1): 2.56  -> 10ml
  049 (2): 2.6 -> 10ml

negative control before induction

OD600 4pm

049 (1): 0.18

049 (2): 0.2

-> let grow until tomorrow

-> reproducibility of growth curve (200ul) with 1000x volume (200ml)?

induction 049 at OD600=1 2x200ml production
  grow main cultivation for purification at 30°C
  Temperatur-dependent production

based on https://link.springer.com/article/10.1186/s12934-023-02065-7

20°C, 25°C and 30°C each 5ml for SDS

**DIENSTAG, 8.9.2026**

- [x] induction 049

induction 049 at OD600=1 2x200ml production (22h)

OD600: 8:39 a clock culture 1: 2.5

culture 2: 2.8

we use all cells and combine them for the wash steps and medium change --> then devide for induction (22h) into two 200ml cultures.

induction 10am

grow main cultivation for purification at 30°C

200ul sample every 3h 1pm, 4pm, 7pm, 9.9. 8am

wash with milliq

resuspend in 200ul milliq

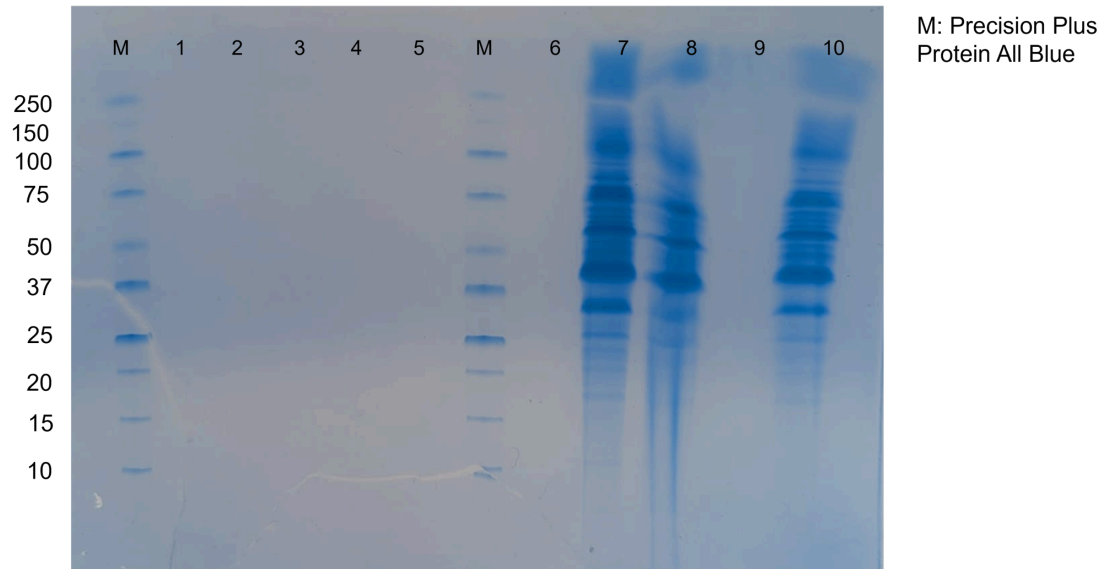
measure fluorescence with tecan

absolute units -> no just in relation

- [x] yeast Trafo pESC-HIS (commercial)

- [x] SDS Gel (last week)

**SDS_gel_induction_annotated.png**




32kDa Encapsulin monomere

26kDa GFP

1 047 nc (preculture)

2 047 induc 200ml

3 047 induc 50ml

4 048 induc

5 049 induc

6 055 induc (nc)

7 after glass beads
8 after 20% precipitation supernatant
9 after 50% precipitation supernatant
10 pellet after 50% precipitation resuspended in PBS

- [x] level 1 cloning of 1.1 NCS and 1.3 NCS (as oligos) backbone was used instead of water

- [x] preculture 055 negative control 4pm

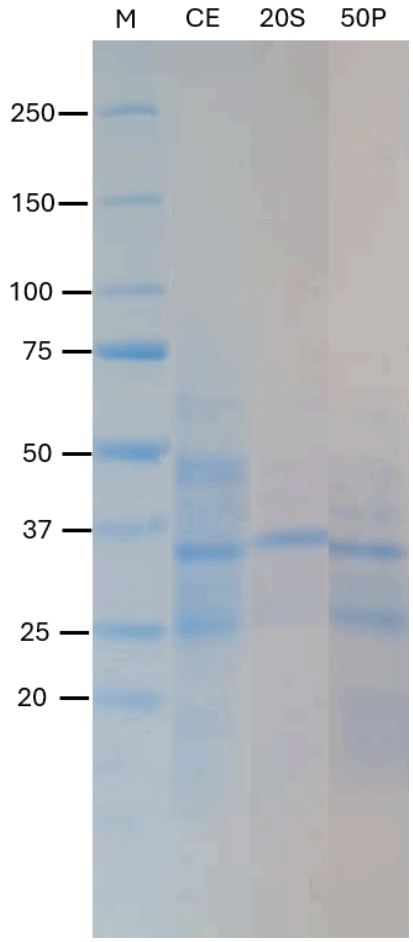
**MITTWOCH, 9.9.2026**

- [x] preculture Lvl2_a_055 negative control induction at 9am (17h)
  centrifuge 10min at roomtemp
  wash 1x
  induction with 5ml induction media at 4pm -> induction until 2pm (10.9) (22h)
  sample at 4pm, 7pm, 9am (10.9) (17h), 12am (20h), 2pm (22h)
- [x] measure GFP from samples: 200ul sample every 3h 1pm, 4pm, 7pm, 9.9. 8am with Tecan
- [x] harvest 9am (-> induction 23h)
- [x] lysis+precipitation according to Purification of Encapsulin (S.cerevisiae)

cell wet weight: 0.8 g --> 2,7 ml PBS added for lysis
C sample taken after Harvest and PBS wash (50 ml falcon was used for this)
after lysis total of 2.7 ml supernatant --> in 2 eppis 1.35 ml each (20 % precipitation: 0.27 g ammonium sulphate)
inverted first after addition of ammonium sulphate
50% precipitation for 1h instead of 10min
1.35ml each -> 0.405g ammonium sulphate

