Large scale expression and purification of full-length huntingtin Q23 with HAP40 from baculoviral expression system production in sf9 insect cells – 2018/05/28

Rationale

To validate our insect cell production system for the generation of physiologically relevant huntingtin constructs, HTT and HAP40 will be co-expressed and purified from sf9 culture. Previously the small-scale culture (~100 mL) promisingly indicated 3:1, 4:1 and 5:1 viral titre ratios of HTT:HAP40 all permitted purification of a complex but insufficient material was obtained to proceed beyond the FLAG tag affinity purification step https://zenodo.org/record/1248166. Therefore 4 L of production will be harvested and HTT:HAP40 purified.

Experiments

1. HAP40 and HTT construct clones

Sample ID	HAP40:TOC011-C01:C241911	huntingtin:TOC009-D01:C240774
Construct PKEY	83963	82809
Clone pkey	241911	240774
Construct ID	HAP40_1-371	BacMam_Cflag_huntingtin_Q23
Cloned AA Seq	MHHHHHHSSGRENLYFQGMA AAAAGLGGGGAGPGPEAGDFL ARYRLVSNKLKKRFLRKPNVAE AGEQFGQLGRELRAQECLPYAA WCQLAVARCQQALFHGPGEAL ALTEAARLFLRQERDARQRLVC PAAYGEPLQAAASALGAAVRLH LELGQPAAAAALCLELAAALRDL GQPAAAAGHFQRAAQLQLPQL PLAALQALGEAASCQLLARDYT GALAVFTRMQRLAREHGSHPV QSLPPPPPPAPQPGPGATPALP AALLPPNSGSAAPSPAALGAFS DVLVRCEVSRVLLLLLLQPPPAKL LPEHAQTLEKYSWEAFDSHGQE SSGQLPEELFLLLQSLVMATHEK DTEAIKSLQVEMWPLLTAEQN HLLHLVLQETISPSGQGV	MATLEKLMKAFESLKSFQQQQQQQQQQQQQQQQQQQQQQQQQPPPPPPPPPPQLPQPPPQAQPLLPQPQPPPPPPPPPPGPAVAEEPLHRPKKELSATKKDRVNH CLTICENIVAQSVRNSPEFQKLLGIAMELFLLCSDDAESDVRMVADECLNKVIKALMDSNLPRLQLELYKEIKKNGAPRSLRAALWRFAELAHLVRPQKCRPYLVNLLPCL TRTSKRPEESVQETLAAAVPKIMASFGNFANDNEIKVLLKAFIANLKSSSPTIRRTAAGSAVSICQHSRRTQYFYSWLLNVLLGLLVPVEDEHSTLLILGVLLTLRYLVPLLQ QQVKDTSLKGSFGVTRKEMEVSPSAEQLVQVYELTLHHTQHQDHNVVTGALELLQQLFRTPPPELLQTLTAVGGIGQLTAAKEESGGRSRSGSIVELIAGGGSSCSPVLS RKQKGKVLLGEEEALEDDSESRSDVSSSALTASVKDEISGELAASSGVSTPGSAGHDIITEQPRSQHTLQADSVDLASCDLTSSATDGDEEDILSHSSSQVSAVPSDPAM DLNDGTQASSPISDSSQTTTEGPDSAVTPSDSSEIVLDGTDNQYLGLQIGQQPQDEDEEATGILPDEASEAFRNSSMALQQAHLLKNMSHCRQPSDSSVDKFVLRDEAT EPGDQENKPCRIKGDIGQSTDDDSAPLVHCVRLLSASFLLTGGKNVLVPDRDVRVSVKALALSCVGAAVALHPESFFSKLYKVPLDTTEYPEEQYVSDILNYIDHGDPQVR GATAILCGTLICSILSRSRFHVGDWMGTIRTLTGNTFSLADCIPLLRKTLKDESSVTCKLACTAVRNCVMSLCSSSYSELGLQLIIDVLTLRNSSYWLVRTELLETLAEIDFRL VSFLEAKAENLHRGAHHYTGLLKLQERVLNNVVIHLLGDEDPRVRHVAAASLIRLVPKLFYKCDQGQADPVVAVARDQSSVYLKLLMHETQPPSHFSVSTITRIYRGYN LLPSITDVTMENNLSRVIAAVSHELITSTTRALTFGCCEALCLLSTAFPVCIWSLGWHCGVPPLSASDESRKSCTVGMATMILTLLSSAWFPLDLSAHQDALILAGNLLAA