SRF-Bio-ASAP XX01ZVNS2B-c001 expression and purification

PAGE23-01623

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Date Started: **2023-Oct-02**
Experiment Started:
Projects: **Expression**;**Purification**;**ASAP**
Related Pages: **PAGE22-01775**;**PAGE23-00292**;**PAGE23-01401**
Referenced by:
Tags: **ASAP**

Info

XX01ZVNS2B-c001

MHHHHHHSSMSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI
ADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDA
LDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSGGGSENLYFQ//SMGKSVDMYIE
RAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEE

WITH TAG:
MW:32812.54
E(red): 51340
PI: 5.47

WITHOUT TAG
NS2B
SMGKSVDMYIERAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEE
MW: 5063.51
E(red): 6990
PI: 3.98
NS3
MAPKEVKKGETTDGVYRVMTRRLLGSTQVGVGVMQEGVFHTMWHVTKGAALRSGEGRLDPYWGDVKQDLVSYCGPWKLDAAWDGLSEVQL
LAVPPGERAKNIQTLPGIFKTKDGDIGAVALDYPAGTSGSPILDKCGRVIGLYGNGVVIKNGSYVSAITQGKREEETPVE
MW: 18095.66
E(red) 30940
NS2B+NS3
MW: 23309.34
E(red): 37930

expect to see three bands after cleavage and rIMAC: ~25kDa, ~18kDa and ~12kDa
Because of coexpression

Previously grown in regular TB, induced at ~OD2 with 5mM IPTG (that's a lot), 18oC overnight expression
33g total pellet wet weight from 2L culture
Purified in standard HEPES buffer. (cleave in 20mM imidazole)
Final buffer: 50 mM HEPEs, pH 7.5, 500 mM NaCl, 20 mM Imidazole, pH 7.0, 5% glycerol, 1 mM TCEP

Clone 6 GST in Mike's page

Expression ID: XX01ZVNS2B-e004
Purification ID: XX01ZVNS2B-p004

Expression

2 Oct 2023
10mL ON culture:
RR3 Glycerol stock from ASAP-1 H06 inoculated in 10mL of 2xLB, grown 37oC 220rpm overnight

3 Oct 2023
Add 1mL of ON culture into 100mL 2xLB+Kan, grow 37oC 220rpm overnight

4 Oct 2023
Add 10mL each into 2x1L TB media (16mL 100% glycerol added after autoclave) + Kan
Grow at 37oC, 250rpm until OD~2
Induce expression with 5mM IPTG, 18oC, 200rpm overnight.

Harvested next day. split into 2x3L: 48 and 51g.

Purification

Buffers:
Base buffer - 50 mM HEPES pH7.5, 500 mM NaCl, 5% glycerol, **1mM TCEP**
Wash buffer 1 - 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, **1mM TCEP,** 30mM imidazole
Wash buffer 2 - 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, **1mM TCEP**, 50mM imidazole
Elution Buffer - 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, **1mM TCEP**, 500mM imidazole
TEV cleavage buffer - 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, **1mM TCEP**, 20mM imidazole
Gel Filtration Buffer (SEC) - 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, **1mM TCEP**, 20mM imidazole

2L pellet thawed and homogenised with lysis buffer to 250mL, supplemented with 1% TX-100, 0.5mg/mL HEWL, 1:4000 benzonase, 20mM imidazole.
Sonicate 40% 10 min 2s on 4s off over ice

Pour clarified supernatant over 3mL Ni Sepharose 6 FF

Wash 50mL 30imi, 50mL 50imi, elute 2x7.5mL 500imi

(used 1OD=1mg/mL)
E1: 18.28 mg/mL
E2: 13.02 mg/mL

after desalt: 9.16 mg/mL, 13mL
STICKY PROTEIN?

