RECOMBINANT HISTONE PREP PROTOCOL

Solutions:

0.2M IPTG, ampicillin, chloramphenicol, PMSF (best added deep into solution), DTT, benzamidine, DMSO, BME, Dialysis membrane 3500MWCO, gradient-capable chromatography apparatus, GE Sepharose Q fast flow (40mL) and SP columns (5mL; separate SP for each histone), nanopure H2O, lyophilizer, sonicator

2xTY: (2L) 32g Bacto-tryptone, 20g Bacto-yeast-extract, 10g NaCl, H20 to 2L, autoclave as 500mL in each of 4x1000mL flasks

Histone Wash T: (250mL for 2L histone induction) 50mM Tris pH=7.4, 1mM EDTA, 100mM NaCl; before use add PMSF, benzamidine and 1mM DTT

Histone Wash TW: (250mL for 2L histone induction) 50mM Tris pH=7.4, 1mM EDTA, 100mM NaCl, 1% v/v Triton X-100; before use add PMSF, benzamidine and 1mM DTT

Unfolding buffer: (40mL for 2L histone induction) 20mM Tris pH=7.4, 10mM DTT, 7M Guanidine HCl. Filter before use.

Urea dialysis buffer: (2x2L changes) 10mM Tris pH=8, 1mM EDTA, ***100mM NaCl for H2A and H2b or 200mM NaCl for H3 and H4***, 7M Urea. Make immediately before use; add PMSF and 5mM BME.

Urea low buffer: (750mL for 2L histone induction) 10mM Tris pH=8, 1mM EDTA, 7M Urea. Make immediately before use, filter and degas; add PMSF and 1mM DTT

Urea high buffer: (250mL for 2L histone induction) 10mM Tris pH=8, 1mM EDTA, 1M NaCl, 7M Urea. Make immediately before use, filter and degas; add PMSF and 1mM DTT.

2M NaCl: (1L for column) 116.88g NaCl, dissolve in dH20 to 1L, filter and degas.

Protocol: For 2L induction

Day One:

Streak out desired BL-21DE3 pLysS strain (CP812 xH2Awt, CP813 xH2Bwt, CP814 xH3wt, CP815 xH4wt, CP1109 xH2AS113C) on fresh LB+Amp+Chlor plates. (NOTE: H4 does not contain pLysS and should therefore be grown on ampicillin media only)

Day Two:

In the morning, inoculate 2x5mL 2xTY-AC cultures with two colonies apiece from the plate, incubate at 37C with rotation. That night, combine 1mL of each of the small cultures into a 50mL 2xTY-AC culture, and leave at 37C overnight without rotation.

Day Three:

In the morning, resuspend 50mL culture and inoculate ~10mL of overnight culture into each of 4x500mL of 2xTY-AC media in ~1000mL flasks. Grow into log phase at 37C with rotation until OD600=0.6, then induce histone expression by adding 0.2mM IPTG. Allow induction to proceed for two (H3 and H4) to three (H2A and H2B) hours. Remember to take pre- and post-induction timepoints (1mL of culture, spin down at 4C 14krpm 5', add 200uL 2xSDS-PAGE sample buffer, boil/spin, run 10uL each on 15% SDS-PAGE). Spin cultures down into 2x1000mL-culture pellets 25' 3000rpm @RT. Resuspend each liter's pellet in 16.7mL Histone Wash T, transfer to a plastic centrifuge tube, and flash-freeze. Good induction cultures yield much whiter bacterial pellets.

Day Four:

Through this step, treat each one-liter pellet separately. Thaw pellet in 30C water bath, mixing occaisionally. Adjust volume to 25mL per liter pellet with fresh Histone Wash T. Keep on ice/cold henceforth. Sonicate (freq level 5) 6x15" or until the solution is no longer viscous (NOTE: H4 should not be sonicated until the Histone Wash TW step) on ice. Spin in JA-17 rotor at 14kRPM (27000g) for 20min at 4C. Decant supernatant, and resuspend pellet in 25mL/liter pellet Histone Wash TW by pipetting up and down until as resuspended as possible. If solution still contains yellowish chunks, or is still viscous, sonicate a little more. Repeat spin and resuspension once more in Histone Wash TW, then twice more after that with Histone Wash T. At the end, discard the supernatant and flash-freeze the resultant pellet ('inclusion body'). The inclusion body when thawed should be whitish and large in proportion to the efficiency of induced histone. A little black or grey discoloration is to be expected.

