SRF-Bio-ASAP CVNSP3A-c004 expression and purification round 2

PAGE23-01317

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Experiment Started:
Projects: **Expression**;**Purification**;**ASAP**
Related Pages: **PAGE23-01211**
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Tags: **ASAP**

Changed expression protocol

Used Mike's autoinduction protocol
(Turns out we've been using different AIM media - this round I used Formedium TB AIM with trace metal, LACTOSE AND GLUCOSE. Autoclaved, 10mL glycerol/L added after autoclave.
Mike uses Formedium TB AIM with trace metal but NO lactorse or glucose, then add 50x AIM mix: 400mL glycerol, 100g lactose, 25g glucose in 1L. There is also commercial trace metal mix for adding into regular TB).
Do this for future AIM protocols cuz adding glucose after autoclave eliminates glucose loss via maillard reaction.

Asked Mike - he did purification in HEPES and SEC in Tris

Expression ID: CVNSP3A-e003
Puridication ID: CVNSP3A-p003

IMAC (round 1)

Base buffer - 10mM HEPES pH7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP
Lysis buffer - Base buffer + 30mM imidazole
Wash buffer - same as lysis buffer
Elution Buffer (IMAC) - base buffer + 500mM imidazole
Gel Filtration Buffer (SEC) **-** 20mM Tris-HCl, 150mM NaCl, 5% glycerol, 1mM TCEP, pH8 at RT

Cell Lysis

1. Add lysis buffer to pellet until total volume is 250mL. Supplement with 1:4000 dilution of benzonase, 0.5mg/mL lysozyme. Mix lysis mixture until homogenous.
2. Sonicated in cold room at 35% amplitude for a total of 10-minute sonication time (2 seconds on 4 seconds off) with thick probe
3. Clarified lysate by centrifugation at 40,000xg , 4°C for 1 hour.

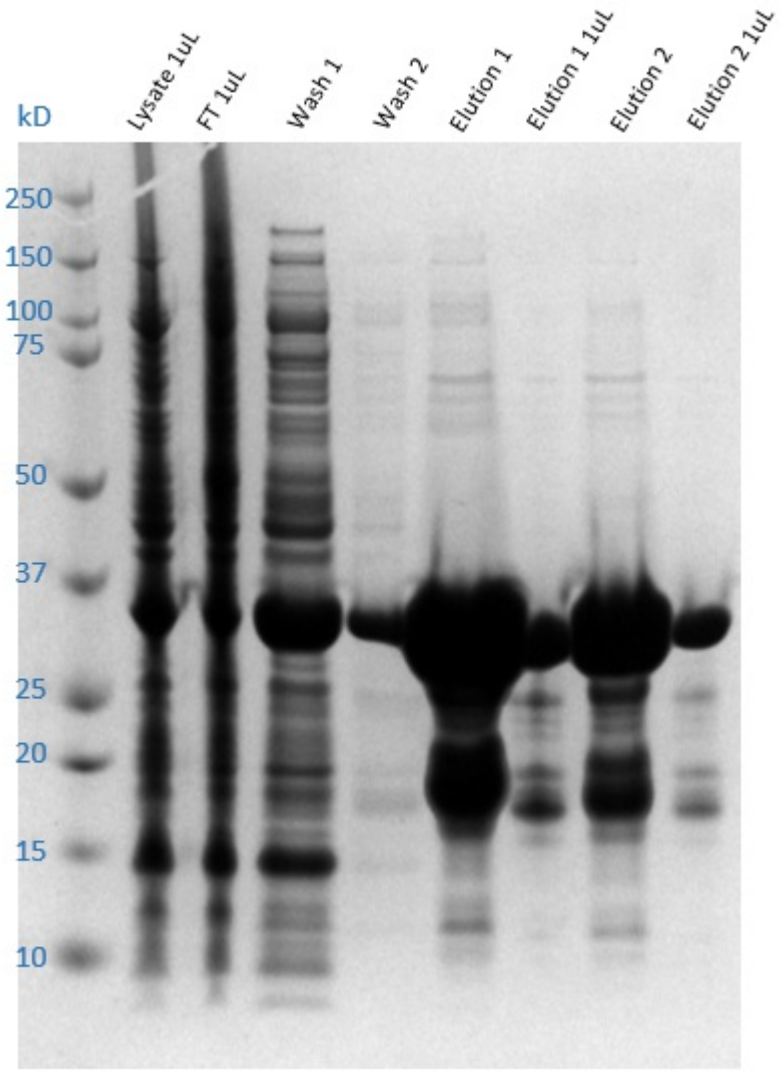
IMAC

1. Wash and equlibrate 1mL bed volume of Ni Sepharose 6 FastFlow resin (regenerated) on gravity flow column, first with distilled water, then with base buffer.
2. Pour supernatant directly onto column containing equilibrated resin. Allow to flow through at ~0.5mL/min (slow drip).
3. Wash resin with 100mL wash buffer twice
4. Elute with 5mL elution buffer, 10min incubation. 2 elutions carried out.

