Assays of nucleosome assembly and the inhibition of histone acetyltransferase activity. (5) Preparation of core histones

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Method Article

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Abstract

Introduction

At present, the best-defined and most useful source of material for reconstitution of chromatin consists of purified core histones. These core histones can be prepared from either solubilized chromatin or H1-depleted chromatin by column chromatography on hydroxyapatite. To maintain the correct stoichiometry of all histones, we recommend preparation from H1-depleted chromatin by single-step elution with 2 M NaCl \(see Note 1). For a detailed introduction to assays of nucleosome assembly and the inhibition of histone acetyltransferase activity, please go here: "http://www.natureprotocols.com/2007/07/30/assays_of_nucleosome_assembly.php":http://www.natureprotocols.com/2007/07/30/assays_of_nucleosome_

Reagents

Preparation of Core Histones by Fast-Performance Liquid Chromatography \(FPLC) Buffer A: 50 mM Na-phosphate \(pH 6.8), 0.1 mM PMSF Buffer B: 50 mM Na-phosphate \(pH 6.8), 2 M NaCl, 0.1 mM PMSF Dialysis membrane \(MWCO 12,000-14,000; Spectrum Laboratories Inc.) Centricon \(MWCO, 30,000 and MWCO 10,000; Millipore Corp.) 20-mL hydroxyapatite column \(Bio-Gel HTP Gel, Bio-Rad Laboratories, Inc.) FPLC system \(Amersham Biosciences Co., Buckinghamshire, UK) **Further Purification and Concentration of Core Histones** Dialysis buffer: 0.2 M NaCl, 10 mM Tris-HCl \(pH 7.5), 1 mM EDTA, 0.1 mM PMSF Elution buffer: 2 M NaCl, 10 mM Tris-HCl \(pH 7.5), 1 mM EDTA, 0.1 mM PMSF 2-mL CM52 column \(5 mg histone/mL bed volume; Whatman International Ltd., Maidstone, England) Dialysis membrane \(MWCO, 6,000 to 8,000; Millipore Corp.)

Procedure

1. Dialyze the pooled H1-depleted chromatin overnight against buffer A at 4 °C. 2. Equilibrate a 20-mL hydroxyapatite column with buffer A and apply H1-depleted chromatin at a flow rate of 2 mL/min in 0% buffer B \(see Note 2). Wash the column with the same buffer under the same conditions for 30 min. 3. Elute non-histone proteins with 25% buffer B \(0.5 M NaCl), at a flow rate of 2 mL/min over 30 min. Elute the core histones as octamers wth 100% buffer B \(2 M NaCl) over 15 min \(collect 2-mL factions). Determine the concentration of core histones in each fraction from the absorbance \(OD_{230nm} = 4.2 corresponds to 1 mg/mL). Analyze the proteins in each fraction on an 18% polyacrylamide gel by SDS-PAGE \(see Note 3). 4. Pool fractions that contain core histones. Concentrate proteins to greater than 0.1 mg/mL for reconstitution of chromatin, if necessary, using a concentrator \(MWCO, 10,000) or a CM52 column. Store at 20 °C in siliconized tubes. **Further Purification and Concentration of Core Histones** 1. Dialyze core histones against dialysis buffer at 4 °C. 2. Prepare a 2-mL CM52 column \(5 mg histone/mL bed volume) and equilibrate with dialysis buffer. 3. Apply core histones to the column, wash thoroughly with 15 mL of dialysis buffer and elute with 5 mL of elution buffer. Measure the absorbance of a small aliquot in water at 230 nm to determine the concentration of histones. Store at 20 °C in siliconized tubes.

Critical Steps

1. When solubilized chromatin is applied to the hydroxyapatite column, wash out H1 first with 0.6 M NaCl in buffer. During this washing, some H2A/H2B is lost, destroying core-histone stoichiometry. Therefore, it is necessary to elute H2A/H2B and H3/H4 separately, with 1 M NaCl and with 2 M NaCl, respectively[1], and to re-adjust the histone stoichiometry for reconstitution of chromatin. 2. The total volume of the preparation of H1-depleted chromatin obtained by protocol A should be large \(~50 mL). In this case, apply sample chromatin to a column using a 50-mL super loop \(Amersham Pharmacia) or after concentrating H1-depleted chromatin to 1 mg/mL with a concentrator \(MWCO, 30,000). 3. Acrylamide:bisacrylamide, 37.5:1; SDS-polyacrylamide separating gel \[18% polyacrylamide \(w/v)] containing 0.375 M Tris-HCl \(pH 8.8); and SDS-polyacrylamide stacking gel \[4% polyacrylamide \(w/v)] containing 0.125 M Tris-HCl \(pH 6.8).

References

1. Kawase, H., Okuwaki, M., Miyaji, M., Handa, H., Ishimi, Y., Fujii-Makata, T., Kikuchi, A. & Nagata, K. NAP-1 is functional homologue of TAF-1 that is required for replication and transcription of the adenovirous genome in a chromatin-like structure. Gene Cells 1, 1045-1056 \(1996).