**Purification of full-length huntingtin (HTT) constructs Q17 and Q46 from HEK293 cells (2017/03/23)**

**Aim:** to purify full-length huntingtin and prepare sample for cryo-EM analysis.

**Expression system:** all proteins were expressed in a stable HEK293 tetracycline-inducibile expression system, kindly provided by Stefan Kochanek. All details are available here: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4370734/>

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NB: all cell culture experiments were completed by Dr. Alma Seitova, head of the Eukaryotic Expression Platform team at SGC Toronto and her team. Cell culture methods used, were as those described within the aforementioned publication.

**3rd February 2017**

* 10 x T-160 flasks of each cell line were harvested. Briefly, the media was removed before cells were washed with 5 mL of PBS. Cells were removed from the flask surface by the addition of 1 mL 0.05 % trypsin (<https://www.fishersci.ca/shop/products/gibco-trypsin-edta-0-05-phenol-red-3/p-4919329>) and then washed 2 x with 100 mL PBS before resuspension in 100 mL aliquots in 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 1x PIs.

**21st March 2017**

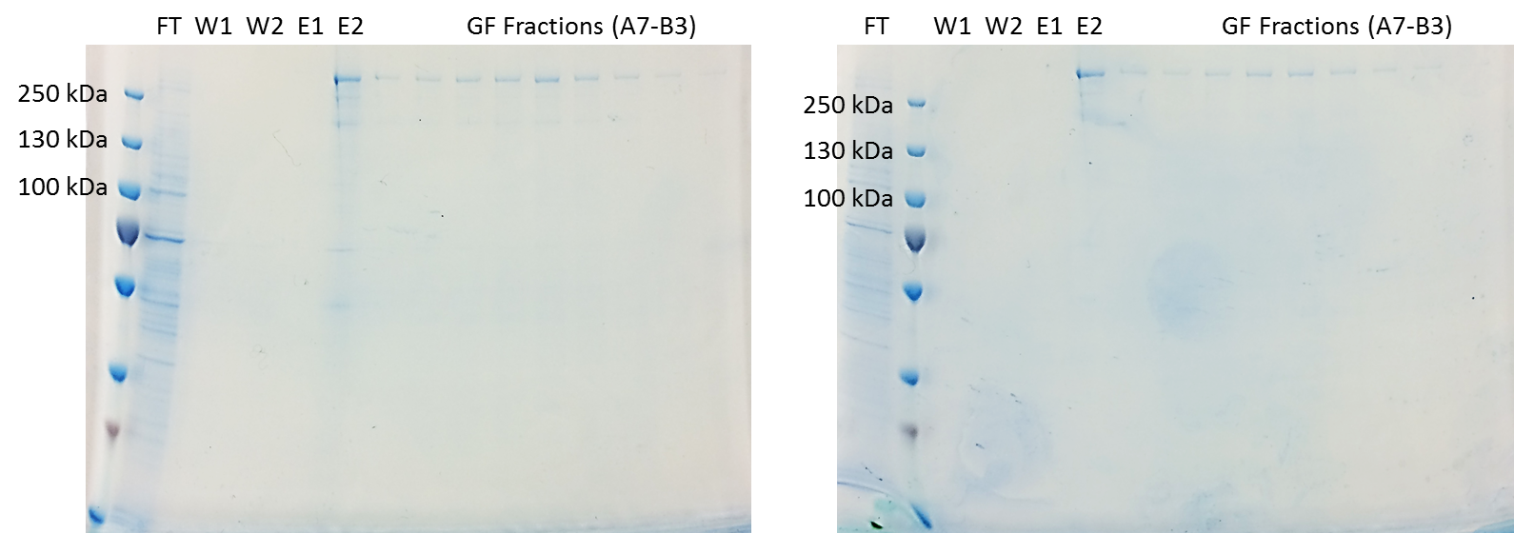
* Cell suspensions of Q17 and Q46 were freeze-thawed x 2 to lyse. Thawed cells resuspended in chilled 250 mL 50 mM Tris-HCl pH8, 500 mM NaCl, 1x PIs with benzonase. Centrifuge at 15,000 rpm, 1 hour, 4 °C for 1 hour (JA 16.25).
* Incubate each clarified lysate each with 2.5 mL equilibrated anti-FLAG M2 resin (Sigma) at 4 °C with rocking for 2 hours in glass bottles.
* Lysate-bead mix loaded into open column (flow through - FT) and then washed with 2 x 200 mL of 50 mM Tris-HCl pH8, 500 mM NaCl (washes 1&2 - W1&W2).
* Huntingtin eluted with 2 x 5 mL 400 µg/mL FLAG peptide in 50 mM Tris-HCl pH8, 500 mM NaCl. Incubate beads with peptide for 5 minutes before eluting (E1). (Bradford assay showed no more protein was eluting).
* Concentrate eluted sample using MWCO 100,000 (E2). Run 1 mL of elution on Superose6 equilibrated in 20 mM Hepes pH7.4, 500 mM NaCl at 0.4 mL/min.

Q17 Q46

Monomer peaks

* Monomer peak of both samples concentrated with MWCO 100,000.
* Q17 final yield was 0.5 mg/mL 7 x 5 μL aliquots, Q46 final yield was 0.4 mg/mL 6 x 5 μL aliquots. Samples flash-frozen in liquid nitrogen and stored at -80 °C.



* Expected yield is 0.5 pg/cell. Each confluent flask ~ 18.4 x 10^6 cells, therefore 10 flasks of each construct ~2 x10^8 cells. Expected total yield ~ 100 μg.
* Actual yields ~15-20 μg. Within expected range of purification.