- [x] Trafo 1.1NCS (Lvl1.1_a_070), 1.3NCS (Lvl1.3_a_071)
- [x] Induction Media Preparation
- [x] SD-HIS plates
- [x] SDS Page of 049 Precipitation

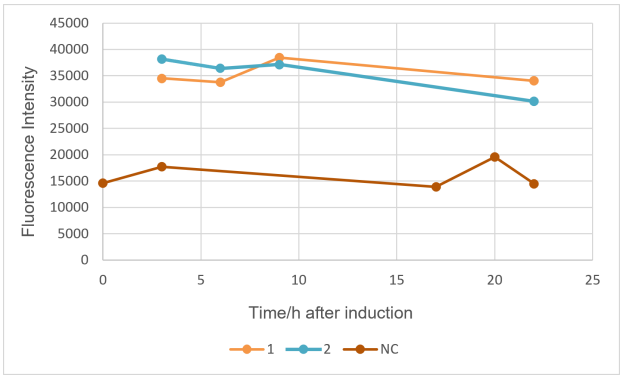
049 Ce and precipitation




**DONNERSTAG, 10.9.2026**

- [x] Glu Media --> SD His autoclave
- [x] measure GFP from 055 INDUCTION EXPERIMENT

**Induction of Qt and negative control (Level 2 Backbone)**




NC: 055 backbone domesticated(ts prple)  1 and 2 are 049 transformed (Qt)

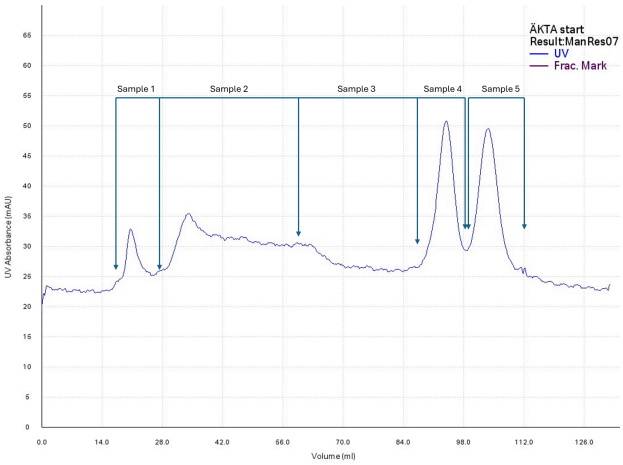
- [ ] SEC of pellet 49 (Qt_GFP)

injection at 13:48

due to pressure alarm the documentation had to be reset after 20 min!

samples were taken manualy with falcons

**SEC 049 with fractions**

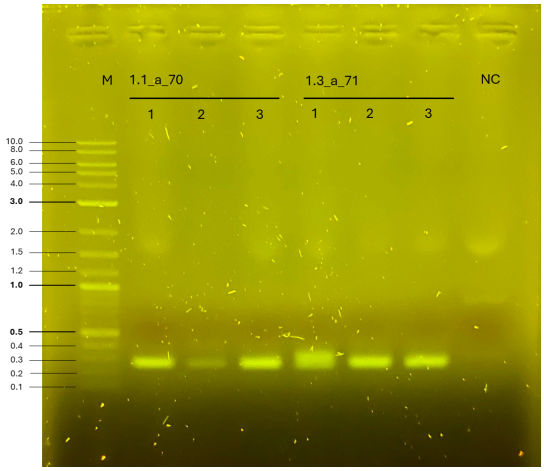



we expect the encapsulin in sample 2

the colume was changed to MiliQ after 112 min (+20)

- [ ] cPCR and gel electrophoresis of 1.1_a_NCS and 1.3_a_NCS casettes Alex John Thottumkal

gel electrophoresis of 1.1_NCS and 1.3_NCS




expected amplicons:
1.1_a_70 NCS 262 bp
1.3_a_71 NCS 262 bp

## FREITAG, 11.9.2026

- [x] documentation SDS from 9.9
- [x] Bradford Assay calibration preperation Ahmed Haj Kacem
- [x] plates from Glycerol 047/055

## SAMSTAG, 12.9.2026

- [x] Miniprep 1.1NCS/ 1.3NCS (70,71) Alex John Thottumkal
- [x] Level 2 Golden gate: with 1.2Gal1/10 (036) Alex John Thottumkal Golden Gate Assembly
  1.1NCS (070) with 1.3Enc Qt (045)
  1.1 NCS with 1.3 Enc Mxpe (044)
  1.1GFP (041) with 1.3 NCS (071)

**Conctruct assembled via Golden Gate level 2**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | **Tube** | **Construct** | **1.1** | **1.2** | **1.3** |
| 2 | 1 | NCS + GAL + EnMxpe | 70 | 36 | 44 |
| 3 | 2 | NCS + GAL + EnQt | 70 | 36 | 45 |
| 4 | 3 | GFP + GAL + NCS | 38 | 36 | 71 |

deviations from protocol because destination lv 2 vector stock was limited:

- reaction volume reduced from 10 µl to 5 µL

    - all component volumes halved because the available destination vector was not sufficient for 3 reactions at 10 µL
- destination vector pooled from three partial stocks
    - the original 055 tsPurple working stock was empty, three residual stocks were found but clone origin/colony was not recorded on the labels, so the pool is of undefined clone/colony composition.
    - all three are labelled 055 Lvl2 tsPurple and are assumed to originate from the same cloning.
- **First master mix discarded.** Enzyme volumes could not be reconstructed after a pipetting error. Reagents for this entry were set up fresh.