ACCIDENTALLY ADDED SENP1 INSTEAD OF TEV

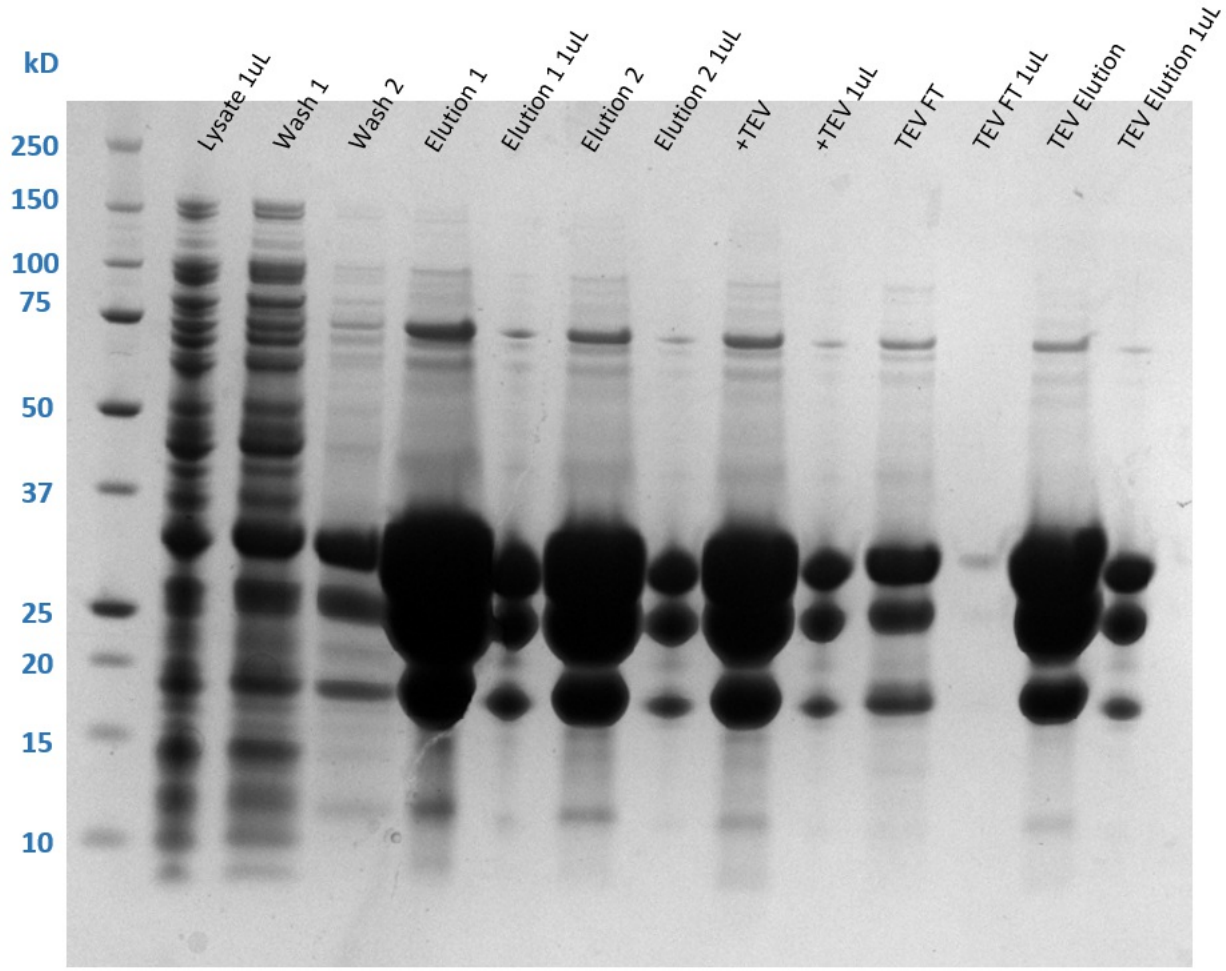
poured over rinsed IMAC resin 2 times, FT collection. Elution with 5mL elution buffer collected.

FT concentrated to ~3mL with 10kDa amicon concentrators and loaded onto s75 column.

