



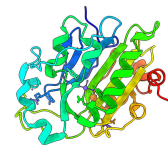
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Version 1

Purification of SerMNucA (Benzonase/Turbonuclease) as 10xHis, MBP or myc-tag fusions V.1

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Protocol status: In development

We are still developing and optimizing this protocol

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Keywords: nuclease, endonuclease, dna, rna, benzonase, turbonuclease, dnase, rnase, purification, protein purification, purification of the sermnuca nuclease, protein purification alongside other commercial enzyme, sermnuca nuclease, purification of sermnuca, enzymatic digestion by nuclease, removal of nucleic acid, purification from either periplasm, common protein purification protocol, large release of nucleic acid, latter enzyme, other commercial enzyme, enzyme, nucleic acid, purified protein, use of the enzyme, nucleic acids research, enzymatic digestion, such as benzonase, stranded dna, rna with high activity, sermnuca endonuclease, untagged versions of the sermnuca endonuclease, protein from either fraction, tag fusions during the lysi, presence of dna, recombinant versions of the serratia, cleavable with tev protease, protein

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Abstract

During the lysis of cells, in particular of eukaryotic origin, there is a large release of nucleic acids. The presence of DNA/RNA increases the viscosity of a lysate and may pose problems during downstream processes. Removal of nucleic acids can be performed in many ways, of which one is by enzymatic digestion by nucleases.

DNase/RNase are commonly added during protein purification alongside other commercial enzymes such as Benzonase (Novagen) and Turbonuclease. The latter enzymes are industrially produced or recombinant versions of the *Serratia marcescens* nuclease. This enzyme, hereafter referred to as SerMNucA, is able to hydrolyze both single- and double-stranded DNA and RNA with high activity.

Reported methods have shown that the nuclease can be made as inclusion bodies followed by refolding or secreted in the extracellular media. Here we report the purification of the SerMNucA nuclease in a soluble form, followed by purification from either periplasm or cell lysate. We find that purified protein from either fraction are active in a salmon sperm degradation assay with the periplasmic fraction slightly more.

This protocol allows for a lab to independently produce tagged and untagged versions of the SerMNucA endonuclease in large quantities that may be required for certain experiments that may be cost prohibitive otherwise.

3 versions are reported here:

10x-His tagged

MBP tagged

myc tagged

The use of the enzyme is in such small quantities that repurification with the tag should not be an issue. The MBP tagged version is cleavable with TEV protease (see also: **10x-His-SuperTEV**). Finally the myc-tagged version should not interfere with any common protein purification protocols.

References:

Benedik and Strych. (1998) FEMS Microbiology Letters

Biedermann et al. (1989) Carlsberg Research Communications

Friedholl et al. (1996) Nucleic Acids Research

Guidelines

This protocol is written with the expectation of standard bacterial culture and basic purification knowledge



Materials

Plasmid #1

Plasmid #2

Plasmid #3

BL21 (DE3) cells (Lucigen)

TB + trace elements (Formedium)

Glycerol (Applichem)

Akta system (Cytiva)

HisPur NiNTA resin (Pierce/Thermo)

Amylose resin (NEB)

Hitrap Q HP 5 mL column (Cytiva)

12-14 kDa dialysis tubing (SpectraPor)

Dialysis clips (SpectraPor)

Sonicator

5 M NaCl

1 M HEPES, pH 7.5

1 M TRIS, pH 8.0

0.5 M EDTA, pH 8.0

2.5 M imidazole, pH 7.5 (We recommend, Sigma #56749-1KG for low background absorbance)

1 M MgCl₂

1 M IPTG

Sucrose

4X LDS loading dye (Genscript)

NuPage 4-20% SDS-PAGE Gels (ThermoFisher)

8M Urea

Salmon testes DNA (Rockland)

Plastic disposable or glass reusable columns (Biorad)

Before start

Recommended to have ready before starting alongside Materials.

