Full-length HTT purification from HEK293T suspension culture using baculovirus transduction protocol for over-expression 2018/04/02

Rationale:

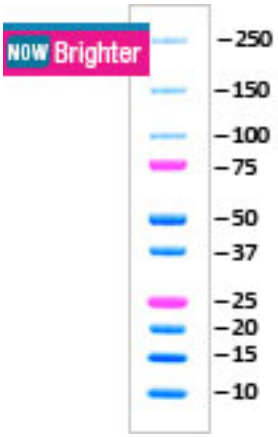
The pBacMam-DiEx-LIC constructs were previously cloned - https://zenodo.org/record/1238914 and used extensively for expression and purification of huntingtin protein from sf9 insect cells e.g. https://zenodo.org/record/1162378. Now, I aim to validate the expression and purification of huntingtin protein from HEK293T suspension culture expressed through baculoviral transduction.

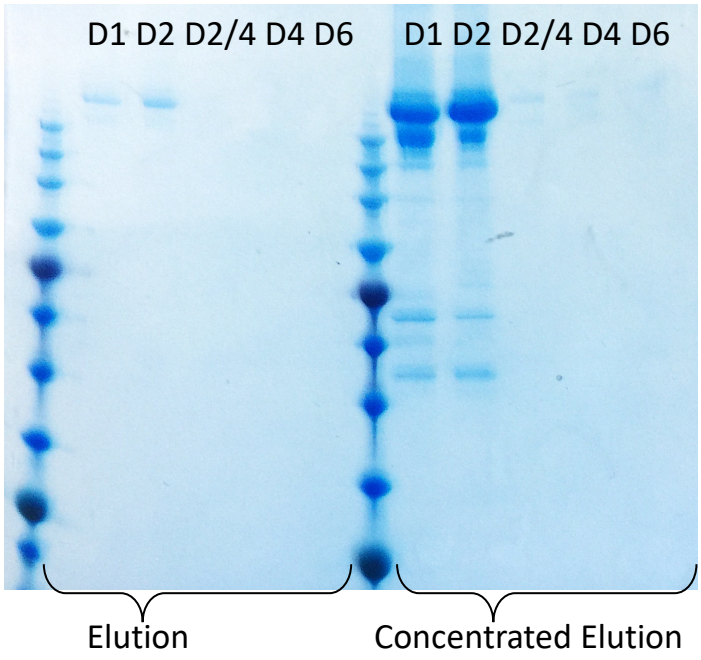
Protocol:

~200 mL HEK293T production for TOC009-D01, D02, (D02/4 – possible construct mix up), D04 and D06 (HTT^{1-3144} pBACMAM C-terminal FLAG – Q23, Q54, Q78 and Q145 respectively) was completed by Alma Seitova using baculoviral transduction to initiate protein expression as per https://zenodo.org/record/1194724. Cells were harvested by centrifugation at 1500 rpm for 30 mins in benchtop centrifuge at 4 °C, resuspended in ~50 mL 50 mM Tris pH 8, 500 mM NaCl supplemented with 1 x protease inhibitors (1 mM PMSF, 1 mM benzamidine-HCl) and then the pastes were stored at -80 ˚C prior to purification.

Cell pastes were thawed and diluted to 250 mL with 50 mM Tris pH 8, 500 mM NaCl and supplemented with 20 µg/mL benzonase. NB: freeze-thaw cycle for cells is sufficient for lysis. The lysate was clarified by centrifugation and then bound to 1 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours. Resin was washed with 500 mL 50 mM Tris pH 8, 500 mM NaCl. HTT protein was eluted with ~10 mL resuspension buffer supplemented with 250 µg/mL 3xFLAG peptide – elution samples. The sample was concentrated to 1 mL – concentrated elution. Samples were run on SDS-PAGE 4-20 % tris-glycine gel of unconcentrated and concentrated FLAG-elution samples.

Biorad prestained ladder.





Conclusions and next steps:

D01 and D02 samples (Q23 and Q54) express well and bands for huntingtin are clearly visible in the elution and concentrated elution fractions analysed by SDS-PAGE. Longer polyQ lengths D04 and D06 (Q78 and Q145) are visible in the concentrated samples but only as faint bands. Next, we will grow large scale productions of the Q23 sample to allow purification of meaningful quantities of huntingtin for analyses.