**Protocols to remove Chaperon from target proteins expressed in Arctic Express cells**

**Suboptimal Urea method to remove the Cpn60 from ArcticExpress cells-**

(Belval et al., 2015, Protein Expression and Purification 109 (2015) 29–34)

Protein purified: Vitis endonuclease, viral movement protein, from Arctic Express cells

**1:** The Ni-column was equilibrated with Lysis-Equilibration-Wash Buffe (LEW1x)r: 300 mM NaCl, 50 mM NaH2PO4 pH 8.0, before loading the bacterial lysate.

**2:** To ensure that all the proteins bound to the column, the recovered flow through was loaded three times on the column.

**3:** After loading the samples, column was washed with 5 volumes of different buffers, but Lysis-Equilibration-Wash Buffer: 300 mM NaCl, 50 mM NaH2PO4 pH 8.0 followed by 5 volumes of the LEW 1x buffer gave maximum purity (>98%).

[It is unclear why these buffers will give lower purity- 2 M urea, 20 mM Tris–HCl pH 6.8, 10 mM ATP, 10 mM MgCl2 **(74%)**; 2 M urea, 20 mM Tris–HCl pH 6.8, 10 mM ATP, 10 mM MgCl2, 5 mM imidazole (**87%**) as urea concentrations are same.

4: The proteins were eluted with commercially available elution buffer. Protein was quantified by nanodrop and purity was assessed by SDS-PAGE and Western blot.

[We have to optimize the minimum amount of Urea required to remove the Cpn60]

**Protocol using [ATP-Mg + denatured bacterial proteins]** (Rial and Ceccarelli; *Protein Expression and Purification* 25 (2002) 503–507): The paper describes the removal of Hsp70 molecular chaperon (DnaK) from fusion protein; authors claim that this strategy can be applied to other chaperons.

Authors used a fusion protein expressed in JM109or BL21 cells in this study. The peptide connecting two proteins in a fusion protein is a potential binding site for Hsp70 chaperon. Authors used Mg-ATP and denatured bacterial lysate to remove the contaminants. One thing is to note that the amount of contaminant (Hsp70) in their experiments is much smaller than our system.

**How to make denatured bacterial lysate**-

In preferred lysis buffer, the bacterial cells were lysed and centrifuged to clarify. The amount of the protein was adjusted to 2 mg/ml in same buffer. The protein was heated at 65OC for 10 min. The samples were centrifuged again, protein was quantified and aliquots were stored in -80 for future use (They don’t mention the quality of denaturation, but I assume they made lot of the denatured protein and stored for all their experiments).

**Protein Purification and removal of DnaK-**

They followed typical His-tag protein purification protocol (I am not listing the buffer etc as it may in our case and can be compared if needed).

Lysis buffer used was 1/10th of original culture e.g. for 10 ml of culture, after centrifugation, they re-suspended the cells in 1 ml of lysis buffer. For His-tag, they did not use any detergent. Lysis was done by sonication (in our case, we may use NP-40 and Lysozyme for lysis, and sonication to break the DNA).

Once the cells were lysed, centrifuged, the supernatant was incubated with Ni-Agarose resin (they did not use column) for 30 min with gentle shaking at 0OC. After incubation, the mixture was centrifuged to remove unbound protein and the resin was washed three times with buffer containing 5 mM Mg-ATP plus 0.1 mg/ml denatured lysate. For all three washes, the resins were incubated with four bed volumes of buffer containing 5 mM Mg-ATP plus 0.1 mg/ml denatured lysate for 10 min each at 0OC. After 3rd wash, the proteins were eluted with buffer containing 100 mM imidazole (in our case, the imidazole concentration will vary, typically upto 500 mM, I never tried 100 mM for Sirt3). A second purification was done, without incubation with denatured lysate +Mg-ATP combination to see the efficiency of the method.