-Autoclaved flasks and bottles

-Autoclaved LB media

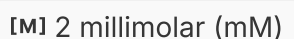
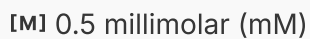
-Autoclaved TB media

-Buffers

-Liquid nitrogen

Growing bacterial cultures (2L) and periplasmic extraction

4d

- 1 Transform the bacterial plasmid expressing the construct of interest (His, MBP or myc fusions) into BL21 (DE3) cells. Plate on to LB-Agar plates + Kanamycin. Grow  Overnight]at Temperature  37 °C or over the weekend at Temperature  25 °C .   1d
- 2 In an afternoon, pick a streak of cells and inoculate into  200 mL of LB + Kanamycin media. Note: for every  1 L of expression culture, you will require  10-20 mL of pre-culture. Grow  Overnight at  37 °C   1d
- 3 The next morning, add MgCl₂ to  2 millimolar (mM) , inoculate  20-40 mL of preculture into 1 flasks containing  2 L of TB media ( 2 L in a  5 L flask is appropriate). Shake in an incubator at  37 °C for 3-4 hours until OD600 ~0.8-1. Take a 1 mL sample of the culture, this will be used as a pre-induction control sample. Immediately change the temperature to  18 °C , add IPTG to  0.5 millimolar (mM) , continue shaking overnight (approximately  20:00:00)    20h
- 4 Take a 1 mL sample of the culture and measure the OD600, this will be used as a whole cell post-induction sample. Harvest the culture by centrifuging at  5000 x g, 10°C, 00:30:00 .  30m
- 4.1 SerMNucA has been designed to be secreted into the oxidizing environment of the periplasm of E.coli to enable proper folding. Proteins from this particular compartment can be extracted by osmotic shock.  10m

Prepare the following:

	A	B	C
	Buffer	Component	Concentration (mM)
	TES 1X	TRIS, pH 8	200
		EDTA, pH 8	0.5



	A	B	C
		Sucrose	500

- 5 For every  2 L of culture, add  20 mL of ice-cold 1X TES buffer and gently rotate or mix for at least  02:00:00 . After mix  30 mL of ice-cold 0.25 X TES buffer was added to generate the osmotic shock. Mixture was rotated and mixed for another  02:00:00 . The periplasmic shocked mixture was recovered by centrifuging at  20000 x g, 10°C, 00:30:00 . The supernatant contains the periplasmic extract. Take a 1 mL sample. The pellet can be saved. Both fractions can be stored at  -20 °C .

5h



- 6 Confirm expression by running an SDS-PAGE gel.

1h

Recommended recipe to prepare SDS-PAGE samples. Mix 1:4, 4X LDS loading dye and 8 M Urea, to make a Urea loading dye.

For samples before and after induction with IPTG, calculate the amount of sample needed to prepare. We use the following formula:

$$1/\text{OD}_{600} \times 100 \text{ uL} = \text{volume in uL to centrifuge down } \img alt="centrifuge icon" data-bbox="570 560 590 580"/> 14000 \text{ rpm, } 00:01:00 .$$

Discard the supernatant, Keep the pellet.