SAPKSLRSSWASEEEANPAATKQEEVWPALGDRALVPMVEQLFSHLLKVINICAHVLDDVAPGPAIKAALPSLTNPPSLSPIRRKGKEKEPGEQASVPLSPKKGSEASAA SRQSDTSGPVTTSKSSSLGSFYHLPSYLKLHDVLKATHANYKVTLDLQNSTEKFGGFLRSALDVLSQILELATLQDIGKCVEEILGYLKSCFSREPMMATVCVQQLLKTLFG TNLASQFDGLSSNPSKSQGRAQRLGSSSVRPGLYHYCFMAPYTHFTQALADASLRNMVQAEQENDTSGWFDVLQKVSTQLKTNLTSVTKNRADKNAIHNHIRLFEP LVIKALKQYTTTTCVQLQKQVLDLLAQLVQLRVNYCLLDSDQVFIGFVLKQFEYIEVGQFRESEAIIPNIFFFLVLLSYERYHSKQIIGIPKIIQLCDGIMASGRKAVTHAIPA LQPIVHDLFVLRGTNKADAGKELETQKEVVVSMLLRLIQYHQVLEMFILVLQQCHKENEDKWKRLSRQIADIILPMLAKQQMHIDSHEALGVLNTLFEILAPSSLRPVD MLLRSMFVTPNTMASVSTVQLWISGILAILRVLISQSTEDIVLSRIQELSFSPYLISCTVINRLRDGDSTSTLEEHSEGKQIKNLPEETFSRFLLQLVGILLEDIVTKQLKVEM SEQQHTFYCQELGTLLMCLIHIFKSGMFRRITAAATRLFRSDGCGGSFYTLDSLNLRARSMITTHPALVLLWCQILLLVNHTDYRWWAEVQQTPKRHSLSSTKLLSPQM SGEEEDSDLAAKLGMCNREIVRRGALILFCDYVCQNLHDSEHLTWLIVNHIQDLISLSHEPPVQDFISAVHRNSAASGLFIQAIQSRCENLSTPTMLKKTLQCLEGIHLSQ SGAVLTLYVDRLLCTPFRVLARMVDILACRRVEMLLAANLQSSMAQLPMEELNRIQEYLQSSGLAQRHQRLYSLLDRFRLSTMQDSLSPSPPVSSHPLDGDGHVSLET VSPDKDWYVHLVKSQCWTRSDSALLEGAELVNRIPAEDMNAFMMNSEFNLSLLAPCLSLGMSEISGGQKSALFEAAREVTLARVSGTVQQLPAVHHVFQPELPAEP AAYWSKLNDLFGDAALYQSLPTLARALAQYLVVVSKLPSHLHLPPEKEKDIVKFVVATLEALSWHLIHEQIPLSLDLQAGLDCCCLALQLPGLWSVVSSTEFVTHACSLIHC VHFILEAVAVQPGEQLLSPERRTNTPKAISEEEEEVDPNTQNPKYITAACEMVAEMVESLQSVLALGHKRNSGVPAFLTPLLRNIIISLARLPLVNSYTRVPPLVWKLGW SPKPGGDFGTAFPEIPVEFLQEKEVFKEFIYRINTLGWTSRTQFEETWATLLGVLVTQPLVMEQEESPPEEDTERTQINVLAVQAITSLVLSAMTVPVAGNPAVSCLEQ QPRNKPLKALDTRFGRKLSIIRGIVEQEIQAMVSKRENIATHHLYQAWDPVPSLSPATTGALISHEKLLLQINPERELGSMSYKLGQVSIHSVWLGNSITPLREEEWDEE EEEEADAPAPSSPPTSPVNSRKHRAGVDIHSCSQFLLELYSRWILPSSSARRTPAILISEVVRSLLVVSDLFTERNQFELMYVTLTELRRVHPSEDEILAQYLVPATCKAAA VLGMDKAVAEPVSRLLESTLRSSHLPSRVGALHGILYVLECDLLDDTAKQLIPVISDYLLSNLKGIAHCVNIHSQQHVLVMCATAFYLIENYPLDVGPEFSASIIQMCGVM LSGSEESTPSIIYHCALRGLERLLLSEQLSRLDAESLVKLSVDRVNVHSPHRAMAALGLMLTCMYTGKEKVSPGRTSDPNPAAPDSESVIVAMERVSVLFDRIRKGFPCE ARVVARILPQFLDDFFPPQDIMNKVIGEFLSNQQPYPQFMATVVYKVFQTLHSTGQSSMVRDWVMLSLSNFTQRAPVAMATWSLSCFFVSASTSPWVAAILPHVI SRMGKLEQVDVNLFCLVATDFYRHQIEEELDRRAFQSVLEVVAAPGSPYHRLLTCLRNVHKVTTCGGSGDYKDDDDK
Construct AA start	1	1
Construct AA end	371	3156
Cloning Method	Ligation-independent cloning using Clontech's In-Fusion enzyme	Ligation-independent cloning using Clontech's In-Fusion enzyme
Vector	pFBOH-MHL (N-terminal tag Hexa-His and TEV cleavage site)	pBacMam-DiEx2-LIC (C-terminal FLAG tag)

