WEB309 - SOMT Expression

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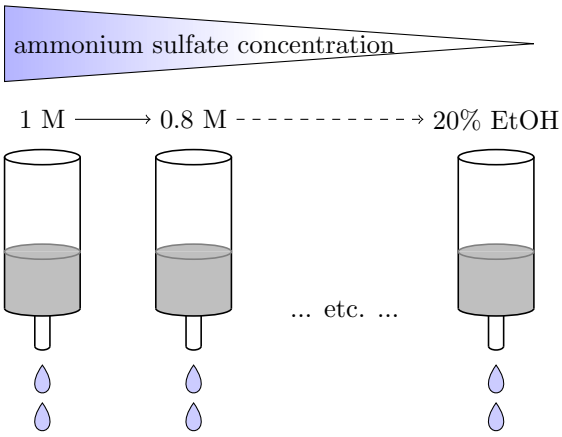


Figure 1: Experimental setup for the HIC.

time	OD^{600}	comment
10.40	0.51	placed at 30°C
11.20	0.592	induced with 1 mM IPTG
17.15	6.98	collected cells (1 mL sample for SDS-PAGE etc.)

1 Cell lysis & Treatment

- cells were resuspended in ca. 40 mL of lysis buffer
- spatula tip of lysozyme was added and incubated at room temp for 15 min on a platform shaker
- three time sonication for 30 s at 70% amplitude
- addition of DNase salts and 1/1000 volume of XX U/mL DNase I
- incubation for 15 min on ice
- centrifugation at 10.000 x g, 4°C for 15 min
- addition of an equal volume (40 mL) of 2 M ammonium sulfate, pH 7.4 to the supernatant (add while stirring on ice) → this takes the supernatant to 1 M ammonium sulfate
  → the solution becomes turbid from precipitating proteins and needs to be centrifuged
- centrifugation at 10.000 x g, 4°C for 30 min
  → **took sample of pellet for SDS-PAGE**

lysis buffer

50 mM Tris/HCl
500 mM NaCl
10 % glycerol
0.1 % Triton X100
pH 7.4

2 Phenyl sepharose column

- Filled self-pack column with 6 mL Phenyl-sepharose suspension in 20 % EtOH → about 4 mL column material
- washed with water (∼4 CV)
- equilibrated with binding buffer (∼3 CV)
- applied 100 mL of cell lysate (@ 1 M ammonium sulfate) from section 1
  → **sample for SDS-PAGE (applied 2 HIC)**
- **started fractionation** (4 mL fractions in 5 mL eppis)
- washed with 2 CV binding buffer
- eluted stepwise with 2 CV each, 0.8/0.6/0.4/0.2/0 M ammonium sulfate, 50 mM KP_i pH 7
- eluted with 2 CV 50 mM KP_i, 10 % EtOH, pH 7
- **ended fractionation**