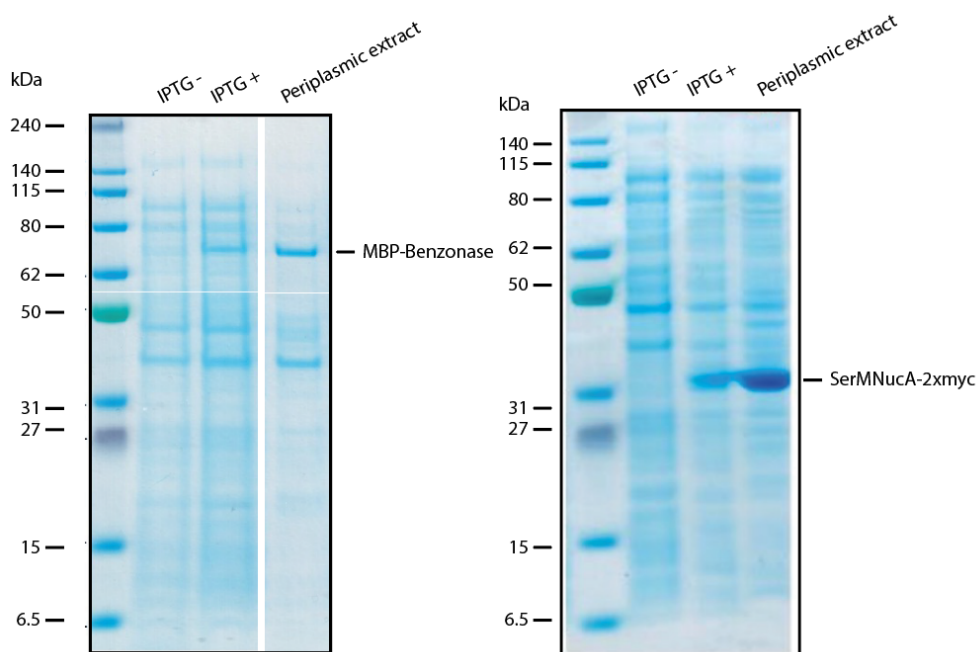
Resuspend pellet in 40 uL of the Urea loading dye.

For the osmotic shock supernatant, mix 30 uL of sample and 10 uL of 4X loading dye

Load 10 uL on to a NuPage 4-12% bis-Tris SDS-PAGE gel.

Expected result

Typical expression pattern and periplasmic extracts are shown here



Prepare purification buffers

- 7 From stock solutions prepare the following as needed :
Filter 0.22 or 0.45 μ m

	A	B	C	D	E
	<i>Buffer</i>	<i>Component</i>	<i>Concentration (mM)</i>		
	Wash buffer (1 L)	NaCl	700		
		HEPES, pH 7.5	20		



	A	B	C	D	E
	Dialysis buffer	NaCl	100		
		TRIS, pH 8	100		
		MgCl ₂	10		
	<i>If performing Ni-NTA</i>				
	Ni-NTA Elution buffer (100 mL)	NaCl	700		
		HEPES, pH 7.5	20		
		Imidazole, pH 7.5	500		
	<i>If performing Amylose</i>				
	Amylose Elution buffer (100 mL)	NaCl	700		
		HEPES, pH 7.5	20		
		Maltose	10		
	<i>If performing anion-exchange</i>				
	Q-Low buffer (1 L)	HEPES, pH 7.5	20		
	Q-High (1 L)	NaCl	1000		
		HEPES, pH 7.5	20		

Buffer lists for purification



Option 1 : Purification by Ni-NTA (10xHis construct)

2h 40m

- 8 Defrost the pellet, and resuspend in  40 mL of wash buffer and glycerol to 10% v/v. Cells are lysed by sonication and clarified by  20000 x g, 4°C, 00:30:00 . Filter the supernatant with a 0.45 um membrane and supplement with imidazole to [IM] 25 Molarity (M) .

40m

Defrost the periplasmic extract and supplement with imidazole to [IM] 25 Molarity (M) .

- 9 Add  5 mL of settled NiNTA resin already equilibrated in wash buffer to the supernatant (SN) and the periplasmic extract. Mix the resin gently for  02:00:00 at  4 °C

2h

- 10 Transfer the resin to a column and collect the flowthrough (FT). The resin is then washed and the target protein eluted as follows:

1h

	A	B	C
	Step	Concentration imidazole (mM) / %Elution buffer	Volume (mL)
	Wash 1	20 mM / 4%	4 × 25 mL
	Wash 2	50 mM / 10%	4 × 12.5 mL
	Wash 3	100 mM / 20%	2 × 12.5 mL
	Elute 1	300 mM / 60%	5 × 10 mL
	Elute 2	500 mM / 100%	1 × 15 mL

Wash and Elute buffers are made by mixing proportionally from the stock Wash and Elution buffers

- 11 Fractions from each step are run on a gel and the purest fractions are pooled. A typical purification is as follows.