Day Five:

Thaw the inclusion body to room temperature. Add 350uL DMSO to each liter pellet and let sit at RT for 30'. Combine both 1L pellets together into one centrifuge tube using a spatula. Add 26.6mL fresh unfolding buffer and mince the pellet as much as possible. Put in a tiny stir bar, cap tubes, and leave them mounted on stir plates for an hour at RT, stirring moderately. Pellet undissolved material by spinning at 14krpm for 20' at RT (you can leave the stir bar in). If you know you had less potent induction, scale down unfolding buffer a little. Save supernatant. Reextract pellet with 9.4mL unfolding buffer after remincing pellet for 30' at RT. Spin again, take supernatant and pool with prior supernatant. Dialyze against two changes of 2L of urea dialysis buffer at 4C, the first for three hours, the second overnight.

Day Six:

Set up the chromatography apparatus such that sample flows first over the Qff column, then over the SP column. Perform all chromatography steps at a flowrate of 2mL/min. Wash columns first with 50mL degassed filtered water, then with 100mL 2M NaCl, then again with 100mL degassed filtered water. Put Urea low buffer through pumphead A and Urea high buffer through pumphead B. Equilibrate the columns with 100mM NaCl (H2A/H2B) or 200mM NaCl (H3/H4) by running the pumpheads at 10% or 20% pumphead B, respectively.

Before loading the dialyzed supernatant onto the columns, spin it at 14krpm for 20' at 4C to remove any precipitate. Transfer the cleared liquid to a new 50mL conical. Load full volume onto the columns (if through the pumpheads, then through pumphead A; subsequently put Urea low buffer back onto pumphead A) and then wash the columns with 100mL of 10% B (or 20% B). Now, remove the QFF column from the chromatography loop. Start running a gradient program (see table 1) and collect 1mL fractions throughout the run. Use the UV absorbance trace of the run to denote fractions of interest, then test all of those fractions via SDS-PAGE. Pool the pure peak histone fractions. Dialyze the pool through 3x2L dialyses against nanopure water containing PMSF and 5mM BME at 4C. Remember to wash all Q and SP columns with 100mL 2M NaCl, and then 100mL filtered degassed water, after use; then, 100mL 20% filtered degassed ethanol for storage.

Day Seven:

Get the concentration of the histone protein post-dialysis by reading absorption at 276nm (see Table II). Use the last change of dialysis water as a blank. Spec at least three different dilutions of histone in dialysis water to ensure linearity. Aliquot into volumes that are convenient for reconstitution (100nmol?). Snap-freeze the aliquots. Lyophilize. Store at -80C.

Table I—Chromatography Gradients

10% B gradient: (2mL/min)	20% B gradient: (2mL/min)
10mL @ 10%B, start fractions (1mL ea)	10mL @ 20%B, start fractions (1mL ea)
Zero UV	Zero UV
10%B->90%B linearly over 50mL	20%B->100%B linearly over 50mL
18mL @ 90%B	18mL @ 90%B

Table II—Histone Properties

Histone	E (276nm, per cm per M)	MW (Da)
xH2A	4050	13960
xH2B	6070	13774
xH3	4040	15273
xH4	5400	11236

Reference:

For the basis of this protocol and also for further steps with recombinant histone proteins, refer to the original Luger methods publication:

Luger K, Rechsteiner TJ, Richmond TJ. *Expression and purification of recombinant histones and nucleosome reconstitution*. Methods Mol Biol. 1999;119:1-16.

Their purification scheme is very similar to this one with the exception that Luger's protocol does not make use of the tandem ion-exchange columns.