Preparation of histone octomer from purified histones

MATERIALS

Unfolding Buffer (50 mL):

	50 mL	Final Concentration
Tris-Hcl, pH 7.5 (1M Stock)	1 mL	20mM
Guanidinium hydrochloride	33.4 g	7M
DTT (1M Stock)	0.5 mL	10mM

Refolding Buffer (3.5 L):

	per L	3.5L	Final Concentration
Tris-Hcl, pH 7.5 (1M Stock)	20 mL	70 mL	20mM
NaCl	117 g	409 g	2M
EDTA (0.5M Stock)	2 mL	7 mL	1mM
2-mercaptoethanol (14.3M Stock)	350 uL	1.22 mL	5mM

6-8 KDa cutoff dialysis membranes
Boil in 1L milliQ water for 5 min and cool to room temperature.

Superdex 200 HR 10/30 column

PROTOCOL

(1) Dissolve each lyophilized histone to a concentration of 2 mg/mL in unfolding buffer. Pipette up and down to dissolve. Do not vortex.

(2) Allow unfolding to proceed for at least 30 min at room temperature and for not more than 3 hrs. (30min – 3 hr)

(3) Mix the four histones so that the molar ratio of H2A: H2B:H3:H4 is 1.2:1.2:1:1.

A slight excess of H2A and H2B is used to ensure complete octamer formation.

This is because octamer and H2A/H2B dimer can be easily resolved on the superdex200 column, but the H3/H4 tetramer cannot be easily resolved from the octamer. (M.W.: H2A – 13,960 Da, H2B – 13,774 Da, H3 – 15,273 Da, H4 – 11,236 Da)

(4) Adjust the final protein concentration of the mix to 1 mg/ml using unfolding buffer.

(5) Dialyze in 6-8 KDa cutoff dialysis bag 3 times against 1 L of refolding buffer. At least one dialysis should be overnight. The rest should be at least 2 hours each.

(6) After final dialysis, spin down sample to remove any precipitated material. Save the supernatant for loading on the column.

(7) Concentrate the supernatant to ~200 uL in a Microcon 10. Save flow-through until the end of the prep.

(8) The previous evening start equilibrating a Superdex 200 HR 10/30 column with refolding buffer.

(9) Load the concentrated sample on the column but save some (~5 uL) for running on a gel. Run the buffer at 0.5 ml/min and collect 0.5 ml fractions. For this particular column, the octamer should elute at between~ 12-13 mls and the dimer between ~15-16 ml.

(10) Run peak fractions on a gel and pool bonafide octamer fractions. Concentrate to greater than 1 mg/mL.

(11) Determine octamer concentration by SYPRO staining using BSA standards. Flash freeze in Liq N2 and store at –80 ^{o}C.