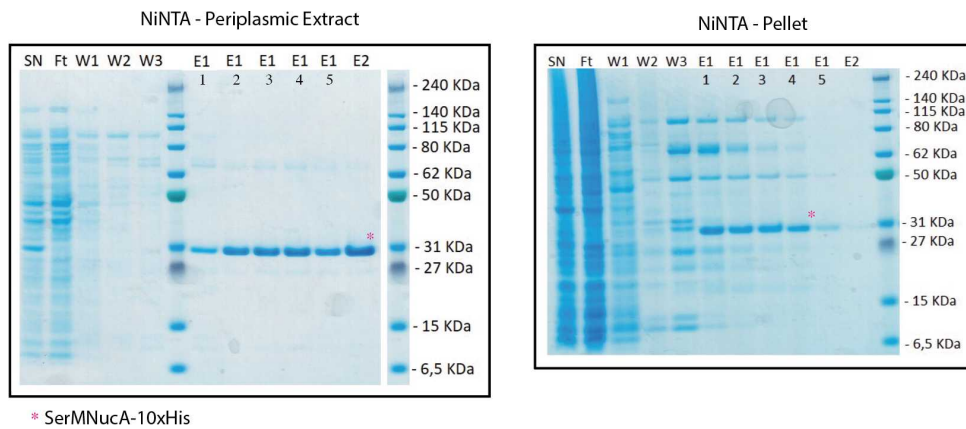
40m



Fractions containing the target protein at a desired purity can be pooled and dialyzed into dialysis buffer overnight at 4 °C .

Expected result

Typical SDS-PAGE gel of a purification from both periplasmic extract and cell pellet.

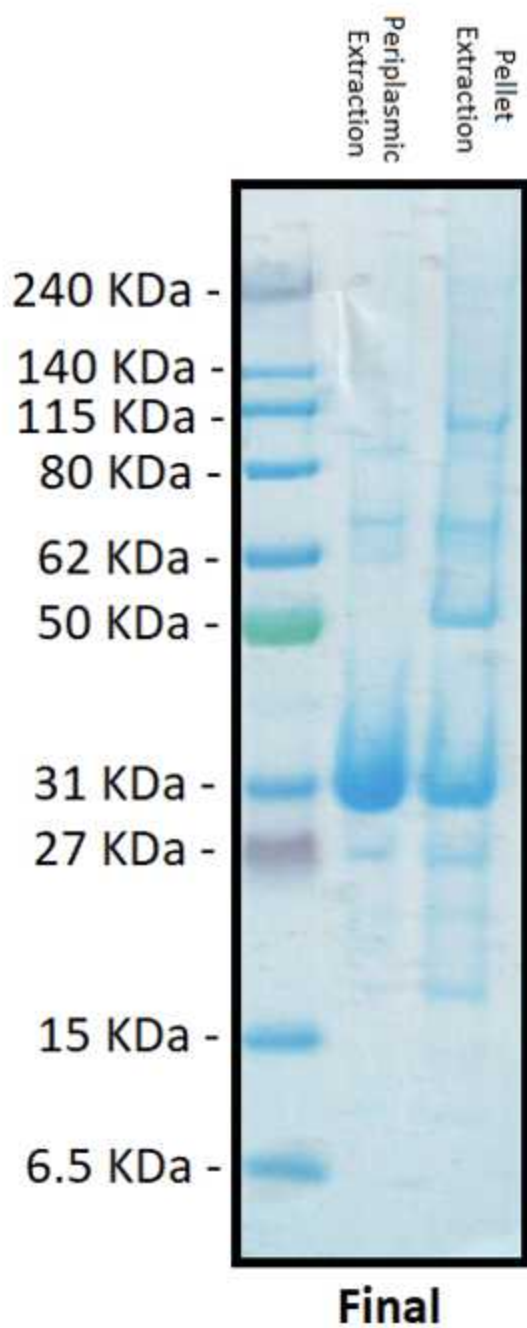


- 12 After dialysis, the sample was concentrated to around 1 mg/mL diluted with glycerol to 50 % (v/v) and run on a gel to verify integrity and purity. The purified SerMNucA-10xHis can be stored at -20 °C .