Desalting

Both elutions are de-salted into lysis buffer with PD-10. Nanodrop = 3.19mg/mL.
WT His-SENP1 added 1:100, incubated in cold room overnight.

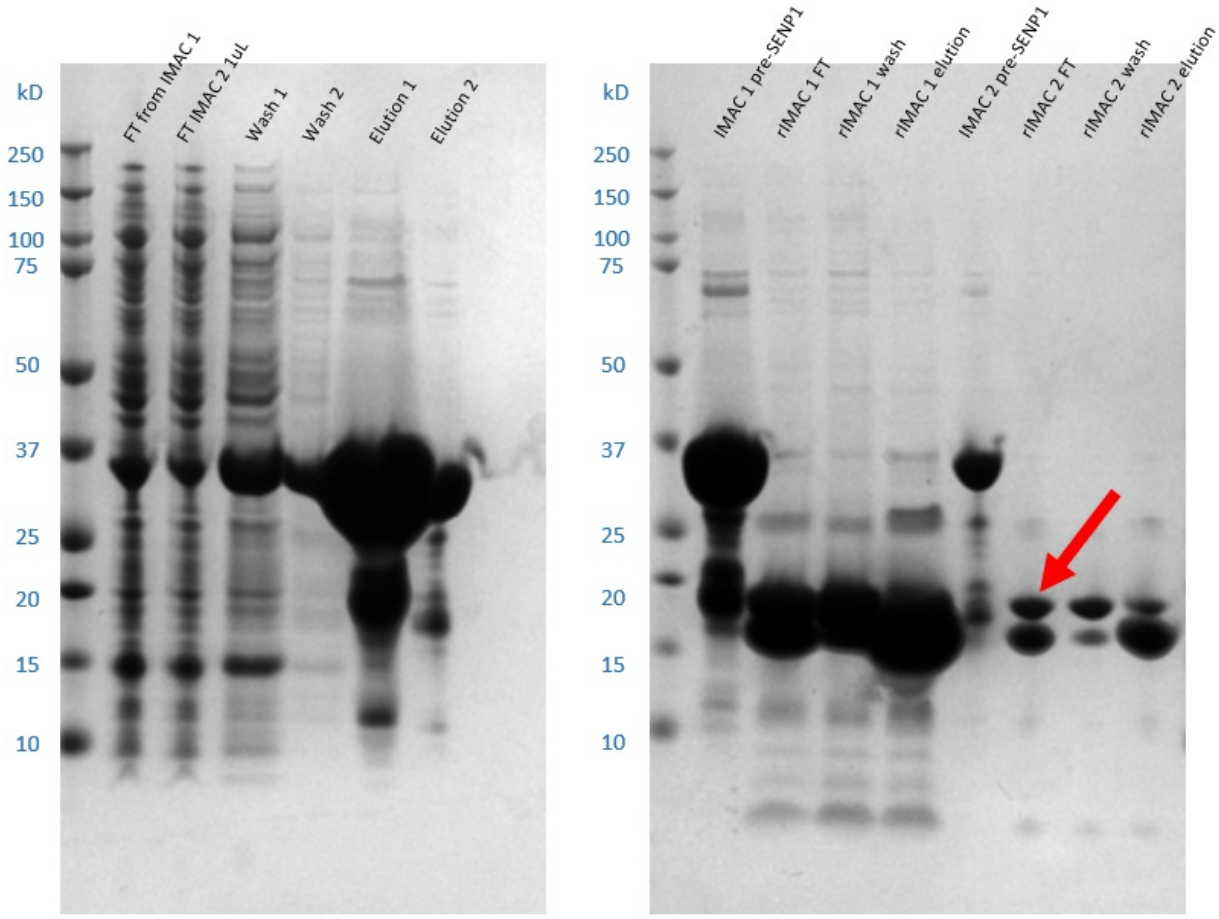
IMAC round 1 result



IMAC (round 2)

