Large scale expression and purification of huntingtin fragment A667-C3140 from baculoviral expression system production in sf9 insect cells – 2018/08/02

Rationale:

Purified huntingtin samples are required for use in structural and functional studies.

Construct information:

Tag + huntingtin protein sequence:

mdykdddkenlyfqgAPLVHCVRLLSASFLLTGGKNVLVPDRDVRVSVKALALSCVGAAVALHPESFFSKLYKVPLDTTEYPEEQYVSDILNYIDH
GDPQVRGATAILCGTLICSILSRSRFHVGDWMGTIRTLTGNTFSLADCIPLLRKTLKDESSVTCKLACTAVRNCVMSLCSSSYSELGLQLIIDVLTL
RNSSYWLVRTELLETLAEIDFRLVSFLEAKAENLHRGAHHYTGLLKLQERVLNNVVIHLLGDEDPRVRHVAAASLIRLVPKLFYKCDQGQADPV
VAVARDQSSVYLKLLMHETQPPSHFSVSTITRIYRGYNLLPSITDVTMENNLSRVIAAVSHELITSTTRALTFGCCEALCLLSTAFPVCIWSLGWH
CGVPPLSASDESRKSCTVGMATMILTLLSSAWFPLDLSAHQDALILAGNLLAASAPKSLRSSWASEEEANPAATKQEEVVWPALGDRALVPMV
EQLFSHLLKVINICAHVLDDVAPGPAIKAALPSLTNPPSLSPIRRKGKEKEPGEQASVPLSPKKGSEASAASRQSDTSGPVTTSKSSSLGSFYHLPS
YLKLHDVLKATHANYKVTLDLQNSTEKFGGFLRSALDVLSQILELATLQDIGKCVEEILGYLKSCFSREPMMATVCVQQLLKTLFGTNLASQFDG
LSSNPSKSQGRAQRLGSSSVRPGLYHYCFMAPYTHFTQALADASLRNMVQAEQENDTSGWFDVLQKVSTQLKTNLTSVTKNRADKNAIHN
HIRLFEPLVIKALKQYTTTTCVQLQKQVLDLLAQLVQLRVNYCLLDSDQVFIGFVLKQFEYIEVGQFRESEAIIPNIFFFLVLLSYERYHSKQIIGIPKI
IQLCDGIMASGRKAVTHAIPALQPIVHDLFVLRGTNKADAGKELETQKEVVVSMLLRLIQYHQVLEMFILVLQQCHKENEDKWKRLSRQIADII
LPMLAKQQMHIDSHEALGVLNTLFEILAPSSLRPVDMLLRSMFVTPNTMASVSTVQLWISGILAILRVLISQSTEDIVLSRIQELSFSPYLISCTVI
NRLRDGDSTSTLEEHSEGKQIKNLPEETFSRFLLQLVGILLEDIVTKQLKVEMSEQQHTFYCQELGTLLMCLIHIFKSGMFRRITAAATRLFRSDG
CGGSFYTLDSLNLRARSMITTHPALVLLWCQILLLVNHTDYRWWAEVQQTPKRHSLSSTKLLSPQMSGEEEDSDLAAKLGMCNREIVRRGALI
LFCDYVCQNLHDSEHLTWLIVNHIQDLISLSHEPPVQDFISAVHRNSAASGLFIQAIQSRCENLSTPTMLKKTLQCLEGIHLSQSGAVLTLYVDRL
LCTPFRVLARMVDILACRRVEMLLAANLQSSMAQLPMEELNRIQEYLQSSGLAQRHQRLYSLLDRFRLSTMQDSLSPSPPVSSHPLDGDGHV
SLETVSPDKDWYVHLVKSQCWTRSDSALLEGAELVNRIPAEDMNAFMMNSEFNLSLLAPCLSLGMSEISGGQKSALFEAAREVTLARVSGTV
QQLPAVHHVFQPELPAEPAAYWSKLNDLFGDAALYQSLPTLARALAQYLVVVSKLPSHLHLPPEKEKDIVKFVVATLEALSWHLIHEQIPLSLDL
QAGLDCCCLALQLPGLWSVVSSTEFVTHACSLIHCVHFILEAVAVQPGEQLLSPERRTNTPKAISEEEEEVDPNTQNPKYITAACEMVAEMVES
LQSVLALGHKRNSGVPAFLTPLLRNIIISLARLPLVNSYTRVPPLVWKLGWSPKPGGDFGTAFPEIPVEFLQEKEVFKEFIYRINTLGWTSRTQFE
ETWATLLGVLVTQPLVMEQEESPPEEDTERTQINVLAVQAITSLVLSAMTVPVAGNPAVSCLEQQPRNKPLKALDTRFGRKLSIIRGIVEQEIQ
AMVSKRENIATHHLYQAWDPVPSLSPATTGALISHEKLLLQINPERELGSMSYKLGQVSIHSVWLGNSITPLREEEWDEEEEEEADAPAPSSPP
TSPVNSRKHRAGVDIHSCSQFLLELYSRWILPSSSARRTPAILISEVVRSLLVVSDLFTERNQFELMYVTLTELRRVHPSEDEILAQYLVPATCKAA
AVLGMDKAVAEPVSRLLESTLRSSHLPSRVGALHGILYVLECDLLDDTAKQLIPVISDYLLSNLKGIAHCVNIHSQQHVLVMCATAFYLIENYPLD
VGPEFSASIIQMCGVMLSGSEESTPSIIYHCALRGLERLLLSEQLSRLDAESLVKLSVDRVNVHSPHRAMAALGLMLTCMYTGKEKVSPGRTSD
PNPAAPDSESVIVAMERVSVLFDRIRKGFPCEARVVARILPQFLDDFFPPQDIMNKVIGEFLSNQQPYPQFMATVVYKVFQTLHSTGQSSMV
RDWVMLSLSNFTQRAPVAMATWSLSCFFVSASTSPWVAAILPHVISRMGKLEQVDVNLFCLVATDFYRHQIEEELDRRAFQSVLEVVAAPG
SPYHRLLTCLRNVHKVTTC