30m

Expected result

Sample after dialysis and dilution with glycerol



**Note**

- 1) Increased purity can be achieved by adding additional steps. (See anion exchange section or by size-exclusion chromatography)
- 2) Both the periplasmic and cell pellet gives active protein (See activity assay section). However the purified protein from the cell pellet is observed to be forming higher-order oligomeric states. This is most likely mediated by improper disulfides formed within the cytosol. The periplasmic extract does not contain these species.

Option 2 : Purification by Amylose (MBP construct)

4h 10m

- 13 Defrost the pellet, and resuspend in  40 mL of wash buffer and glycerol to 10% v/v. Cells are lysed by sonication and clarified by  20000 x g, 4°C, 00:30:00 . Filter the supernatant with a 0.45 um membrane.
- Defrost the periplasmic extract.
- 14 Add  5 mL of settled amylose resin already equilibrated in wash buffer to the supernatant (SN) and the periplasmic extract. Mix the resin gently for  02:00:00 at  4 °C
- 15 Transfer the resin to a column and collect the flowthrough (FT). The resin is then washed and the target protein eluted as follows:

30m

2h

1h

	A	B	C
	Step	%Elution buffer	Volume (mL)
	Wash	0	4 × 25 mL
	Elute	100	5 × 10 mL



	A	B	C

Wash and Elute buffers are made by mixing proportionally from the stock Wash and Elution buffers

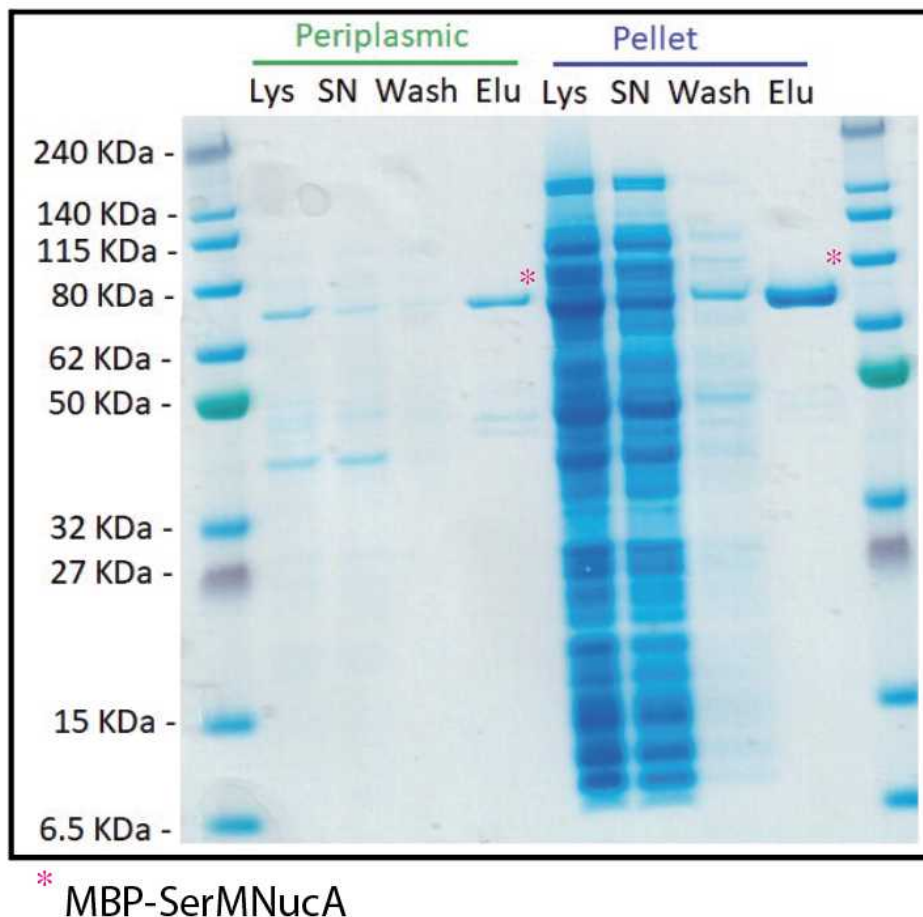
- 16 Fractions from each step are run on a gel and the purest fractions are pooled. A typical purification is as follows.