Significant amount of target protein still present in FT and washes.
FT, wash 1 and 2 pooled together. Passed over 5mL fresh Ni Seph resin at slow drip rate.
Same IMAC procedure carried out otherwise. Elute 1x10mL with elution buffer.
Also de-salted and SENP1 added.
Nanodrop after desalting = 5.23 mg/mL

IMAC round 2 and cleavage



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rIMAC and SEC

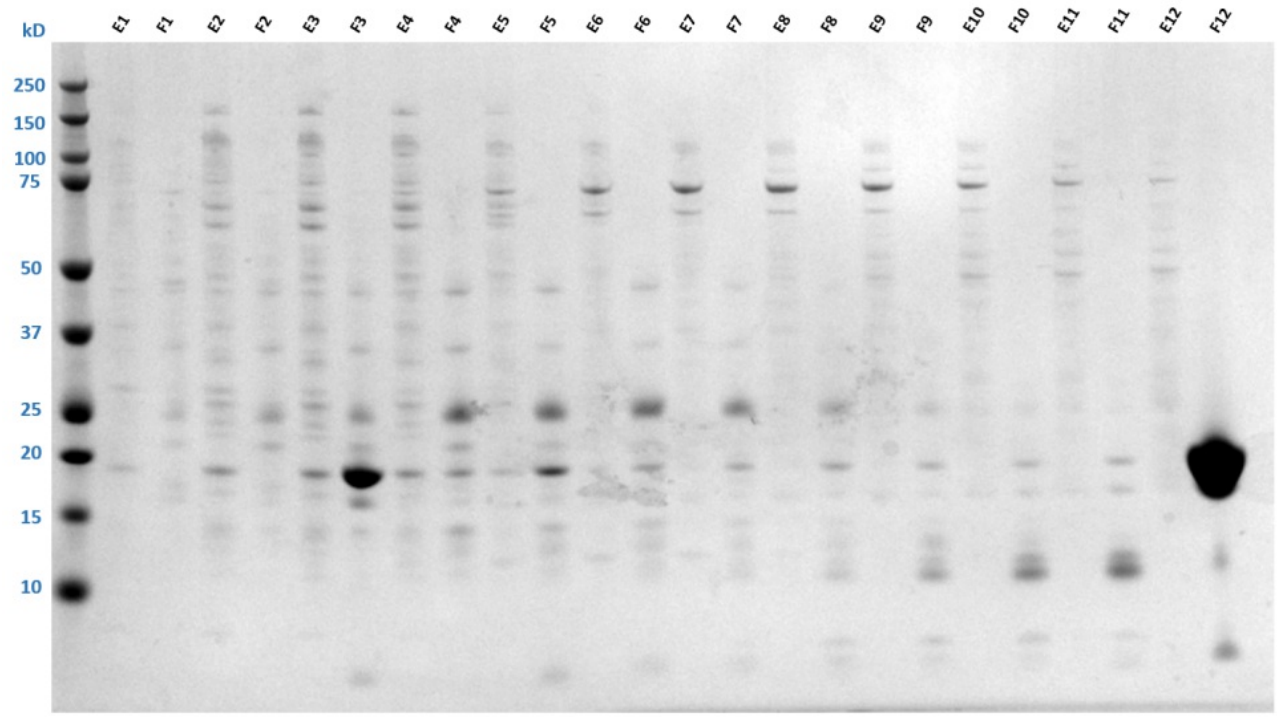
Both overnight samples were passed 3x through the original Ni column (washed with 1cv of wash buffer)
FT was collected and pooled for concentration with 10kDa MWCO Amicon concentrators
rIMAC resin was eluted to check what bound on the resin.

FT concentrated to ~3mL and injected onto Superdex s75 16/60 column. Run at 1mL/min