**pooling of three almost empty stocks of Level 2 destination vector**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | **Stock** | **Conc. (ng/µl)** | **Conc. (nM)** | **Volume used (µl)** |
| 2 | 1 | 115 | 27.0 | 2 |
| 3 | 2 | 66 | 15.5 | 9 |
| 4 | 3 | 45.6 | 10.7 | 20 |
| 5 | Pool | 56.2 | 13.2 | 31 |

the pool was used undiluted at 13.2 nM instead of the standard 15 nM
DNA insert components pipetted individually. onlybuffer and enzymes were premixed in MM
Enzyme mix (3.5x): 1.7 µl 10x T4 Ligase Buffer, 0.85 µl T4 DNA Ligase, 0.34 µl BbsI. Total 2.9 µl.

**set up per tube**

| | A | B |
|---|---|---|
| 1 | **Component** | **µl** |
| 2 | 055 lv2 BB pool, 13.2 nM | 2.65 |
| 3 | 1.2_036, 15 nM | 0.5 |
| 4 | fixed insert (070 or 071), 15 nM | 0.5 |
| 5 | variable insert, 15 nM | 0.5 |
| 6 | enzyme / buffer MM | 0.8 |
| 7 | Total | 4.95 |

**MONTAG, 14.9.2026**

- [x] SDS Page purification SEC 49
    - [x] Bradford Assay without dilution

Table1

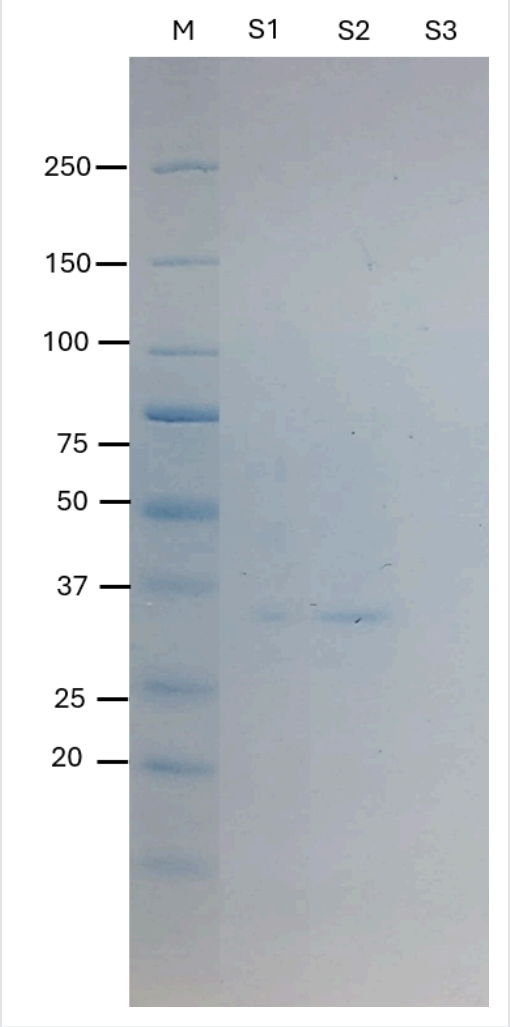
| | A | µg/ml | absorbance | volume ml | total amount µg | volume after concetration µl |
|---|---|---|---|---|---|---|
| 1 | | 0 | 0.62702821 | | | |
| 2 | | 10 | 0.74358452 | | | |
| 3 | | 20 | 0.88667953 | | | |
| 4 | | 30 | 1.01082934 | | | |
| 5 | | 40 | 1.17155862 | | | |
| 6 | | 50 | 1.32477754 | | | |
| 7 | | 60 | 1.50546299 | | | |
| 8 | | 70 | 1.63826694 | | | |
| 9 | | 80 | 1.84233147 | | | |
| 10 | | 90 | 1.99636829 | | | |
| 11 | | 100 | 2.19933731 | | | |
| 12 | sample1 | 11.4909889 | 0.75265762 | 10 | 114.9 | |
| 13 | sample2 | 18.3985219 | 0.86179665 | 33 | 607.134 | |
| 14 | sample3 | 3.255562 | 0.62253788 | | | |
| 15 | sample4 | 3.57065243 | 0.62751631 | 10 | 35,7 | |
| 16 | sample5 | 3.23320555 | 0.62218465 | | | |

- [x] concentration of samples

we will use the samples 1,2 and 5 for concentration with centrifugation Purification of Encapsulin (S.cerevisiae)

try with water first to see if the filter is intact. 2 min 3000 g --> around 0.5 ml

SEC sample 1,2 and 4




- [x] Yeast Wt Overnight for Trafo 3ml YPD + Chloramp

## Temperatur-dependent Induction Experiment/ Precipitation Optimization

preculture 4pm (23h) 20ml 047 / 7ml 055 inoculated from old plates
for 3 replicates 20°C, 25°C and 30°C each 10ml
total 90ml main culture
3 replicates at 30°C 055
total 30ml main culture

**DIENSTAG, 15.9.2026**

measure OD600 at 11am: diluted 1:10 for measurement
049 culture 1: 3,7
049 culture 2: 2,79
055 cuture: 0,045

the first 049 culture was diluted by using 10 ml additional selection medium to see how the growth will be effected, the second culture was placed in the cold room till inoculation of the main culture

- [x] measure OD600 at 3pm (1:10)

80 µl sample and 720 µl H2O

measured :
049: 0,175 --> 1,75
055: 0,135 --> 1,35

- [x] inoculation mainculture **4pm** (17h) OD: 0.15

for 3 replicates 20°C, 25°C and 30°C each 10ml
total 90ml main culture
3 replicates at 30°C 055
total 30ml main culture

use rest of the culture for glycerol stock of 049 and 055 --> centrifuge and resuspend in 0,5 ml medium add 0,5 ml glycerol

- [x] Miniprep Level 2 Backbone 055

**Miniprep 15.09.2026**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | name | concentration / ng/µL | A260/A280 | A260/A230 |
| 2 | 55 | 222.1 | 1.81 | 2.29 |

