Small scale test-expression of full-length huntingtin Q23 with HAP40 in baculoviral expression system production in sf9 insect cells – 2018/05/14

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.

Experiments

1. HAP40 construct clone

Sample ID	HAP40:TOC011-C01:C241911
Construct PKEY	83963
Clone pkey	241911
Construct ID	HAP40_1-371
Cloned AA Seq	MHHHHHHSSGRENLYFQG**MAAAAAGLGGGGAGPGPEAGDFLARYRLVSNKLKKRFLRKPNVAEAGEQF GQLGRELRAQECLPYAAWCQLAVARCQQALFHGPGEALALTEAARLFLRQERDARQRLVCPAAYGEPLQ AAASALGAAVRLHLELGQPAAAAALCLELAAALRDLGQPAAAAGHFQRAAQLQLPQLPLAALQALGEAAS CQLLARDYTGALAVFTRMQRLAREHGSHPVQSLPPPPPPAPQPGPGATPALPAALLPPNSGSAAPSPAAL GAFSDVLVRCEVSRVLLLLLLQPPPAKLLPEHAQTLEKYSWEAFDSHGQESSGQLPEELFLLLQSLVMATHEK DTEAIKSLQVEMWPLLTAEQNHLLHLVLQETISPSGQGV**
Construct AA start	1
Construct AA end	371
Cloning Method	Ligation-independent cloning using Clontech's In-Fusion enzyme
Vector	pFBOH-MHL (N-terminal tag Hexa-His and TEV cleavage site)

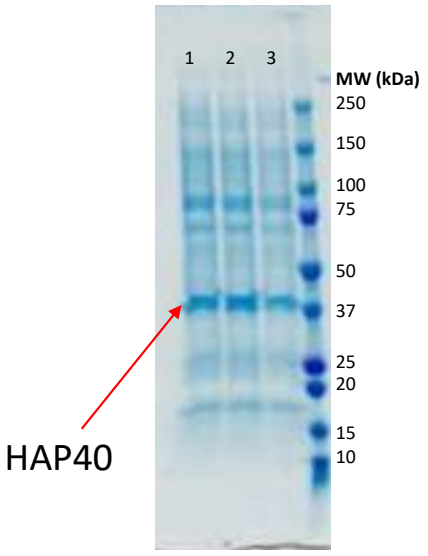
2. HAP40 test expression in sf9 culture:

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

~3 mL sf9 production in triplicate
Protein purified using Talon beads on May 1 2018

Lysis Buffer: 25 mM tris pH 8.0, 300 mM NaCl, 2.5 % (v/v) glycerol, 2.5 mM imidazole, 0.6 % (v/v) NP40
Wash Buffer: 25 mM tris pH 8.0, 300 mM NaCl, 2.5 % (v/v) glycerol, 15 mM imidazole
Elution Buffer: 25 mM tris pH 8.0, 300 mM NaCl, 2.5 % (v/v) glycerol, 300 mM imidazole

HAP40:TOC011-C11



HAP40 over-expression is indicated on the SDS-PAGE.

3. HTT-HAP40 co-expression in sf9 culture:

~100 mL sf9 production HTT Q23 (TOC009-D01) and HAP40 (TOC011-C01) in 3:1, 4:1 and 5:1 of the respective viruses. See above link for full BVES methods.

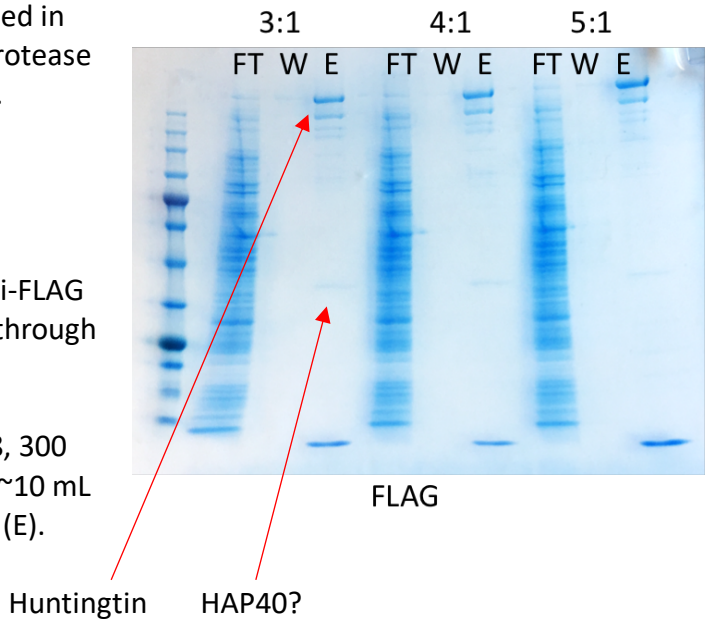
Cells were harvested by centrifugation on May 7 2018 at 1500 rpm, 30 mins, 4 °C and stored at -80 °C until purification.