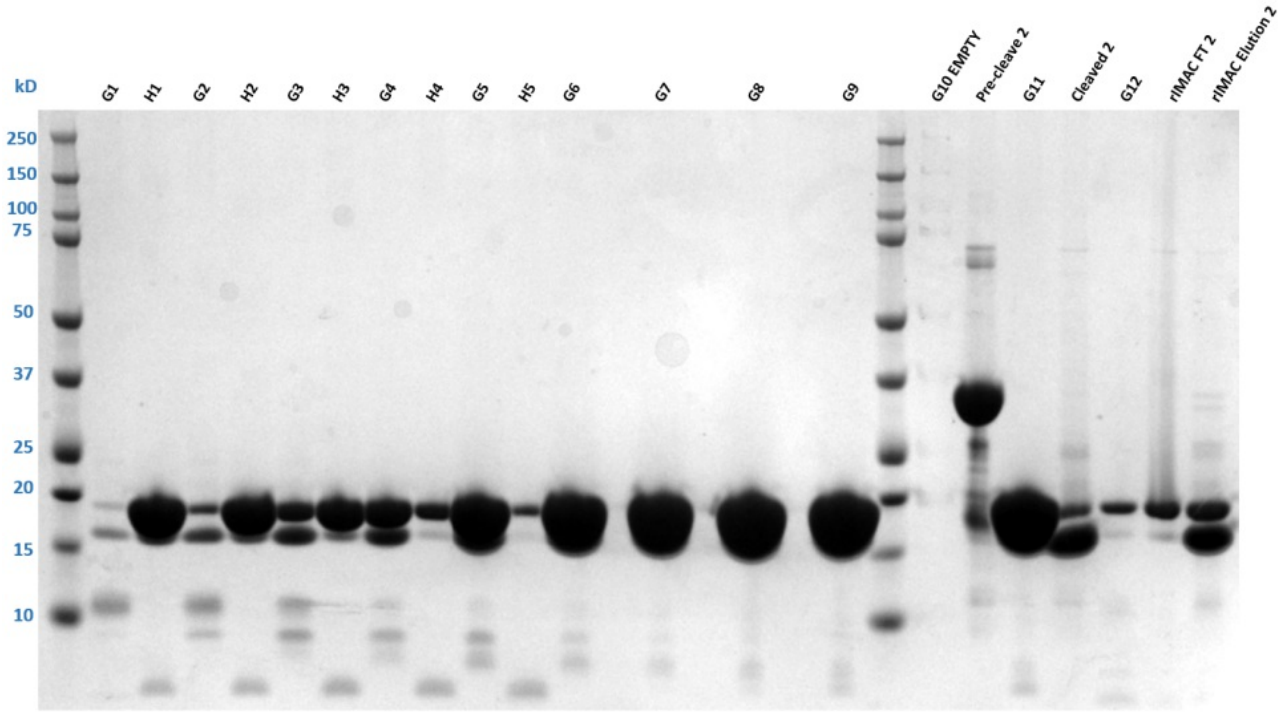
FRACTION COLLECTOR MALFUNCTION: liquid fispensed from either side of arm rather than from the nozzle. This caused the sample to go into adjacent wells and got mixed up.

Ran fraction E1-H5.

