**Protein purification schedule (Sirt3 102-399)**

* **Current status ( up to 7/26/16):**
* Purified 4 x 800 ml batches of Sirt3 using FPLC (currently purifying 4th batch).
* Approximately 1.1 ml protein obtained from each batch (total ~ 4.4 ml).
* Each batch was individually frozen in -80 C (~100 ul of each was aliquoted for activity assay).
* **7/27/16 – 7/29/16:**
* Will measure the activity and concentration of each batch (from aliquot).
* Combine the batches and re-measure the concentration and activity of the combined large batch.
* Run a gel of the combined batch relative to Enzo Sirt3 to compare purity of bulk enzyme.
* Determine total amount of Sirt3 available (in terms of Units).
* **8/1/16 – 8/5/16:**

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* Start preparing media, overnight cultures to grow a new set of 4 x 800 ml cell cultures.
* Will grow and harvest cells all week and store batches in -80 C.
* **8/8/16 - :**
* Make fresh buffers for a new round of bulk purification using FPLC.
* Re-condition HisTrap column.
* Purify protein from frozen cell batches.

**Schedule for truncated Sirt3 (118-399)**

• Current status: We have the sequencing results for the truncated Sirt3 plasmid. 4 out of 6 constructs are positive, one of which will be used for overexpression and purification.

• According to my schedule up to the week of 8/25/16, I will be busy with growing and purifying the 2nd large batch (consisting of 4 smaller batches) of Sirt3 - AE (102-399) for XG and AU experiments.

• I can resume work on the truncated Sirt3 beginning the week of 8/29/16.

• Transformation of the plasmid into Rosetta 2 (DE3) cells, screening of positive colonies and small scale overexpression would take at least 2 weeks (approximately by 9/12).

• Small scale purification (without optimization) and measurement of activity would take another two weeks (approximately by 9/23).

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