

## Supplementary Data

### Expression of Protein Complexes Using Multiple E. coli Protein Co-expression Systems: A Benchmarking Study

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#### Protocol for co-expression of selected complexes

##### *Expression and purification of his-Cdt:Geminin complex*

Transformed cells were grown at 37 °C and when the optical cell density at 595 nm (OD<sub>595</sub>) was around 0.6, temperature was lowered to 30 °C and protein expression was induced by addition of 0.1 mM IPTG (final concentration). After 3 hours cells were collected by centrifugation and the weight of cell pellet was measured. Cells were resuspended in buffer A (50mM Tris-HCl pH 7.5, 200mM NaCl, 5mM β-mercaptoethanol) supplemented with 20mM Imidazole, Lysozyme (1 mg Lysozyme per ml of resuspended cells), DNaseI (5μg/ml of resuspended cells). Cells were disrupted by sonication and cell debris and insoluble proteins were removed by centrifugation at 10,000 g for 30 min at 4 °C. The soluble fraction was loaded onto 250 μl of Ni<sup>2+</sup>-beads and incubated for 15 minutes at 4 °C. Beads were washed with 10 Column volumes (CV) of buffer A containing 20mM Imidazole and bound proteins were subsequently eluted in a single step with 2 CV of buffer A containing 500mM Imidazole.

##### *Expression and purification of his-TFII Ea:TFII Eβ complex*

Transformed cells were grown at 37 °C and when OD<sub>595</sub> was around 0.8, temperature was lowered to 22 °C and protein expression was induced by addition of 0.4 mM IPTG (final concentration). Cells were kept at 22 °C during o/n incubation. Cells were collected by centrifugation and the weight of cell pellet was determined. Cells were resuspended in buffer B: (20 mM Tris pH 7.5, 250 mM NaCl, 2 mM β-mercaptoethanol) supplemented with protease inhibitor cocktail. Cells were disrupted by sonication and cell debris and insoluble proteins were removed by centrifugation at 10,000 g for 30 min at 4 °C. The soluble fraction was loaded onto 250 μl of Ni<sup>2+</sup>-beads and incubated for 60 minutes at 4 °C. Beads were washed 3 times with 4CV of buffer B and bound proteins were subsequently eluted in a single step with 2 CV of buffer B containing 250 mM Imidazole.

##### *Expression and purification of his-PB2:Importin α5*

Transformed cells were grown at 37 °C and when OD<sub>595</sub> was around 0.8, temperature was lowered to 20 °C and protein expression was induced by addition of 0.2 mM IPTG (final concentration). After o/n expression at 20 °C, cells were harvested by centrifugation and the weight of cell pellet was measured. Cells were resuspended in buffer C: 30 mM Tris (pH 7), 200 mM NaCl, 5 mM β-mercaptoethanol supplemented with protease inhibitor cocktail. Cells were disrupted by sonication and cell debris and insoluble proteins were removed by centrifugation at 10,000 g for 30 min at 4 °C. The soluble fraction was loaded onto 250 μl of Ni<sup>2+</sup>-beads and incubated for 60 minutes at 4 °C. Beads were washed 3 times with 4 CV of buffer D (10 mM Tris pH 7.0, 200 mM NaCl, 5 mM β-mercaptoethanol) supplemented with 50mM Imidazole. Bound proteins were subsequently eluted in a single step with 2 CV of buffer D containing 500 mM Imidazole.

*Expression and purification of his-NFYC:NFYB:NFYA complex*

Transformed cells were grown at 37 °C and when OD<sub>595</sub> was around 0.6, temperature was lowered to 25 °C and protein expression was induced by addition of 1 mM IPTG (final concentration). After 4 hrs, cells were harvested by centrifugation and the amount of cell pellet was weighed. The weight of cell pellet was measured and quantified. Cells were resuspended in buffer E: 20 mM Tris pH 7 and 400 mM NaCl. Cells were disrupted by sonication and cell debris and insoluble proteins were removed by centrifugation at 10,000 g for 30 min at 4 °C. The soluble fraction was loaded onto 250 µl of Ni<sup>2+</sup>-beads and incubated for 60 minutes at 4 °C. Beads were washed 3 times with 4 CV of Buffer F (20 mM Tris pH 8.0, 200 mM NaCl, 10 mM β-mercaptoethanol) containing 15 mM Imidazole. Bound proteins were subsequently eluted in a single step with 2 CV of buffer F containing, 200 mM Imidazole.