SEC faulty frac collector 1



SEC faulty frac collector 2 and round 2 rIMAC

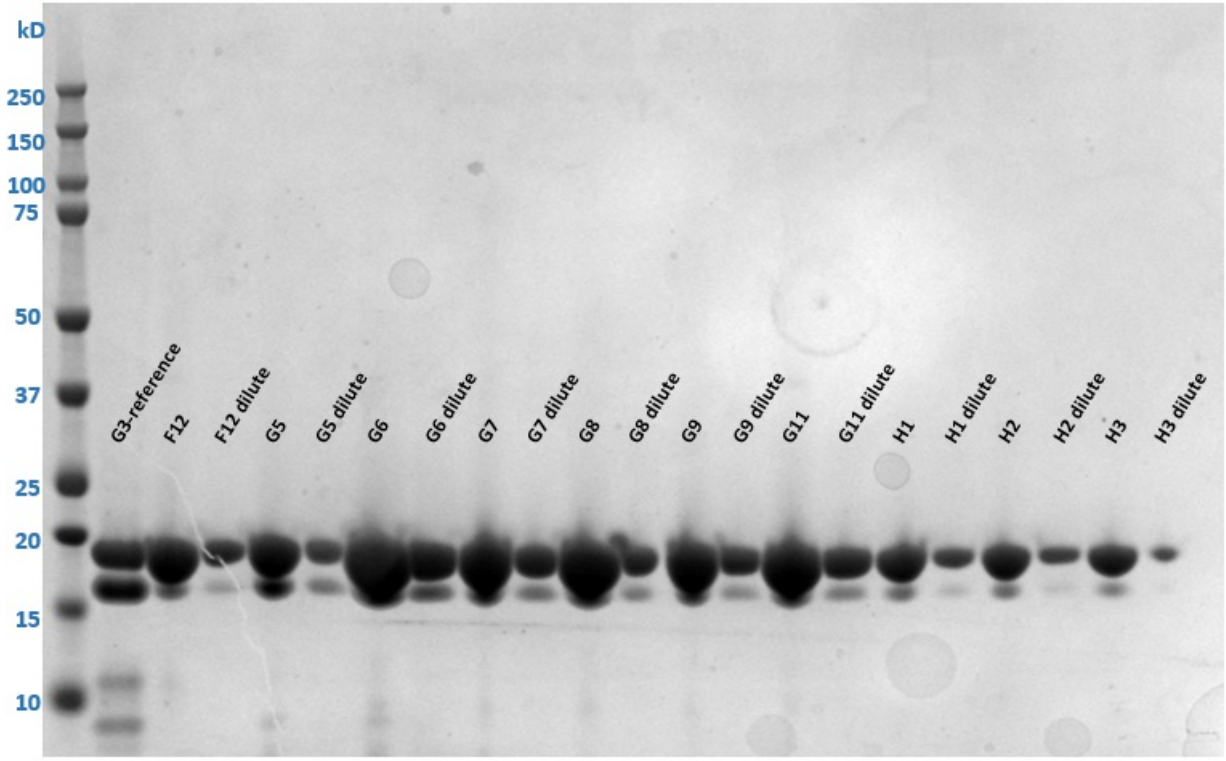


re-run

Some of the fractions were too concentrated to tell if there is double band present:
F12, G5, G6, G7, G8, G9, G11, H1, H2, H3
(fraction G10 was empty)

Run 1uL of boiled sample.
Dilute 1uL boiled sample in 4uL fresh LPS buffer. Load 1uL.
Use G3 as reference for the location of the two bands (higher is target protein, lower is SUMO)

Rerun



Fraction pooling, additional rIMAC, and final concentration

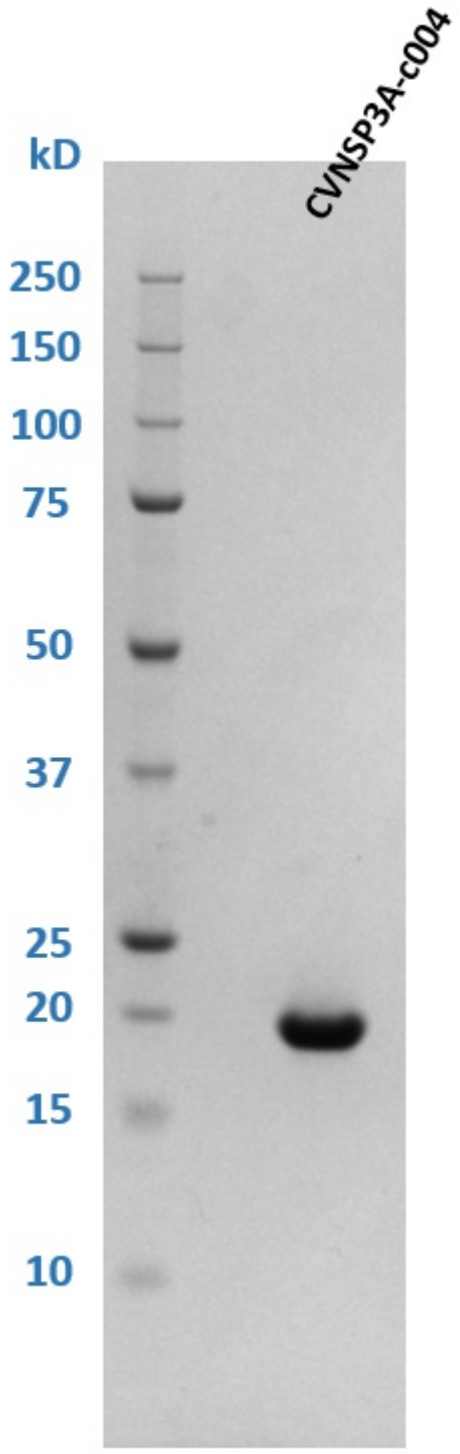
The following fractions were pooled:
F12, G5, G6, G7, G8, G9, G11, G12, H1, H2, H3, H4, H5

Concentrated in Amicon 10kDa MWCO concentrators.

Final sample: 56x50uL + 40uL extra, 21.3mg/mL
50uL ones in blue and extra is in pink tube
+15mL SEC buffer

60.5mg total yield

Final Sample



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16/8/2023 shipped 15 aliquots to Jasmin from Diamond, along with 15mL buffer