Preparation of various nucleosomes for biochemical and structural analyses

Genomic DNA, which encodes genetic information, is three-dimensionally organized in the cell as chromatin. Its basic unit is the nucleosome, a disc-like structure containing two copies each of histones H2A, H2B, H3, and H4, with DNA wound around it. Chromatin is composed of various types of nucleosomes that are involved in different biological phenomena. The variability is notably illustrated by the presence of nucleosomes containing non-allelic histone isoforms, named histone variants. The *in vitro* reconstitution of these atypical nucleosomes requires specific steps, as compared to canonical nucleosomes.

Here we describe the methods for the preparation of canonical histones and histone variants, as well as the methods for the reconstitution of histone complexes, canonical nucleosomes, and heterotypic nucleosomes.

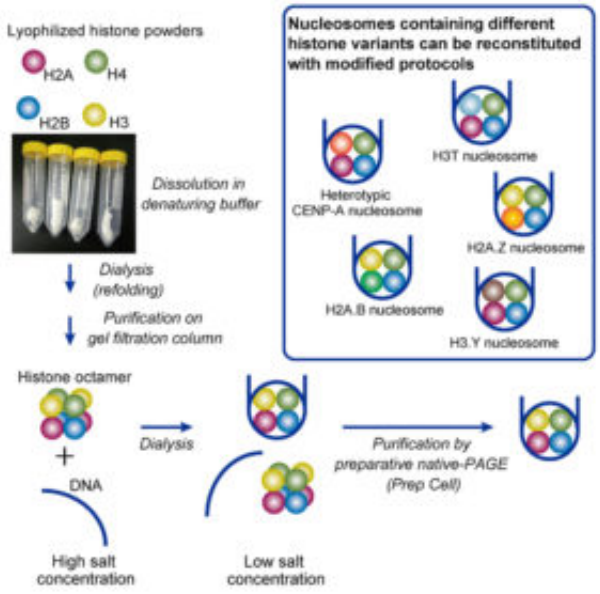

Fig. 1. Schematic representation of the reconstitution of nucleosomes containing histone variants.

His_6-tagged histones of interest are expressed in *E. coli*. The bacterial cells are harvested and disrupted by sonication, and then the proteins are denatured. The histones are purified by nickel-affinity chromatography, which captures His_6-tagged proteins. The His_6-tag peptide is removed by thrombin protease at room temperature for 3h (n.b.: for CENP-A and H3.Y, the thrombin digestion protocol differs slightly). Lastly, the histones are purified on a cation-exchange column. The purified

fractions are collected, dialyzed against water, and lyophilized. The histone powder is stored at 4°C.

The histone octamer, composed of histones H2A, H2B, H3, and H4 in equal stoichiometry, is then reconstituted. The lyophilized histone powders are mixed together and dissolved in denaturing buffer. The histones become correctly assembled during four consecutive sets of dialyses against high salt buffer. The histone octamer is then purified on a gel filtration column. After concentrating the fractions, the octamer is stored at -80°C.

When the four histones are not compatible for reconstitution as an octamer, H2A and H2B can be reconstituted as a dimer and H3 and H4 as a tetramer. The H2A-H2B dimer and H3-H4 tetramer are used for the nucleosome reconstitution, instead of the histone octamer. This is the case for H2A.B and H3T.

The reconstitution of the H2A.B-H2B dimer differs slightly from other dimer reconstitution protocols: after purification by Ni-NTA affinity chromatography, His_6-tagged H2A.B is mixed with the lyophilized H2B powder and then dialyzed against the denaturing buffer. The His-H2A.B-H2B dimer becomes reconstituted after four sets of dialyses with decreasing salt concentrations. The His_6-tag is then removed by thrombin protease. The H2A.B-H2B dimer is purified following the same procedures as used for the other histone complexes.

The nucleosome reconstitution is performed using the salt dialysis method with a histone octamer. Briefly, DNA and the histone octamer are mixed in an appropriate molar ratio in 2 M KCl high salt buffer (minor modifications are required when the nucleosome is reconstituted using the H2A-H2B dimer and H3-H4 tetramer). The sample mixture is then dialyzed until a 250 mM KCl salt concentration is achieved. The reconstituted nucleosome is finally purified by preparative native-PAGE, using a Prep Cell apparatus. The selected fractions are collected, concentrated, and stored at 4°C.

Using the heterotypic H3.3/CENP-A nucleosome as an example, the additional steps required for the reconstitution of heterotypic nucleosomes are presented below:

> The lyophilized H2A, H2B, His_6-SUMO tagged H3.3, CENP-A, and H4 are mixed together and dissolved in denaturing buffer. The octamer is reconstituted as described above and purified on a gel filtration column.
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> Nucleosomes are reconstituted with the octamers in the three possible combinations, H3.3/H3.3, H3.3/CENP-A, and CENP-A/CENP-A, and the heterotypic H3.3/CENP-A nucleosome is purified with a Prep Cell apparatus.
> The His_6-SUMO tag attached to H3.3 is then cleaved by PreScission protease. The sample is finally purified with a Prep Cell apparatus.

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