Pellets were thawed on May 8 2018 and resuspended in ~200 mL 20 mM Tris pH 8, 300 mM NaCl with 1 x protease inhibitor mix, 0.5 % (v/v) Tween-20 and benzonase.

Cell lysates were spun at 15000 rpm, 1 hour, 4 °C (JLA16.2500, Beckman).

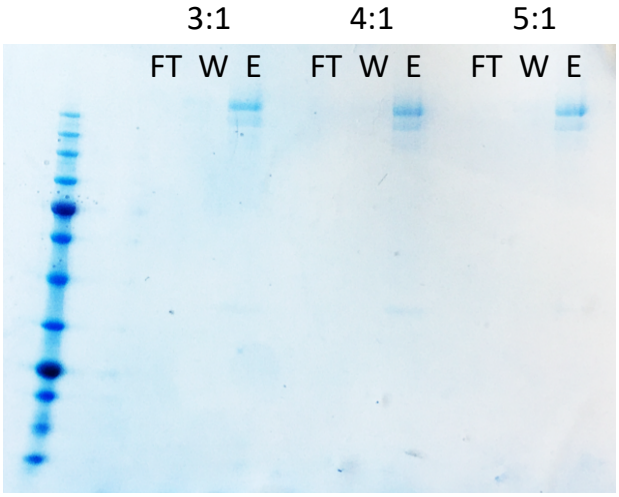
Clarified lysate was incubated with ~ 1 mL each anti-FLAG resin (Sigma) and rocked at 4 °C for 2 hours – flow through (FT).

Resins were washed with ~500 mL 20 mM Tris pH 8, 300 mM NaCl – wash (W). The protein was eluted with ~10 mL wash buffer + 250 µg/mL 3xFLAG peptide – elution (E).

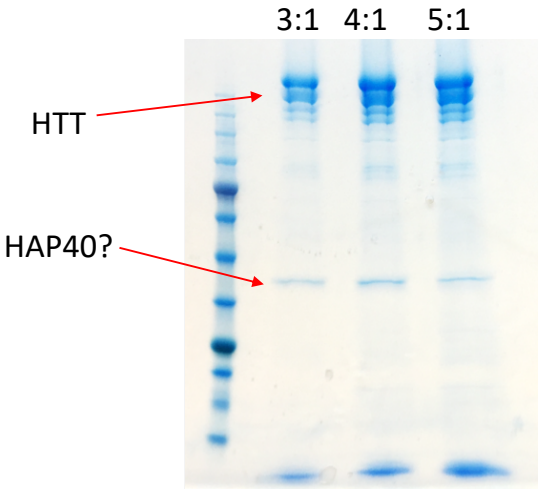


FLAG

FLAG-elution samples were bound to ~1 mL Ni-NTA resin for 30 mins – flow through (FT). The resin was washed with ~50 mL wash buffer + 15 mM imidazole – wash (W). Proteins were eluted with ~5 mL wash buffer + 300 mM imidazole – elution (E). Elution samples were concentrated in MWCO 100,000 spin concentrators.



Ni



Concentrated Ni Elution

Conclusions

A band of ~40 kDa is visible in the FLAG and Ni elution fractions. However, wherever this is HAP40 is not clear. A key finding by Guo et al (2018) is that the HTT-HAP40 complex resolves as a discrete peak on a Superose6 column run. I will scale the production to ~4 L of the 3:1 HTT:HAP40 to prep this complex and hopefully have sufficient material to purify via gel filtration in addition to affinity chromatography. I will also excise the bands in the gels of the concentrated Ni elution and digest the protein to identify by mass spectrometry.