**Expression analysis and Sirt3 purification using Arctic Express cells:**

**Expression analysis:**

**1:** The plasmid containing SIRT3 gene was transformed in Arctic Express cells and was grown on LB-plate containing Ampicillin at 37°C.

**2:** Several transformants were picked up and were grown overnight in LB medium containing gentamycin (20ug/ml) and ampicillin (100ug/ml) antibiotic at 37°C.

**3:** Next morning the cells were inoculated (2%) in LB-media without antibiotic selection for 3 hours at 30°C.

**4:** The protein expression was induced by adding different concentration of IPTG 15°C for 24 hours.

**5:** Expression of protein was analyzed in induced and un-induced cultures by SDS-PAGE.

Sirt3-**His Tag protein purification (All the steps at 4OC unless mentioned)**

Add 1 mg/ml lysozyme to lysis buffer + protease inhibitor, use 1ml per gram pellet

Re-suspend bacterial pellet in lysis buffer, try to mix so that it is homogenous

Keep on ice for up to 20-30 minutes then briefly sonicate to break DNA

Transfer lysate into Eppendorf’s tubes, and centrifuge max speed for 10 minutes at 4 OC

(While this is going on, wash the Ni-column with 10 ml of wash buffer in the cold room)

Load sample (e.g. sample amount 5 mL), let it pass through column

Wash the column 5 times, with wash buffer, amount equivalent to sample volume, collect each 5 ml separately

Elute protein by Elution Buffer with 500 µL fractions (about 10 fractions)

Run SDS-PAGE to see the purity

Pool fractions; dialyze against dialysis buffer with 10 % Glycerol overnight

**Buffers for purification:**

**1: Lysis Buffer:**

20 mM Sodium Phosphate Buffer pH 7.4

500 mM NaCl

20 mM Imidazole

1mM PMSF

0.5% NP-40

1 mg/ml lysozyme

**2: Wash Buffer (Binding Buffer):**

20 mM Sodium Phosphate Buffer pH 7.4

500 mM NaCl

20 mM Imidazole

**3: Elution Buffer:**

20 mM Sodium Phosphate Buffer pH 7.4

500 mM NaCl

500 mM Imidazole