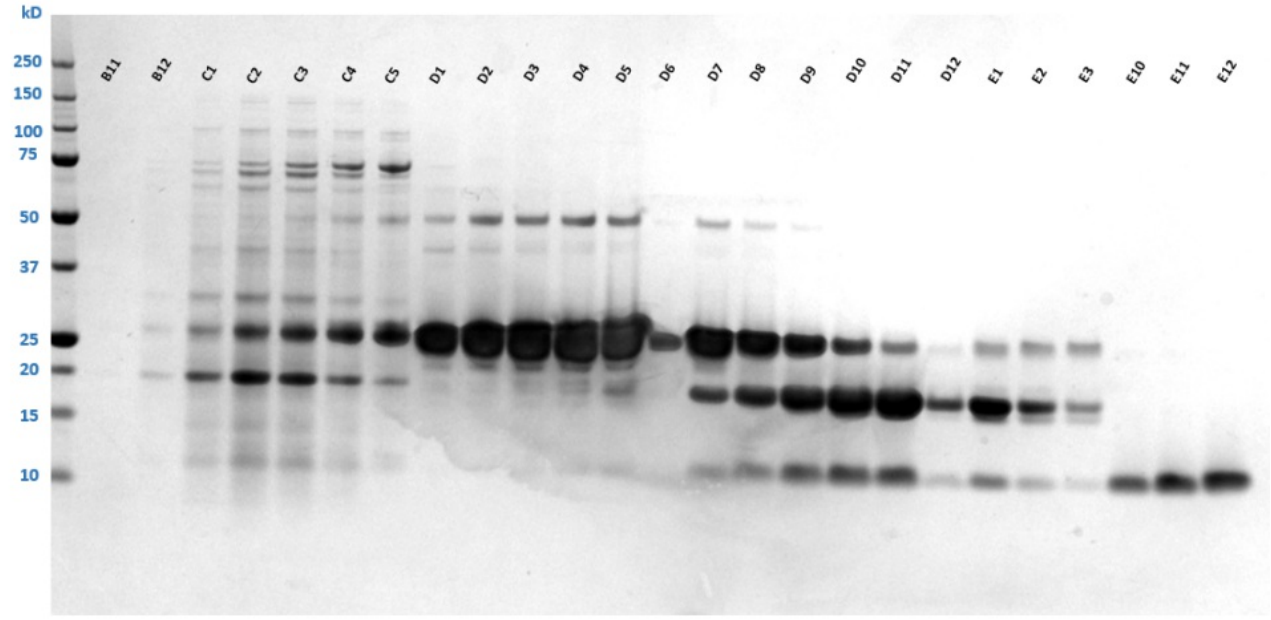
To rescue: pool all protein- containing fractions from s75
rIMAC elution (5mL) diluted to 50mL to reduce imidazole concentration to 50mM. Add to the s75 fractions.
TEV added 1:100. Incubated overnight in cold room.