2. Protein expression and purification:

Full BVES methods can be found here: https://zenodo.org/record/154611 in file BVES_protocols.docx

3:1 HTT Q23 (FLAG-tagged, TOC009:D01) to HAP40 (His-tagged, TOC011:C01) virus ratios used for 4 L BVES sf9 production. Cells were harvested by centrifugation, resuspended in ~200 mL 50 mM Tris pH 8, 300 mM NaCl and were stored at -80 ˚C prior to purification.

Cell pastes were thawed and resuspended in ~400 mL total 50 mM Tris pH 8, 300 mM NaCl, 0.5 % (v/v) Tween-20 supplemented with 1 x protease inhibitors supplemented with benzonase. The lysate was clarified by centrifugation at 15,000 rpm for 1 hour (JLA16.2500) and then bound to 2 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT). Resin was washed with 2 x 250 mL 50 mM Tris pH 8, 300 mM NaCl (wash 1 and 2 – W1 and W2). HTT-HAP40 protein was eluted with ~12 mL resuspension buffer supplemented with 200 µg/mL 3xFLAG peptide (elution – E).

The sample was then rocked with 1 mL Ni-NTA at 4 ˚C with rocking for 30 mins. Ni-NTA beads were washed with 50 mM Tris pH 8, 300 mM NaCl, 15 mM imidazole and then eluted with 50 mM Tris pH 8, 300 mM NaCl, 300 mM imidazole.

The elution was concentrated to 1 mL (elution concentrated – EC) and run on Superose6 column.