40m

Fractions containing the target protein at a desired purity can be pooled and dialyzed into dialysis buffer overnight at  4 °C .

Expected result

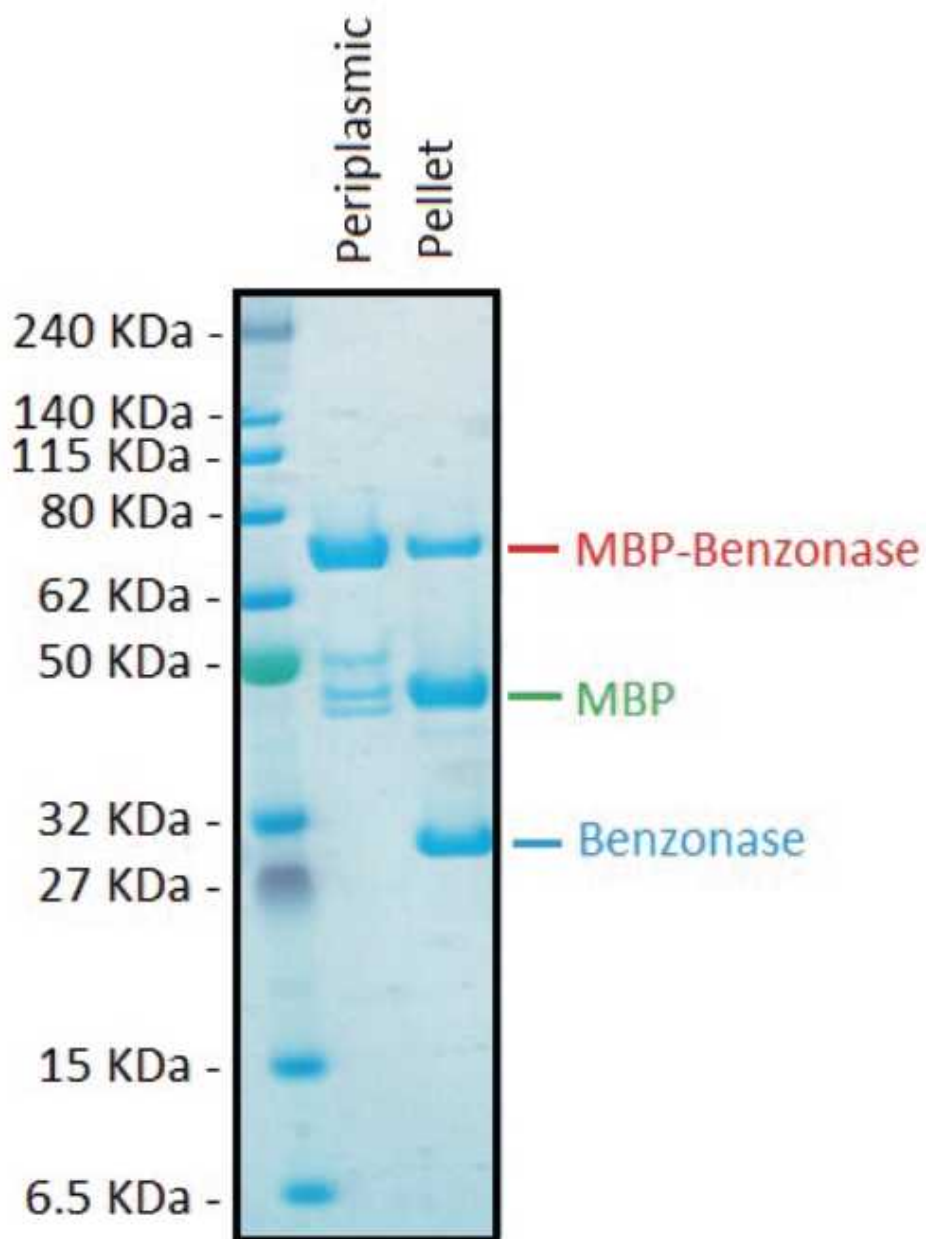
Typical SDS-PAGE gel of a purification from both periplasmic extract and cell pellet.



