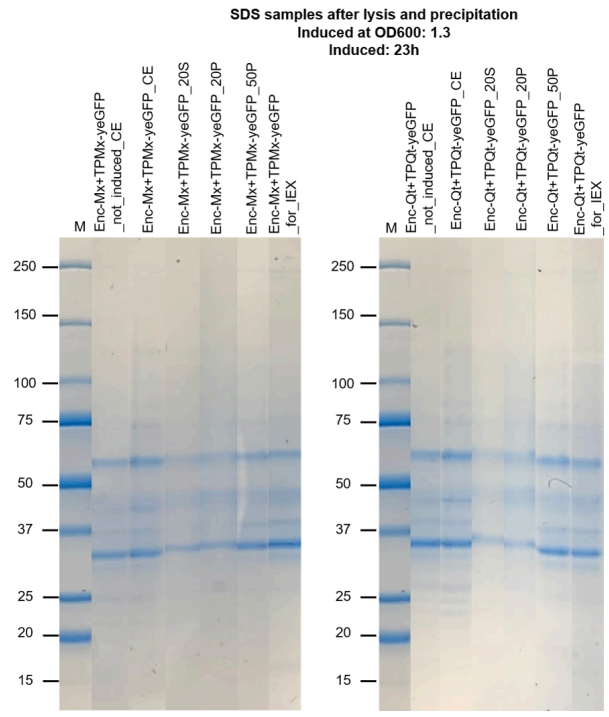
pipetting sceme for SDS

	A	B	C	D	E	F
1		für SDS	1mg/ml			
2			µg/ml stock		use volume µl	water µl
3	1	CE 47 ni	2177		2.3	2.7
4	2	CE 47	2377		2.1	2.9
5	3	20S 47	1663	0.60132291	3	2
6	4	20P 47	2019	0.4952947	2.5	2.5
7	5	50P 47	4794	0.20859408	1	4
8	6	IEX 47	3968	0.25201613	1.25	3.75
9	7	CE 49 ni	2000	0.5	2.5	2.5
10	8	CE 49	1756	0.56947608	2.85	2.15
11	9	20S 49	1084	0.92250923	5	0
12	10	20P 49	1710	0.58479532	3	2
13	11	50P 49	3639	0.27480077	1.35	3.65
14	12	IEX 49	2979	0.33568312	1.5	3.5
15						

SDS lysis and purification



- [x] Mx 047 in liquid culture
- [x] Qt 049 from glycerol onto plate

SONNTAG, 27.9.2026

Negative Controls Purification

- [x] preculture 055
- [x] preculture 047,049

in 20 ml SD His inoculated with liquid culture from friday

MONTAG, 28.9.2026

- [x] prepare SD His Glucose and Galactose ! autoclave at 12:30 am
- [x] decide which fractions need to be pooled concentrated from IEX 47 and 49 to be analysed on SDS (silver stain)

Negative Controls Purification

preculture OD at 2:30pm:

47: 2.7

49: 2.5

55: 2.9

- [x] mainculture 055, 047, 049 200ml inoculation with OD 0,1 at 7pm

47: 7,4 ml plus 192,6ml SD His Glucose

49: 8 ml plus 192ml SD His Glucose

55: 6,9ml plis 193,1 ml SD His Glucose

- [x] liquid culture 055,047,049 with rest medium

- [x] SDS of IEX samples pooled and silver stained

how to pool for silver stain

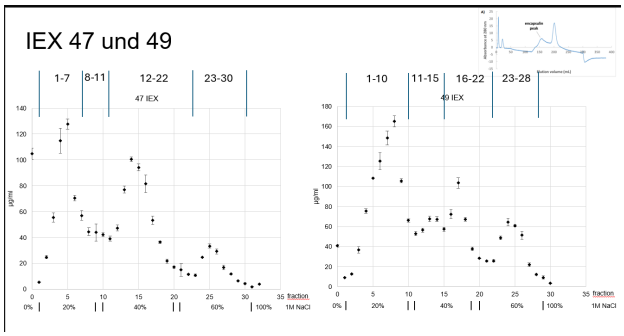
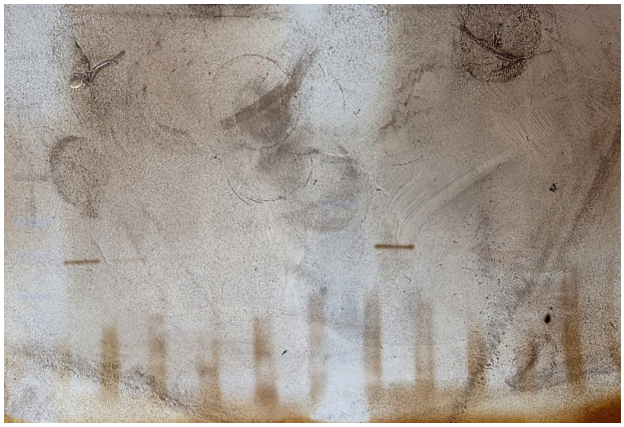


image.png



- [x] yeast culture for trafo with new strain

DIENSTAG, 29.9.2026

- [x] concetration of IEX samples pooled to 500 µl

fraction volume before concentration

	fraction	047	049
1	1	7	6,5
2	2	2,5	3,75
3	3	8	4
4	4	7	4

30kDA cut off columes

- [x] repeat Bradford

test 1:5 and 1:10 dilution with bradford --> in the future 1:10 dilution

- [x] new calibration curve

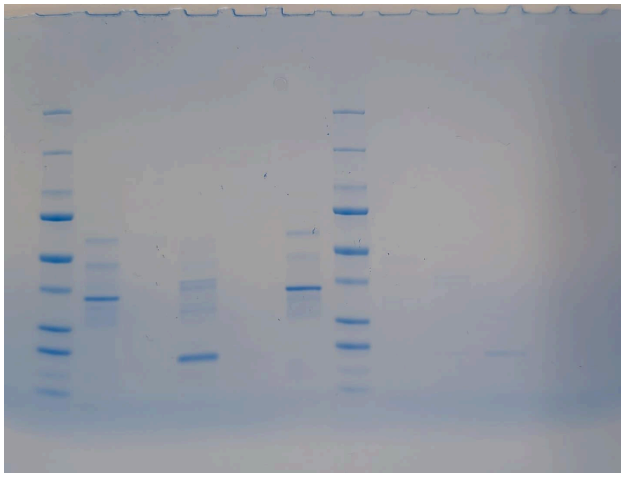
Table5

	A	B	C	D
1		absorbance 590/450	µg/ml	total µg/ml
2	47_1:5_F1	2.15796829	86.1705914	430.852957
3	47_1:5_F2	0.94033106	16.1914403	80.9572015
4	47_1:5_F3	2.32419109	95.723626	478.61813
5	47_1:5_F4	0.98976619	19.0325394	95.1626969
6	47_1:10_F1	1.41678668	43.573947	435.73947
7	47_1:10_F2	0.80198227	8.24036025	82.4036025
8	47_1:10_F3	1.52244385	49.6461983	496.461983
9	47_1:10_F4	0.85337199	11.1937924	111.937924
10	47_df_F1	0.72784658	3.97968844	3.97968844
11	47_df_F2	0.71898006	3.47011863	3.47011863
12	47_df_F3	0.71789694	3.40786985	3.40786985
13	47_df_F4	0.67365395	0.86516943	0.86516943
14	49_1:5_F1	2.49660264	105.632336	528.161678
15	49_1:5_F2	0.99233632	19.1802482	95.9012409
16	49_1:5_F3	1.11851358	26.4318151	132.159075
17	49_1:5_F4	1.15975428	28.8019703	144.009852
18	49_1:10_F1	1.5783065	52.8566954	528.566954
19	49_1:10_F2	0.86288089	11.7402813	117.402813
20	49_1:10_F3	0.89613507	13.6514406	136.514406
21	49_1:10_F4	0.95605699	17.0952294	170.952294
22	49_df_F1	0.74878072	5.18279981	5.18279981
23	49_df_F2	0.71411931	3.19076492	3.19076492
24	49_df_F3	0.70812365	2.84618672	2.84618672
25	49_df_F4	0.6855851	1.55086788	1.55086788

based on bradford little amount of protein passed through the filter while concentrating