- [x] Golden Gate Level 2 1.1NCS + 1.3 Qt, 1.1NCS+1.3Mxpe, 1.1yeGFP+1.3NCS

**Conctruct assembled via Golden Gate level 1**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | **Tube** | **Construct** | **1.1** | **1.2** | **1.3** |
| 2 | 1 | NCS + GAL + EnMxpe | 70 | 36 | 44 |
| 3 | 2 | NCS + GAL + EnQt | 70 | 36 | 45 |
| 4 | 3 | GFP + GAL + NCS | 38 | 36 | 71 |

- [x] Yeast Trafo pESC empty (repeat)

- [x] prepare SD His Medium
- [x] prepare Chloramp stock

**MITTWOCH, 16.9.2026**

wash mainculture start at 9am
OD600 49 9am: 2.3
55 9am: 2.1

we tried two differend methods for induction. solution one with cells not in the exponential phase but diluted to a 1 OD and the second solution with starting a new culture inoculating with mainculture. the time between mesurments/ probes is the same for both if they are colored red. the other probes were taken to gain more mesurment and insight on the gfp signal after differend time points. the probes will be compared using TECAN GFP mesurement

**frist solution:** half of mainculture for OD=1
induction 1:20pm, 9 ml per flask
take 200ul samples at
t0 1:20pm (0h)
t1 4:20 (3h) Marina Schreidl Alex John Thottumkal
t2 6:50 (5.5h) Alex John Thottumkal
t3 9:20 (8h) Alex John Thottumkal
spin down, decant supernatant, freeze -20°C

**second solution:**-> fresh media with 0.5 (marked with an X)
grow for 2h OD = 0.48 for both (11:00am) --> due to recoveray after beeing in stationary phase
keep cultivating
15:00 OD600: 049= 0.88 and 055:= 1,01 (place 055 in freezer)
16:30 OD600 049= 1,25 and 055 = 1,1 --> use for induction
pellet for 10 min 4700rpm wash with Water 10 min 4700rpm resuspend in Induction media (049 90 ml and 055 30ml)
distribute in flasks (9ml each) and take t0 sample
t0 6:30 (0h)
t1 9:30 (3h)

**DONNERSTAG, 17.9.2026**

- [x] take 200ul samples at

**first solution:**
t4 8:20 (19h)
t5 11:20 (22h)

**second solution:**
t2. 8:30 (14h)
t3 10:30 (16h)
t4 13:30 (19h)
t5 16:30 (22h)

spin down, decant supernatant, freeze -20°C!

- [x] Tecan GFP Fluorescence intensity of **solution 1**

resuspend pellet in 200ul H2O and mesure

**Pipetting scheme induction experiment**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | H2O | H2O | H2O | | | | | | | | | |
| B | t0 (20°C) | t0 (20°C) | t0 (20°C) | t0 (25°C) | t0 (25°C) | t0 (25°C) | t0 (30°C) | t0 (30°C) | t0 (30°C) | t0 nc (30°C) | t0 nc (30°C) | t0 nc (30°C) |
| C | t1 (20°C) | t1 (20°C) | t1 (20°C) | t1 (25°C) | t1 (25°C) | t1 (25°C) | t1 (30°C) | t1 (30°C) | t1 (30°C) | t1 nc (30°C) | t1 nc (30°C) | t1 nc (30°C) |
| D | t2 (20°C) | t2 (20°C) | t2 (20°C) | t2 (25°C) | t2 (25°C) | t2 (25°C) | t2 (30°C) | t2 (30°C) | t2 (30°C) | t2 nc (30°C) | t2 nc (30°C) | t2 nc (30°C) |
| E | t3 (20°C) | t3 (20°C) | t3 (20°C) | t3 (25°C) | t3 (25°C) | t3 (25°C) | t3 (30°C) | t3 (30°C) | t3 (30°C) | t3 nc (30°C) | t3 nc (30°C) | t3 nc (30°C) |
| F | t4 (20°C) | t4 (20°C) | t4 (20°C) | t4 (25°C) | t4 (25°C) | t4 (25°C) | t4 (30°C) | t4 (30°C) | t4 (30°C) | t4 nc (30°C) | t4 nc (30°C) | t4 nc (30°C) |
| G | t5 (20°C) | t5 (20°C) | t5 (20°C) | t5 (25°C) | t5 (25°C) | t5 (25°C) | t5 (30°C) | t5 (30°C) | t5 (30°C) | t5 nc (30°C) | t5 nc (30°C) | t5 nc (30°C) |
| H | | | | | | | | | | | | |

- [x] lysis solution 1

pool 3x samples (30,25 and 20 °C  049 and 055 as NC) of each temperatur condition for harvest note wet dry wate and resuspend in PBS 3,5 ml per g wet cell --> each in 0,7 ml resuspened for lysis

**wet cell mass**

| | condition | wet weight |
|---|---|---|
| 1 | 049 30°C | |
| 2 | 049 25°C | |
| 3 | 049 20°C | |
| 4 | 055 30°C | |
| 5 | | |

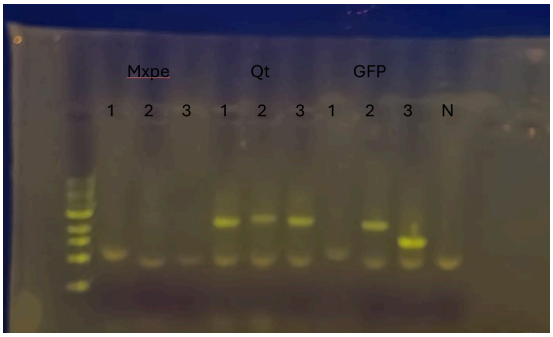
based on the TECAN mesurement we will decide with cultures (solution 1 or 2) we will use for the precipitation experiment!