Construct ID	TOC005-B12
Residues	A677-C3140 (assuming Q19 – template: http://www.kazusa.or.jp/kop/vd/FHC15881/)
Protein MW (Da)	275989
Protein pI	6.06
Abs 0.1% (=1 g/l)	0.913
Tag type	FLAG
Vector	pFB-Nflag-LIC

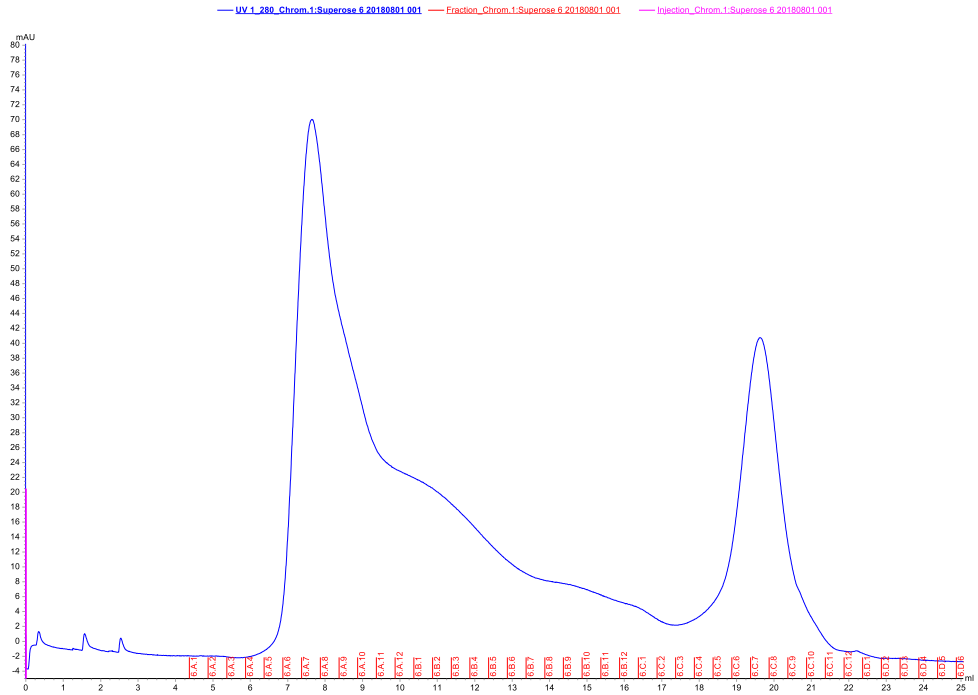
Materials and methods:

Growth: TOC005-B12 (A677-C3140) P3 virus used for 8 L BVES sf9 production. Cells were harvested by centrifugation at 4000 rpm, 10 mins, 4 °C (Beckman JLA 8.1000) on 26th July 2018. HTT cell pellets were resuspended in ~160 mL of 50 mM Tris pH 8, 500 mM NaCl. Cell resuspensions were spiked with protease inhibitor mix and then stored at -80 ˚C prior to purification. Full BVES production methods are here: https://zenodo.org/record/154611

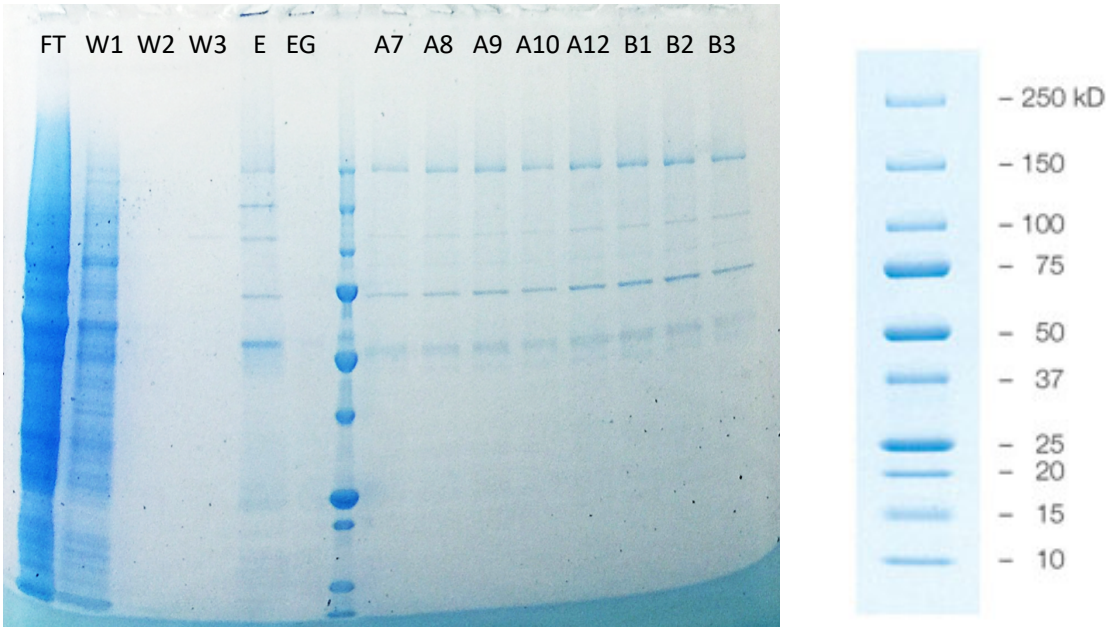
Purification: Cell pastes were thawed and diluted to 500 mL with 50 mM Tris pH 8, 500 mM NaCl and supplemented with benzonase and 1 x protease inhibitors. NB: freeze-thaw cycle for cells is sufficient for lysis. Lysates were clarified by centrifugation at 15,000 rpm, 1 h, 4 °C and then bound to 10 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT). The resin was washed with 3 x 250 mL 50 mM Tris pH 8, 500 mM NaCl per column (wash – W1 and W2). HTT protein was eluted with ~15 mL resuspension buffer supplemented with 200 µg/mL 3xFLAG peptide per

column (elution – E). Samples were concentrated and run as 1 mL injection on Superose 6 10/300 GL column in 20 mM Hepes pH 7.4, 300 mM NaCl, 1 mM TCEP, 5 % (v/v) glycerol. Samples were analysed by 4-20 % tris-glycine SDS-PAGE.

Gel filtration:



SDS-PAGE analysis of samples throughout the purification:



Yield and comments about sample:
Samples not of sufficient quality to use in further experiment. Multiple bands in sample seen by SDS-PAGE suggest degradation of sample or clipping at protease exposed regions of disorder. The gel filtration trace does not show a clear monomer peak (full-length huntingtin monomer elutes ~14 mL on Superose6 10/300 column) indicating that the sample also aggregates and is not stable.