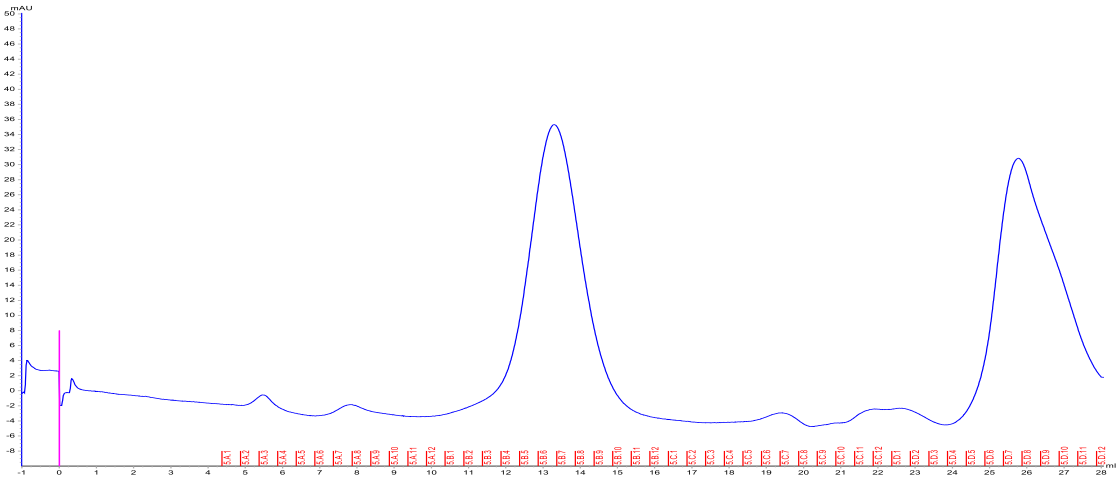

Figure 1 - Single peak seen on analytical gel filtration indicating sample is monodisperse

Samples were run on SDS-PAGE 4-20 % tris-glycine gel of the purification process.

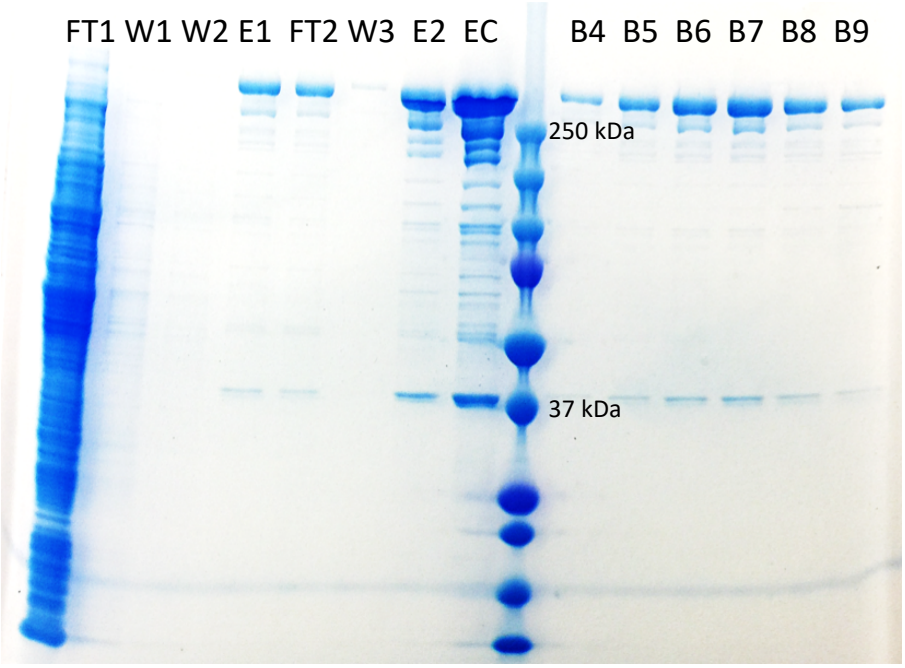

Figure 2 - SDS-PAGE of fractions taken throughout purification process.

Conclusions

Peak shape from elution is NOT typical huntingtin distribution indicating stable HTT-HAP40 complex has formed. SDS-PAGE shows bands ~350 kDa and ~40 kDa likely corresponding to HTT and HAP40 respectively. As HTT is ~8-9 times larger than HAP40, the band will be 8-9 times more intense, this is observed in the gel filtration fractions.

Yields good and sample clean -> 3.0 mg/mL – ~100 µL aliquots.