- 17 After dialysis, the sample was concentrated to around $[M] 1 \text{ mg/mL}$ diluted with glycerol to $[M] 50 \% (v/v)$ and run on a gel to verify integrity and purity. The purified MBP-SerMNucA can be stored at -20°C .

Expected result

Sample after dialysis and dilution with glycerol



Note

1) We observed degradation of the fusion construct. Increased purity can be achieved by adding additional steps. (See anion exchange section or by size-exclusion chromatography).

2) TEV cleavage by TEV protease can also be performed. It should be noted that one should remove the protease if downstream use are with protein samples that contain TEV or 3C protease cleavage sites.

3) Both the periplasmic and cell pellet gives active protein (See activity assay section).

Option 3 : Purification by Anion-Exchange Chromatography (myc tagged)

30m

- 18 Defrost the pellet, and resuspend in  40 mL of Q-Low buffer and glycerol to 10% v/v. Cells are lysed by sonication and clarified by  20000 x g, 4°C, 00:30:00 . Filter the supernatant with a 0.45 um membrane.

30m

Defrost the periplasmic extract.

- 19 Using an AKTA system, load the sample on to a 5 mL HiTrap Q column (5 mL = 1 CV) equilibrated with Q-Low buffer.

Once loaded, run a gradient from 0 to 100% Q-High buffer over 16 CV.

The protein should elute between **20-30 mS/cm of conductivity**

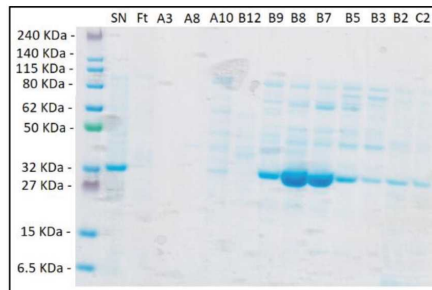
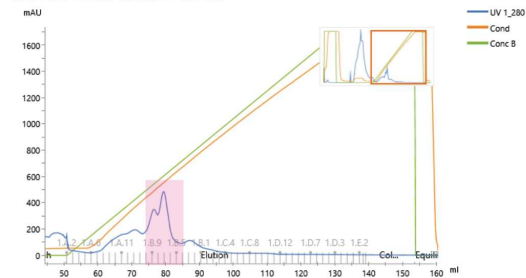
Fractions from each step are run on a gel and the purest fractions are pooled. A typical purification is as follows.

Fractions containing the target protein at a desired purity can be pooled and dialyzed into dialysis buffer overnight at  4 °C .