- [x] SDS of concetrated samples (5 µl each)

WhatsApp_Image_2026-10-01_at_12.06.16.jpeg



Negative Controls Purification

- [x] measure OD 600 of 047,049 and 055 if OD 1 continue otherwise let it grow longer

8:10: 1:10 dilution
47: 0,085
49: 0,067
055: 0,082

8:50: 1:10 dilution
47: 0,107 --> transfer to fridge

9:20 1:10 dilution
49: 0,114
055: 0,118

- [x] continue with wash:

47 and 49 with PBS (unsterile) --> harvest of not induced samples as controle
055 with Mili Q (sterile) --> medium exchange to induction Medium (11 am)
induced in 200ml SD his Galactose --> 1L flask shake 22h 30°C 180rpm
take 5 ml sample of 055 not induced --> pellet (not washed yet)

- [x] harvest 047, 049 --> store pellet in feezer after PBS wash
- [x] induction 055 with SD His Galactose 200ml at 11 am --> let it produce till next day at 9 am
- [x] take 055 not induced sample --> pellet

- [x] yeast trafo

MITTWOCH, 30.9.2026

Negative Controls Purification

- [x] OD600 of 055 induced 1:10 OD 0,098 ; Harvest at 10 am
- [x] lysis, precipitation 047, 049, 055 take samples for SDS Purification of Encapsulin (S.cerevisiae)

047 and 049 already washed with PBS --> need to be weight and resuspendet in PBS
055 need to be harvested and washed with PBS, weight, resuspendet
055ni need to be washed with PBS, weight, resuspended

wet cell weight
047: 0,6567g: 2,4 ml
049: 0,6744g: 2,4 ml
055i: 0,361g: 1,3 ml
055ni: 0,112g: 0,4 ml

lysis using glass beads
--> CE sample 50µl
precipitation: 20 and 50% --> take SDS samples

- [x] Bradford CE and precipitation samples

Bradford results 47 and 49ni 055i and ni for SDS

	A	B	C	D	E	F
1		für SDS	1mg/ml			
2			µg/ml stock		use volume µl	water µl
3	1	047Ce	2109.29016	0.47409314	2.37	2.63
4	2	04720S	1754.30533	0.5700262	2.85	2.15
5	3	04720P	983.617427	1.01665543	5.00	0.00
6	4	04750P	4203.05484	0.23792219	1.19	3.81
7	5	049Ce	2022.25078	0.49449851	2.47	2.53
8	6	04920S	1684.32558	0.59370944	2.97	2.03
9	7	04920P	1721.55259	0.58087101	2.90	2.10
10	8	04950P	3499.08017	0.28578939	1.43	3.57
11	9	055Ce	1858.20842	0.53815276	2.69	2.31
12	10	05520S	1448.1837	0.69052013	3.45	1.55
13	11	05520P	1164.16234	0.85898673	4.29	0.71
14	12	05550P	1850.17766	0.54048864	2.70	2.30
15	13	055Ce	3917.49324	0.25526528	1.28	3.72