Table 1: Fractions collected from stepwise elution of HIC

Fraction	1 2	3 4	5 6	7 8	9 10	11 12	13 14
AS [M]	1	0.8	0.6	0.4	0.2	0	20 % EtOH

Binding Buffer

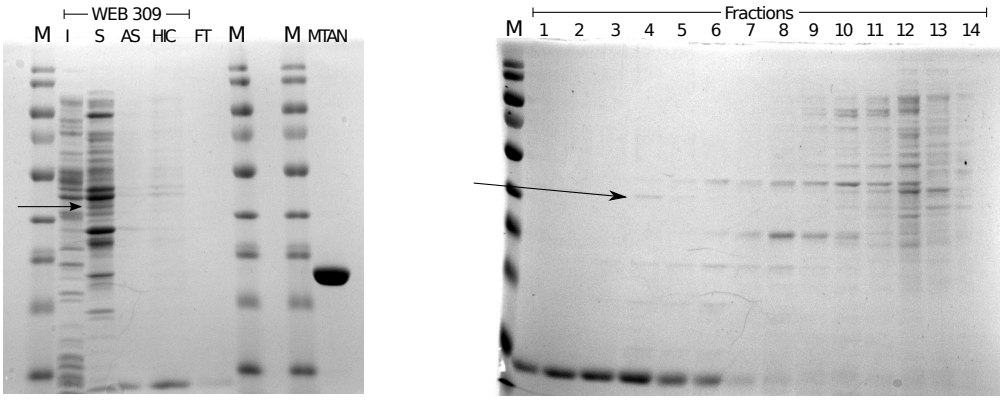
50 mM Phosphate, 1 M $(NH_4)_2SO_4$

Elution buffer

stepwise lowering of $(NH_4)_2SO_4$ concentration (0.2 M steps)

3 SDS-PAGE

- samples from fractions were analyzed by UV-VIS (A^{280}) and Bradford for protein estimation
- dilution factor for Bradford was 5
- 10 μl of each sample were applied directly on SDS-PAGE (10% acrylamid)
- 500 μl of each fraction were also precipitated by TCA and resuspended in 100 μl PBS + 20 μl SDS loading dye for further analysis, due to low protein concentration on first SDS-PAGE (see below) → these samples were stored at -20°C for later analysis
- the sample taken from culture prior to harvesting were lysed with B-PER II reagent and subfractioned into soluble and insoluble fraction for SDS-PAGE



(a) Various samples from during workup.

(b) Fractions eluted during HIC.

Figure 2: SDS-PAGE of WEB309. I – insoluble fraction, S – soluble fr., AS – pellet after ammonium sulfate addition, HIC – supernatant after ammonium sulfate addition (this was applied to HIC), FT – flowthrough. M – Marker, MTAN – E.coli methylthioadenosyl nucleosidase.

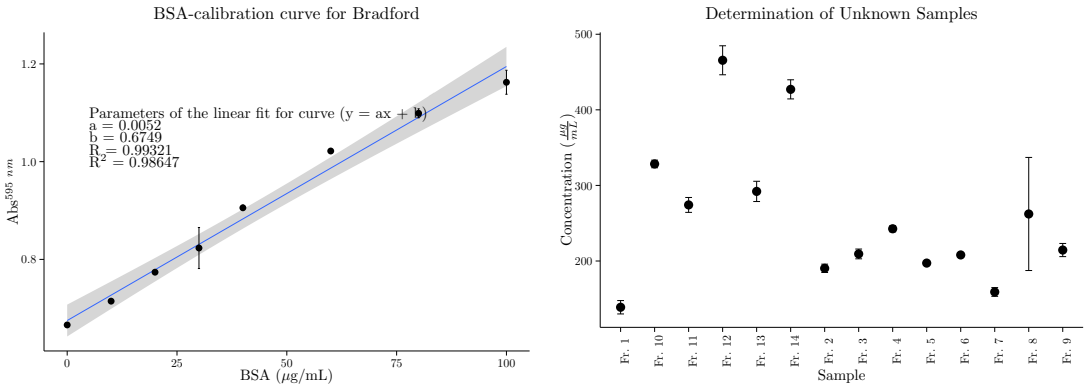


Figure 3: Protein determination by bradford

Table 2: Setup of the gels for SDS-PAGE analysis

lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Fraction	M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Gel 2	M	insoluble	soluble	AS pellet	applied 2 HIC	flowthru	M		M	MTAN (5μg)						

	Sample	protein concentration ($\frac{\mu g}{mL}$)	SD
1	Fr. 1	138.82	8.84
2	Fr. 10	328.40	5.03
3	Fr. 11	274.20	9.86
4	Fr. 12	465.56	19.18
5	Fr. 13	292.09	13.40
6	Fr. 14	427.09	12.65
7	Fr. 2	190.33	5.51
8	Fr. 3	209.37	6.46
9	Fr. 4	242.65	4.01
10	Fr. 5	197.25	1.02
11	Fr. 6	208.03	2.11
12	Fr. 7	159.07	5.78
13	Fr. 8	262.23	74.81
14	Fr. 9	214.52	8.71