Expected result

Typical chromatograph and gel of fractions

260623 Untagged SerMNucA Periplasmic Extract 5mL Q HP 0to100percent over 16CV 96 well Outlet1 FT 001 001

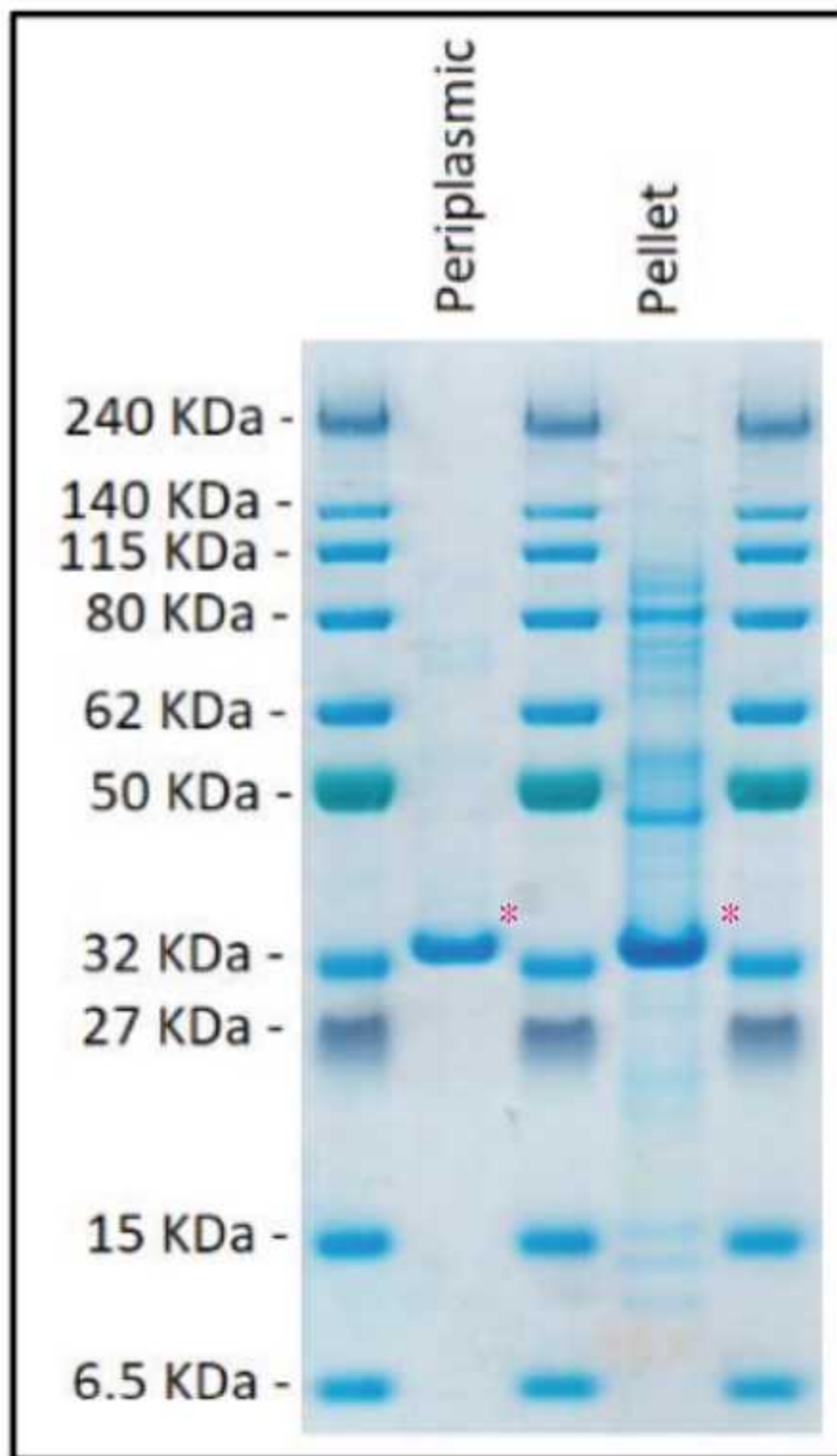


- 20 After dialysis, the sample was concentrated to around [M] 1 mg/mL diluted with glycerol to [M] 50 % (v/v) and run on a gel to verify integrity and purity. The purified SerMNucA-2xmyc can be stored at -20°C .



Expected result

Sample after dialysis and dilution with glycerol



* SerMNucA-2xmyc



Note

1) The purification from the pellet is not shown. What is shown in the final sample was an anion exchange step as for the periplasmic fraction, followed by a size exclusion on fractions that were enriched with the band of correct size.

2) Both the periplasmic and cell pellet gives active protein (See activity assay section).

Activity Assay

21

- 1) Prepare Assay buffer (10 mL) : 50 mM TRIS, pH 8, 1 mM MgCl₂
- 2) Dilute Salmon testes DNA to 2 mg/mL in Assay Buffer