- [x] calibration preperation bradford! --> 1ml each Bradford Assay
- [x] E. coli Trafo Level 2 1.1NCS + 1.3 Qt, 1.1NCS+1.3Mxpe, 1.1yeGFP+1.3NCS

**FREITAG, 18.9.2026**

- [x] cPCR Dummies Level 2 1.1NCS + 1.3 Qt, 1.1NCS+1.3Mxpe, 1.1yeGFP+1.3NCS
  lvl2_a_098 Qt-Dummy: 2617 bp
  lvl2_a_099 Mxpe-Dummy: 2771 bp
  lvl2_a_100 GFP-Dummy: 2617 bp
  bei alex in geneious unter lvl1-> dummy

cPCR 21.09.26




**SONNTAG, 20.9.2026**

- [x] 11:30am preculture 047 and 049 20ml one culture includes 10ml SD HIS Gluc medium. yeast was taken from old plates for inoculation

shake at 180 rpm 30°C

**MONTAG, 21.9.2026**

- [x] OD measurement preculture 1:10

047:
c1 0.232
c2 0.261
049
c1 0.241
c2 0.27
measure again in 2h
047:
c1 0.248
c2 0.271
049
c1 0.232
c2 0.267
measure again in 1.5h
047:
c1 0.264
c2 0.278
049
c1 0.258
c2 0.301
transfer to freezer.
Measure OD after combining both cultures
047 0,275
049 0,274

- [x] 7pm mainculture 049 and 047 400ml in 2L colums OD 0.1

## Temperature-dependent Induction Experiment

- [x] Lysis Solution 2 4xCE

- [x] lysis (CE) of **solution 2**

**wet cell mass solution 2**

| | condition | wet weight |
|---|---|---|
| 1 | 049 30°C | 0.13 |
| 2 | 049 25°C | 0.121 |
| 3 | 049 20°C | 0.124 |
| 4 | 055 30°C | 0.134 |
| 5 | | |

- [x] Bradford 049 +055
- [x] SDS-PAGE 049+055 S1 4xCE, S2 4xCE -> 8 samples
- [x] Bradford 049 Purification repeat

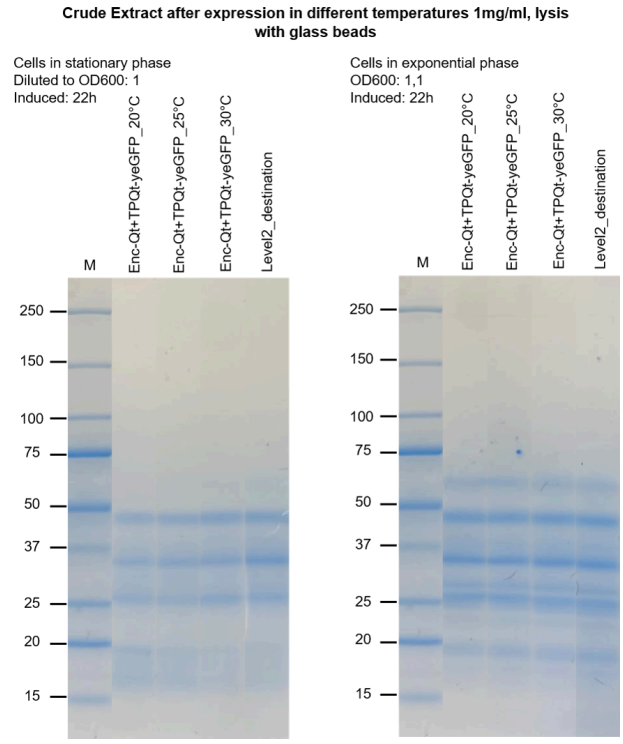
**Table4**

| | A | extinction | µg/ml diluted | µl/ml |
|---|---|---|---|---|
| 1 | s1 1:10 20 | 1.96164927 | 78.8380267 | 788.380267 |
| 2 | s1 1:10 25 | 2.04565563 | 83.6111155 | 836.111155 |
| 3 | s1 1:10 30 | 2.01696563 | 81.9810018 | 819.810018 |
| 4 | s1 1:10 055 | 2.0683499 | 84.9005622 | 849.005622 |
| 5 | s1 1:50 20 | 0.95057242 | 21.3904785 | 1069.52393 |
| 6 | s1 1:50 25 | 0.88434758 | 17.6277036 | 881.38518 |
| 7 | s1 1:50 30 | 0.89045157 | 17.9745213 | 898.726064 |
| 8 | s1 1:50 055 | 0.8769153 | 17.2054148 | 860.27074 |
| 9 | s2 1:10 20 | 4.29290051 | 211.295484 | 2112.95484 |
| 10 | s2 1:10 25 | 4.16652061 | 204.114807 | 2041.14807 |
| 11 | s2 1:10 30 | 3.34977128 | 157.708595 | 1577.08595 |
| 12 | s2 1:10 055 | 0.63942922 | 3.7118873 | 37.118873 |
| 13 | s2 1:50 20 | 2.05184649 | 83.962869 | 4198.14345 |
| 14 | s2 1:50 25 | 1.90182869 | 75.4391304 | 3771.95652 |
| 15 | s2 1:50 30 | 1.82377117 | 71.0040436 | 3550.20218 |
| 16 | s2 1:50 055 | 1.48383747 | 51.6896288 | 2584.48144 |
| 17 | peak 2 1:20 | 0.78233755 | 11.8316792 | 236.633584 |

learning: 1:50 for CE and concetration works but is not 100% perfect. we have a total of 118 µg/ml in the tube of 049. (only 1/5 from what expected)
for SDS the samples of solution 2 had to be diluted:
20, 25, 30 --> 1:4
and 055 --> 1:2