the next day: concentrated to ~3mL and injected to s75 again

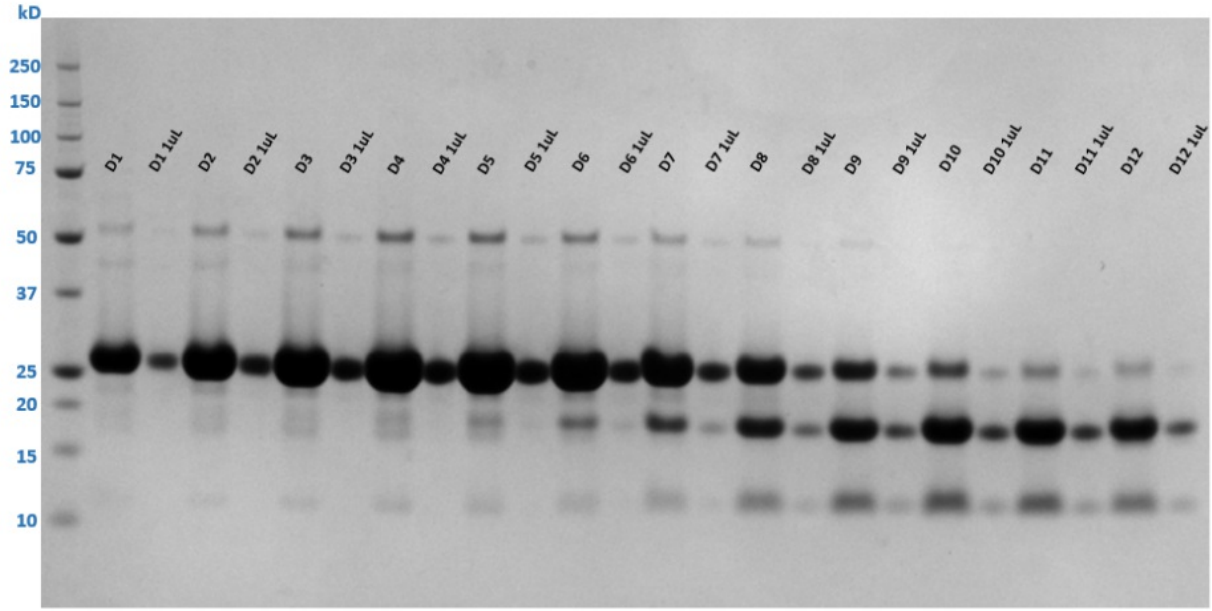
IMAC result



Second round TEV - SEC result



SEC rerun with more DTT and longer boiling

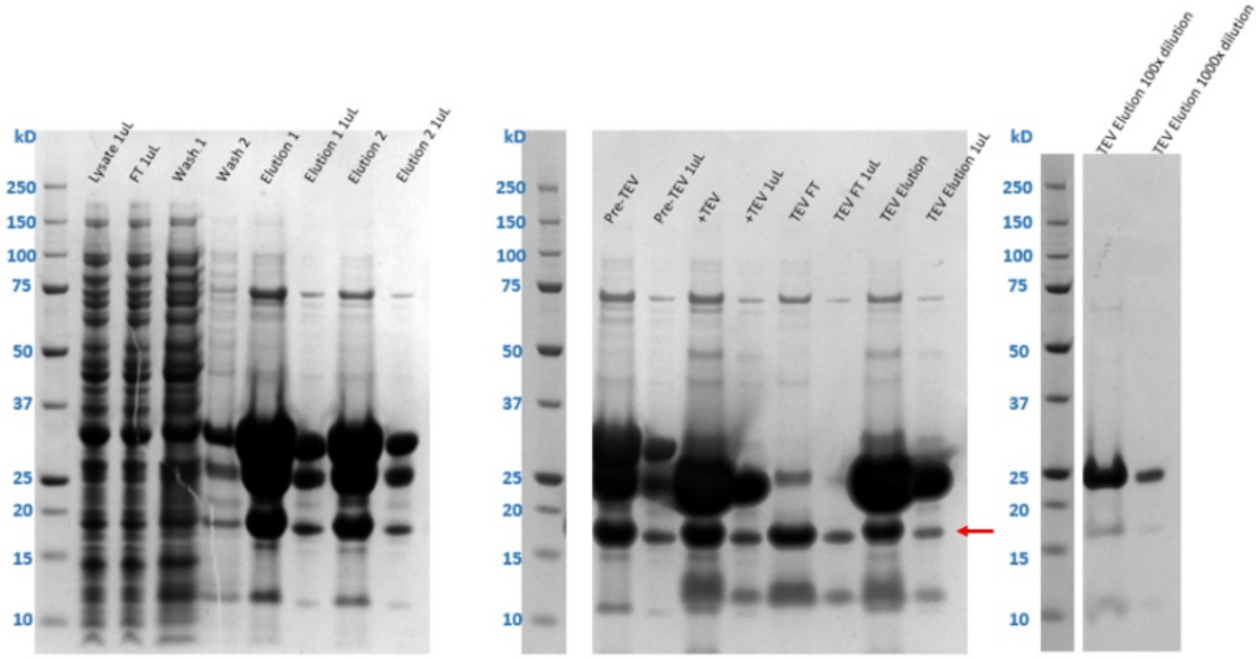


Batch 2

Second 3L also processed with following modification:
- rIMAC is carried out by pouring cleavage mix over the used resin (washed) 3x

This helped clean up the FT from TEV and uncleaved products significantly.
The protein seems to stick to the resin?
A280 decreased by almost 1/5 this time as well
Bands corresponding to cleaved protein also appear in TEV elution (500imi) and is quite dark.