- [x] SDS

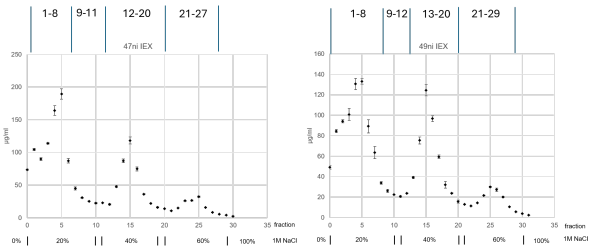
- [x] IEX 47 ni, 49ni, 055 i

DONNERSTAG, 1.10.2026

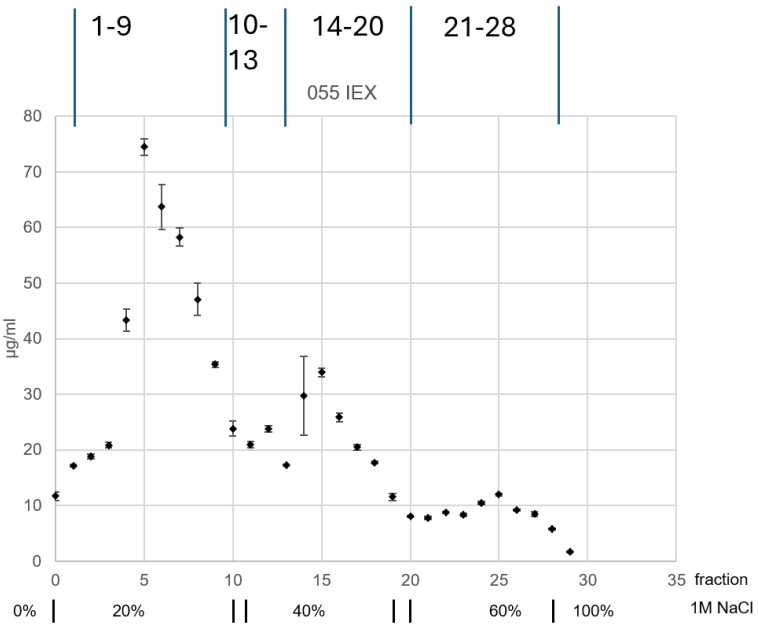
Negative Controls Purification

- [x] Bradford IEX samples 50 µl for each fraction

IEX of not induced controles



IEX of 055 induced controle



- [x] concentrate with 30kDA cut off columes to 500 µl (F2 to 250µl)

fraction volume before concentration1

	fraction	047ni	049ni	055
1	1	6.5	6	7
2	2	2	3.5	3.5
3	3	8	6.5	7
4	4		7	5.5

- [x] Bradford of concetrated sampels: 1:10 dilution

Bradford results 47ni 49ni and 055 IEX fractions

	A	B	C	D
1				µg/ml
2	47_1	2.28222651	101.865	1018.65
3	47_2	0.77801004	8.435406	84.35406
4	47_3	1.38854064	46.3565617	463.565617
5	47_4	0.94821822	19.0073431	190.073431
6	49_1	2.11081989	91.2186267	912.186267
7	49_2	0.845752	12.6429815	126.429815
8	49_3	1.57297289	57.8119805	578.119805
9	49_4	0.93363131	18.1013239	181.013239
10	55_1	1.25647284	38.1535928	381.535928
11	55_2	0.8790071	14.7085156	147.085156
12	55_3	0.81810271	10.9256344	109.256344
13	55_4	0.84612232	12.6659828	126.659828

- [x] SDS with 5 µl sampel each

FREITAG, 2.10.2026

- [x] glycerol 055,049 and 047 out of 10ml rest from the cultivation of this week. resuspended in 500µl SD His

MONTAG, 5.10.2026

- [x] concentrate IEX 47 F2 and 49 F2, 3and 4 from last week again to 250 µl
- [x] Bradford again
- [x] SDS for comparision
- [x] liquid culture 055, 047, 049, 098, 099, 100 1ml

DIENSTAG, 6.10.2026

mainculture 055, 047, 049, 098, 099, 100 10ml 6pm
(14h) - 8am

MITTWOCH, 7.10.2026

OD 8:30am
047: 3.2
049: 3.2
055: 3.6
098: 3.0
099: 3.0
100: 3.2
main (10ml) with OD 0.1
measure at 1:30pm ~0.3

measure at 4:30pm

take sample 047/ 049 not induced 1,5 ml

induction 055, 047, 049, 098, 099, 100 10ml
22h

DONNERSTAG, 8.10.2026

move culture to cold room.

FREITAG, 9.10.2026

microscopy 1pm
1ml of culture, wash 2x with MilliQ
technical replicate
glycerol from new strain