- [x] SDS PAGE -> one gel

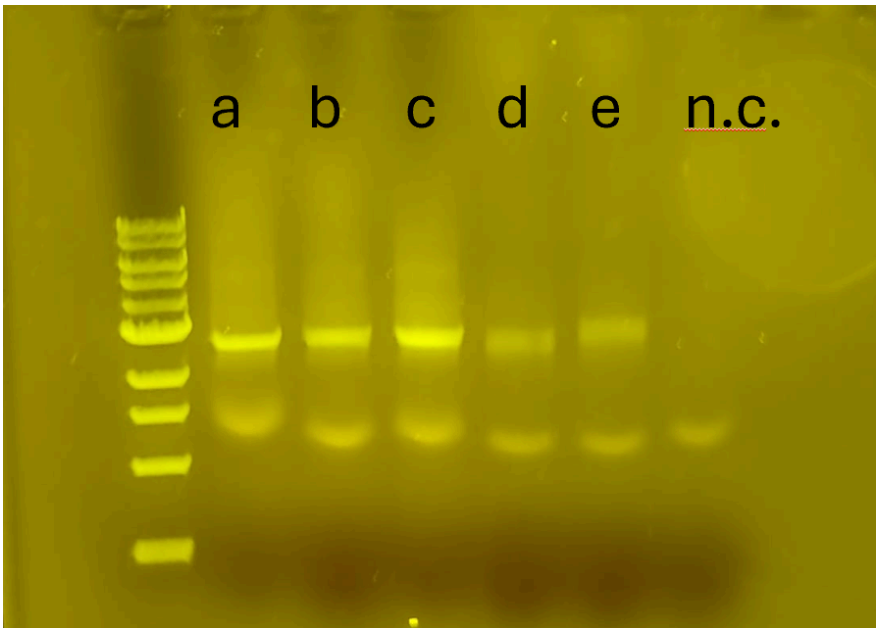
**SDS temperature experiment**




- [x] 2x 3ml preculture for yeast transformation Wt in YPD

cPCR Lvl2_a_099 1.3NCS-E-Mxpe

**cPCR_21.09.2026.png**



## DIENSTAG, 22.9.2026

- [x] 7am OD measurement for induction 049 and 047 400ml

1:10
047: 0.066 --> 0,660
049: 0,061 --> 0,610
has to grow longer
measure again at 9:15 1:10
047: 0.13 --> 1.3
049: 0.13 --> 1.3

- [x] take a not induced sample as negative control--> CE 50 ml
- [x] induction 049 and 047 350 ml SD His Galactose

- [x] native PAGE 049
  3x samples
- [x] Sample and running Buffer nativePAGE

image.png

**Sample buffer (2x):**

| |
|---|
| 62.5 mM Tris-HCl, pH 6.8 |
| 25% glycerol Glycerol |
| 1% Bromophenol Blue |

Running Buffer:

| |
|---|
| 25 mM Tris |
| 192 mM glycine |

## MITTWOCH, 23.9.2026

- [x] lysis/precipitation 047 and 049 (use inverter)

- [x] 4l PBS buffer (steril + degased) Ahmed Haj Kacem

- [x] yeast trafo pESC-HIS 001
  repeat of denaturation of carrier DNA

- [x] overnight E. coli 055

## DONNERSTAG, 24.9.2026

- [x] IEX column preperation

- [x] change buffer of Sample 047 and 049 and peak2 to Tris buffer (Buffer A) Roti spin Maxi (1ml )

The samples: P2, 047, 049
Filter: Amicon MWCO 30 kDa vom Merck

1. Wash the filter falcons with 5 mL MilliQ water by centrifuging the falcon at 3000 g for 5 min, to damp the filter membrane.
2. After the washing step, discard the water (take the filter out of the falcon first!)
3. Pipette the sample (protein in PBS) onto the filter falcon and 5 mL Tris buffer. (PBS will be exchanged with Tris in two cycles)
4. 1 cycle: centrifuge the falcons at 3000 g for 3 min → was enough: 5 mL solution flowed through, which could mean PBS was partly exchanged.

After the 1st cycle: volumes on top of the filter:

- P2: 1 mL
- 047: ca. 2 mL
- 049: ca. 2 mL

1. Because we thought that most of PBS was exchanged, we used only 2 mL Tris buffer for the sample 047 & 049 and 4 mL for the P2 sample instead of 5 mL to avoid that more volume flows through the filter and potentially more proteins get lost. Centrifuge them for 1 min

→ 1 min wasn't enough; we centrifuged them for 2 min more.

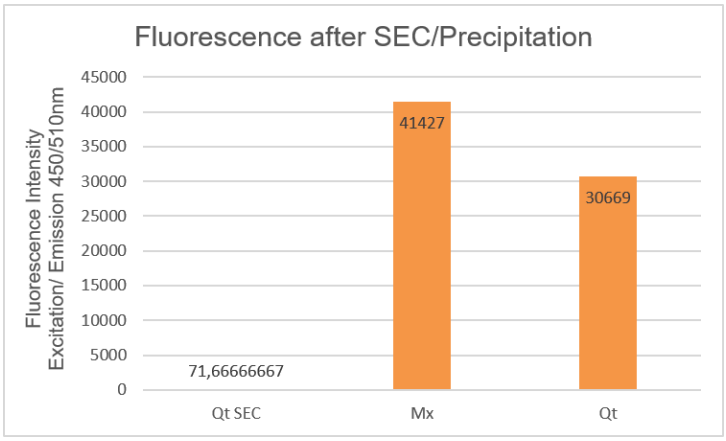
- Samples 047 & 049 had a volume of 1.5 mL on top of filter, but the wanted volume is 1 mL. They were centrifuged again for 2 min → it worked for 049 but not for 047 (probably due to little suspension, the encapsulins probably clogged the filter) → always resuspend for centrifuging

1. Before taking sample, resuspend the solution well. Take 50 µL from the sample on top of the filter, which has the proteins (incl. encapsulins) dissolved in Tris (labelled: IEX). Take 200 µL (labelled DES) from the flow through, which might potentially include encapsulins as well; we were not sure of the filter's retention. Put these samples in PCR stripes with the corresponding label; they will be used for SDS-PAGE.