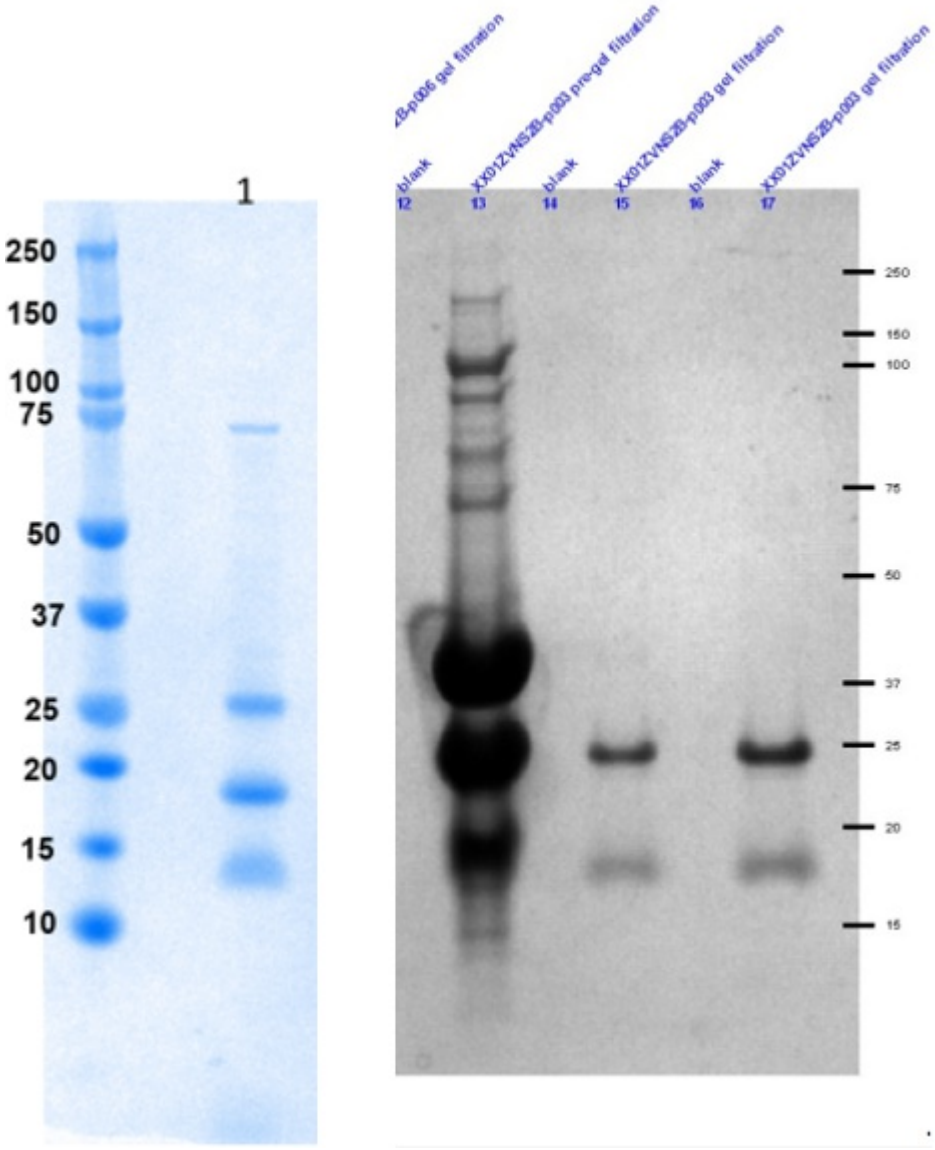
Batch 2 IMAC and cleavage results



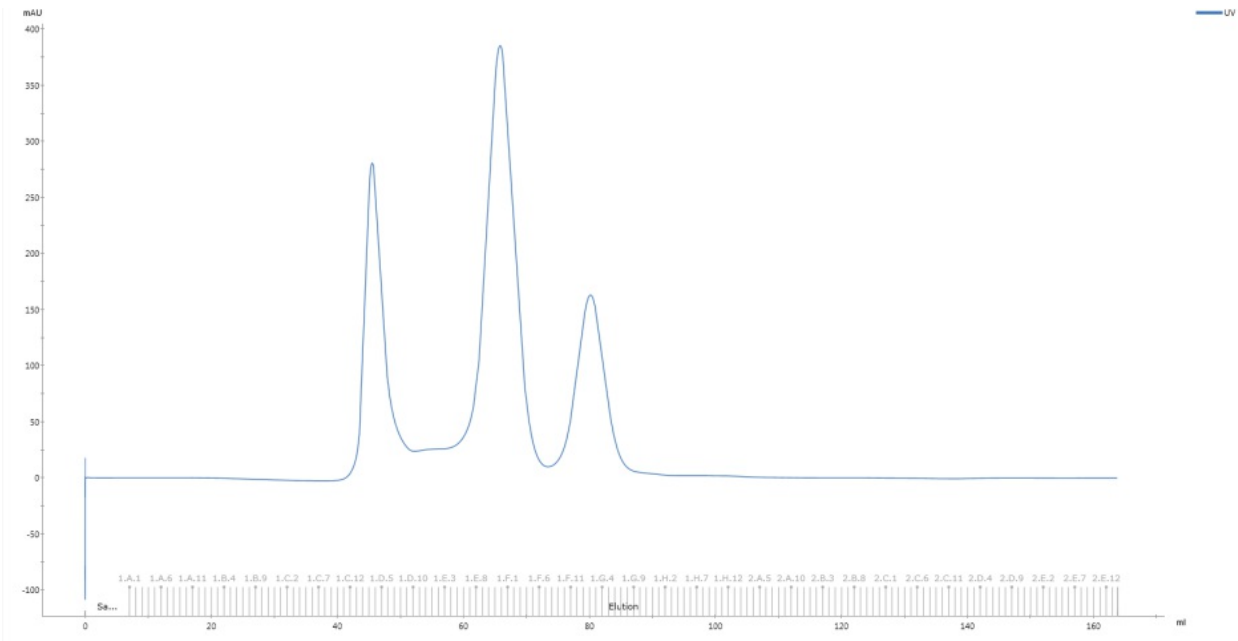
Batch 2 IMAC and cleavage results arrow

Red arrow = cleaved NS3

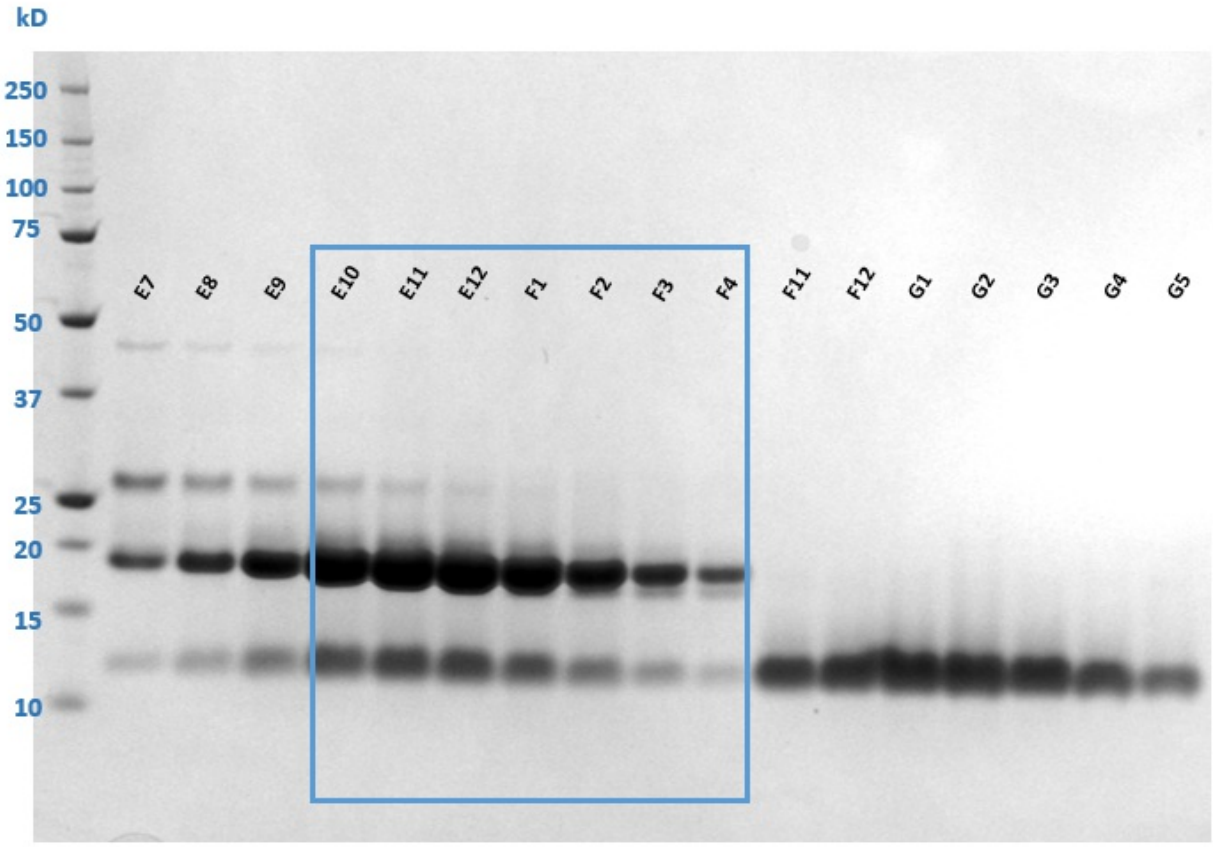
Comparison with Mike and Mel's previous purifications



Chromatogram



Batch 2 SEC



Concentration and final sample

Fraction D12-E1 and fraction E10-F4 from SEC 2 were pooled and concentrated in 10kDa MWCO amicon concentrators, then Vivaspin 500 10kDa concentrators.

Final concentration is 42.25 mg/mL, 10x10uL aliquots.
Yield = 4.23 mg