**Huntingtin Exon 1 Q16 and Q46 pET32a thioredoxin tagged expression and purification 2018/04/09**

**Rationale:**

To express and purify huntingtin exon 1 protein samples to investigate DNA binding properties of these portions of the huntingtin protein. Previous attempts expressed a lot of protein, but that protein was misfolded/aggregated and appeared in the void peak of the gel filtration profile. Change buffers to improve protein stability.

**Constructs:**

Huntingtin exon 1 Q16 <https://www.addgene.org/11487/> and huntingtin exon 1 Q46 <https://www.addgene.org/11515/> from Bennet et al (2002) PNAS, Pubmed ID: 12193654.

**Protocol:**

6 L each LB culture grown of each exon 1 construct in LEX system at 37 ˚C until OD600 ~ 0.8 then induced with 1 mM IPTG and grown at 25 ˚C. Cells were harvested by centrifugation and then the pastes stored at -80 ˚C prior to purification.

Cell pastes were resuspended in 500 mL 50 mM Tris⋅HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 1 mM TCEP supplemented with 20 benzonase µg/mL and 1 mM PMSF and 1 mM benzamidine. Cells were lysed by sonication. The lysate was clarified by centrifugation at 15000 rpm in JLA16.250 for 60 mins, supplemented with 5 mM imidazole and then bound to 5 mL Ni-NTA resin per lysate at 4 ˚C with rocking for 1 hour. Resin was washed with 500 mL lysis buffer supplemented with 25 mM imidazole. Exon 1 proteins were eluted with ~20 mL lysis buffer supplemented with 300 mM imidazole. The sample was concentrated to 5 mL and run on S75 16/60 in 50 mM Tris⋅HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 1 mM TCEP.

Q16



Q46



Fractions of the samples were concentrated and flash frozen:

Q16 – 14.4 mg/mL 50 uL x 19

Q46 – 8.5 mg/mL 80 uL x 18

**Concluding remarks:**

Changing the buffer conditions allow purification of sample of higher purity which is not aggregated.