- [x] cPCR CE 055,047,049,047ni,049ni with cPCR S. cerevisiae Taq skipping the lysis step because the cells are already lysed
  fw: 118, rv: LP034
  - [x] 2µl direct sample as template
  - [x] 1:10 dilution (2µl used for PCR)
  - [x] 2% gel

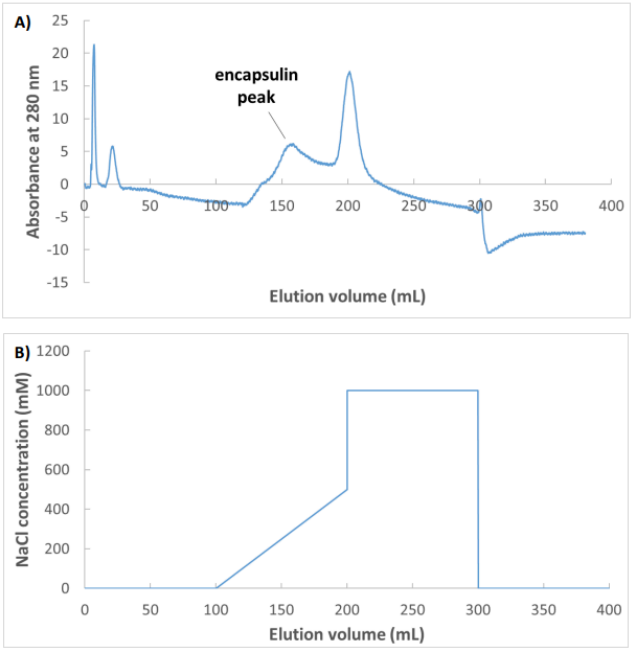
- [x] Bradford 047,049
  - [x] CE
  - [x] precipitation samples
- [x] GFP measurement of samples after precipitation 049,047, and peak 2 (in Tris buffer) buffer in first three wells!
  - [x] measurement and then put back for IEX

Fluorescence Measurement




- [x] purification of 047 and 047ni + 049 and 049ni as well as peak 2, with IEX 20mM Tris-Base + 0%, 20%, 40%, 60%, 100% NaCl

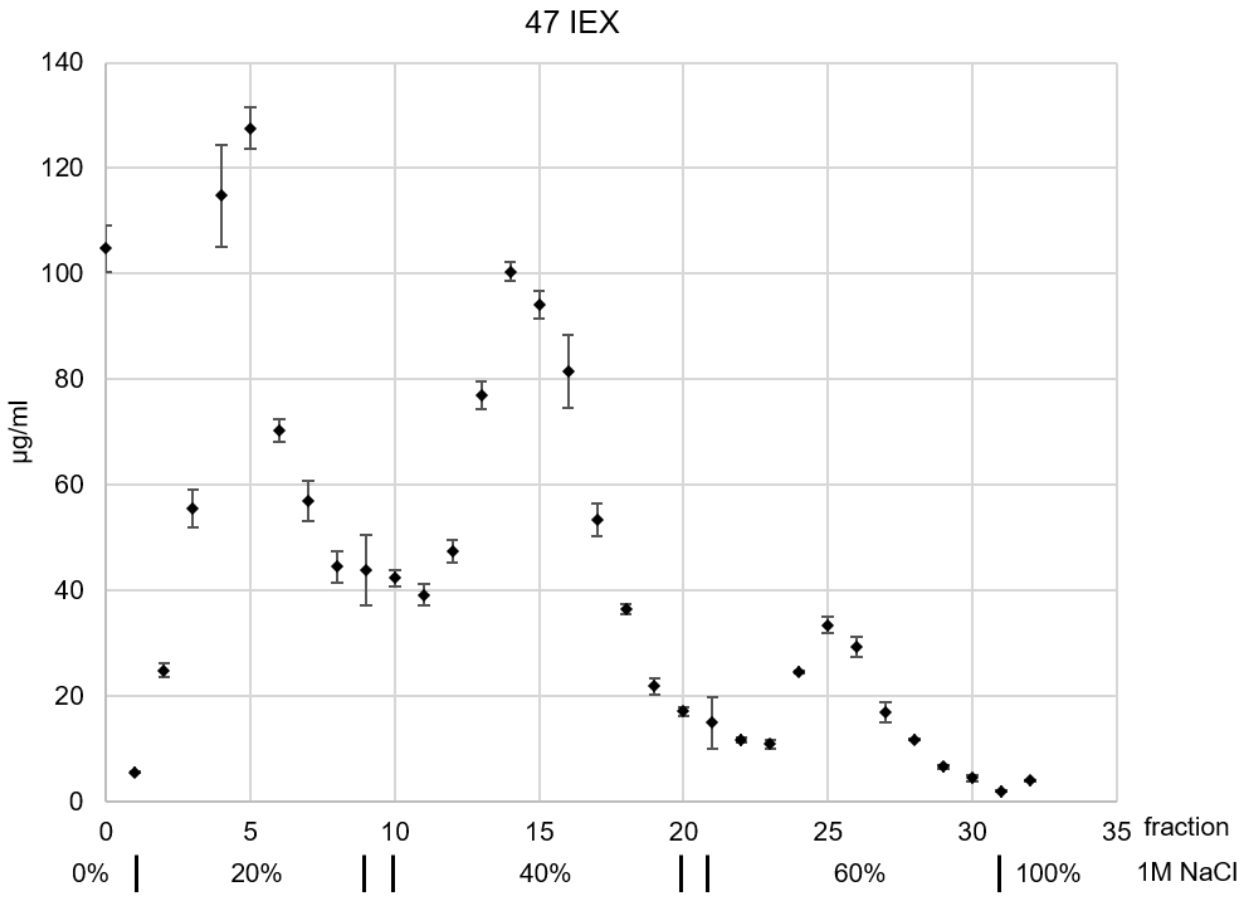
IEX (Lau et. al, 2018)



