Jacob McAuley
Date Performed: May 14, 2019

RAB1A Expression and Purification

Rationale:

As a result of a BioID experiment done to identify possible interaction partners of Huntingtin, RAB1A was flagged. This purification aims to provide a RAB1A sample for future experiments to confirm the possible interaction between these proteins.

Growth:

3 x 2 L LB media inoculated with *E. coli* BL21 codon plus RIL transformed with the plasmid detailed in the table, were grown at 37°C in LEX system. The cells were induced at OD_{600}~0.52 with 0.5mM IPTG. Cultures were grown overnight at 18°C.

Construct ID	Vector	Cloned AA Sequence	Mol. Weight	PI
HPC021:A01	pET28a-LIC	MGSSHHHHHHSSGLVPRGSMSSMNPEYDYLFKLLLIGDS GVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKT IKLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQES FNNVKQWLQEIDRYASENVNKLLVGNKCDLTTKKVVDYT TAKEFADSLGIPFLETSAKNATNVEQSFMTMAAEIKKRM GPGATAGGAEKSNVKIQSTPVKQSGGGCC	24.7kDa (including tag)	7.05

Purification:

Collect cells from media culture using centrifugation at 4000 rpm for 10 mins at 4°C in JLA 8.1000. Resuspend cell pellet volume (31.5 mL) in a 1:10 dilution with lysis buffer (20 mM HEPES pH 7.4, 300 mM NaCl, 1 mM TCEP, 2.5% glycerol [v/v]). Add 5 µL of benzonase and 1 mL of protease inhibitors. Lyse cells using sonication and clarify lysate via centrifugation at 16000 rpm for 60 mins at 4°C in JLA16.250. Rock clarified lysate with equilibrated NiNTA (4 mL) for an hour at 4°C. Benchtop centrifuge the samples at 1000 rpm for 10 mins at 4°C to collect beads at the bottom of the container, collect supernatant (flow through - FT). Resin washed in open column (BioRad) with buffer + 20 mM Imidazole (wash - W). Elute beads with buffer + 300 mM Imidazole (elution - E). Concentrate elution to 5 mL with spin concentrator (MWCO 10,000). Load sample into AKTA using Superdex s75 16/60 column and run at 1 ml/min in buffer (20 mM HEPES pH 7.4, 300 mM NaCl, 1 mM TCEP, 2.5% glycerol [v/v]). Fractions purity was assessed by SDS Page. The peak fractions were then collected and concentrated. The concentration was calculated using a nano-drop. Identity was confirmed by mass spectrometry.

Results:

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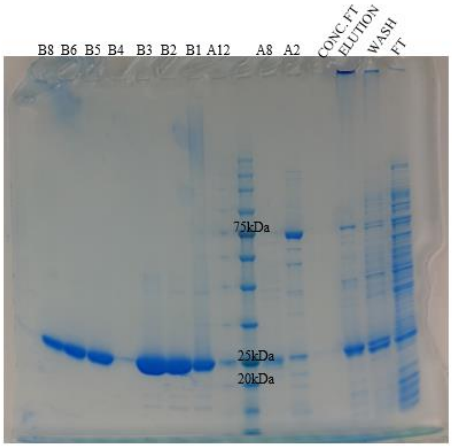

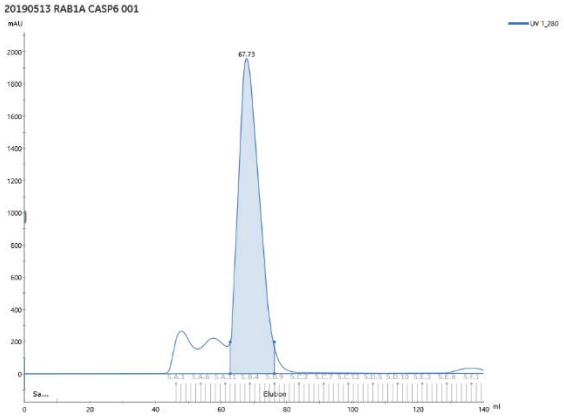

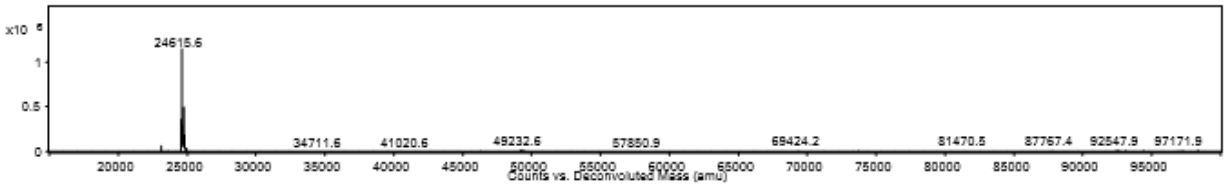

Yield: Final concentration of 110 mg/mL aliquoted into 80 x 10µL and 20 x 20µL for a total yield of 132mg of protein.

Conclusion:

The margin of error on the concentration calculation is quite high as the nano-drop used shows considerable variance in its output. This issue seemed to be accentuated by the concentration of this protein as it was above the capacity of the nano-drop to read accurately. To combat this issue, a 1:20 dilution was tested. The concentration was corrected for abs 0.1% value and dilution factor. Aside from this, the purification of RAB1A was successful. The purity and quantity of protein produced will be more than sufficient for its use in future experiments.