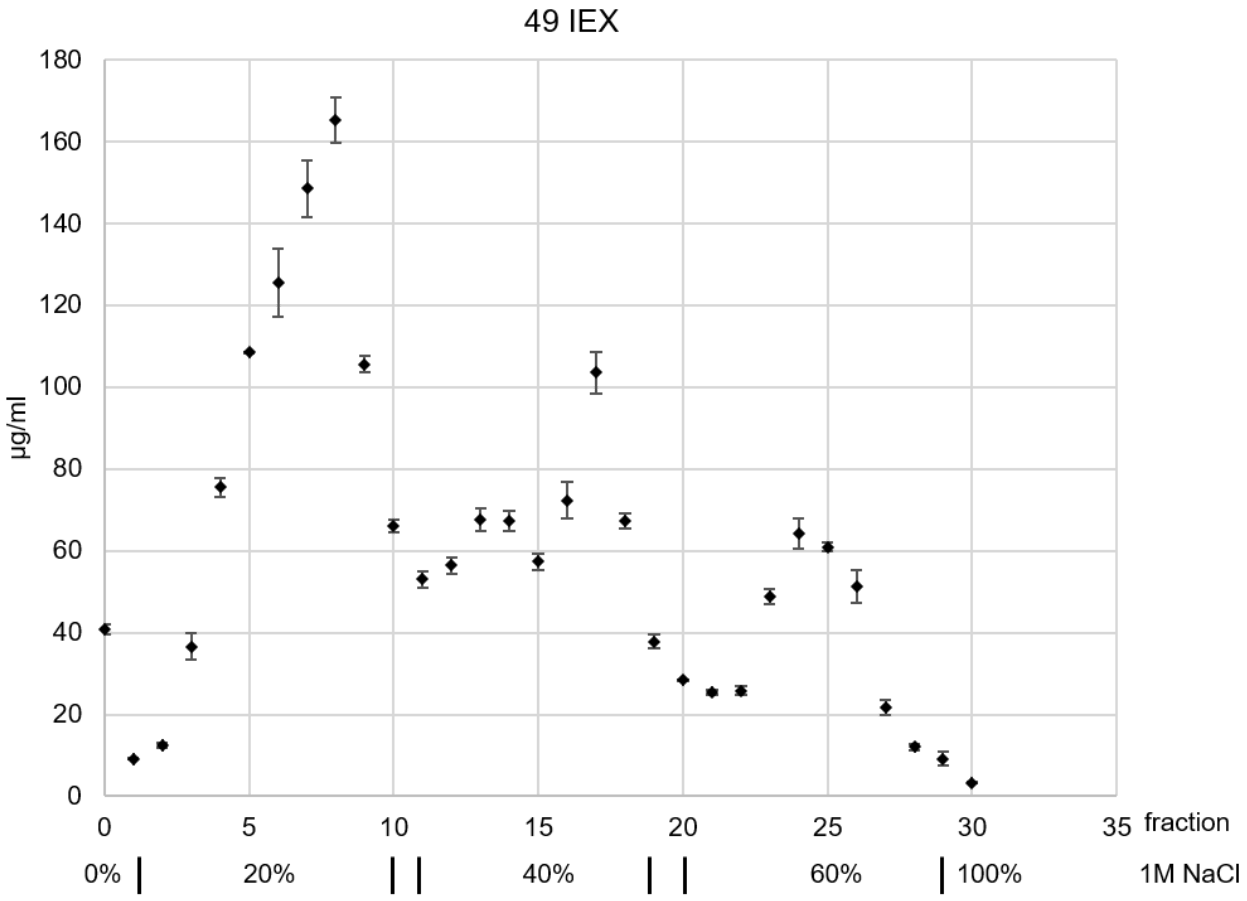

**Supplementary Figure 14.** A) Example FPLC chromatograph of ion-exchange chromatography purification of encapsulins, with the encapsulin peak eluting at 145-170 mL. This is the second column-based step in the purification process, leading to completely homogeneous encapsulin samples. B) The salt gradient used for the ion-exchange process, with Tris pH 8 as the base buffer.

- [x] collect sample for SDS
- [x] Bradford of all fractions IEX 47 and 49 as well as the Ce and precipitation samples

IEX results 47 generated with Bradford measurement



IEX results 49 generated with Bradford measurement




- [x] Miniprep 055 @Marina
- [x] yeast preculture

**FREITAG, 25.9.2026**

- [x] SDS of 47 and 49 lysis and precipitation including 47 not induced and 49 not induced as negativ controle. All samples were leaded with 1mg/ml protein (according to Bradford measurement)

**Bradford results 47,1**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | name | extinction | µg/ml diluted | µg/ml |
| 2 | CE 47 ni 1:100 | 0.97652027 | 21.77766317 | 2177.76632 |
| 3 | CE 47 1:50 | 1.41454656 | 47.54391537 | 2377.19577 |
| 4 | CE 47 1:100 | 1.10936185 | 29.59187344 | 2959.18734 |
| 5 | 20S 47 1:20 | 2.02004815 | 83.16165576 | 1663.23312 |
| 6 | 20P 47 1:10 | 4.04016161 | 201.9918592 | 2019.91859 |
| 7 | 50S 47 | 4.0826376 | 204.490447 | 204.490447 |
| 8 | 50P 47 1:50 | 2.23645827 | 95.89166269 | 4794.58313 |
| 9 | IEX 47 1:50 | 1.95549055 | 79.36415006 | 3968.2075 |
| 10 | DES47 | 0.80334818 | 11.59106952 | 11.5910695 |
| 11 | CE 49 1:50 | 1.23314249 | 36.87308783 | 1843.65439 |
| 12 | CE 49 1:100 | 0.90490949 | 17.56526408 | 1756.52641 |
| 13 | 20S 49 1:20 | 1.52812089 | 54.22475843 | 1084.49517 |
| 14 | 20P 49 1:10 | 3.51373384 | 171.0255197 | 1710.2552 |
| 15 | 50S 49 | 4.19562472 | 211.1367484 | 211.136748 |
| 16 | 50P 49 1:50 | 1.84371036 | 72.78884447 | 3639.44222 |
| 17 | IEX 49 1:50 | 1.61931203 | 59.58894289 | 2979.44714 |
| 18 | DES49 | 0.77020013 | 9.641183915 | 9.64118391 |
| 19 | IEX P2 1:50 | 0.66541603 | 3.477413346 | 173.870667 |
| 20 | DES P2 | 0.66061646 | 3.195085987 | 3.19508599 |