Assays of nucleosome assembly and the inhibition of histone acetyltransferase activity. (4) Preparation of histone H1-depleted chromatin

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Method Article

Keywords: histone chaperone, nucleosome assembly, inhibition of HAT, transcription factor, AP-1

Posted Date: July 30th, 2007

DOI: https://doi.org/10.1038/nprot.2007.336

Abstract

Introduction

The most efficient method for solubilizing the chromatin in isolated nuclei is to digest nuclei with Micrococcal nuclease \(see Note 1). Linker histones are then removed from the solubilized chromatin by FPLC on a hydroxyapatite column \(protocol A) or by a batch method with a cation-exchange resin \(protocol B). 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

 Nucleus-isolation buffer: 10 mM Tris-HCl \(pH 7.5), 1.5 mM $MgCl_2$, 1.0 mM $CaCl_2$, 0.25 M sucrose, 0.1 mM PMSF Micrococcal nuclease \(Worthington Biochemical Corp., Lakewood, NJ. U.S.A.; cat. no. LS004797), dissolved at 15,000 U/mL in water and stored frozen at -20°C 100 mM ethyleneglycol-bis-\(2-amino ethyl ether) N,N,N',N'-tetraacetic acid \(EGTA; Sigma-Aldrich Japan; cat. no. E3889) Lysis buffer: 10 mM Tris-HCl \(pH 6.8), 5 mM EDTA, 0.1 mM PMSF Protease inhibitor cocktail \(Roche Diagnostic GmbH, Mannheim, Germany; cat. no. 04-693-132-001) Dialysis bag \[molecular weight cutoff \(MWCO), 12,000-14,000; Spectrum Laboratories, Inc., Rancho Dominguez, CA, U.S.A.] Solution A: 0.1 mM PMSF Solution B: 1 M potassium phosphate \(K-phosphate; pH 6.8), 0.1 mM PMSF 20-mL hydroxyapatite column \(Bio-Gel HTP gel; Bio-Rad Laboratories Inc., Hercules, CA, U.S.A.) Fast protein liquid chromatography \(FPLC) system \(Amersham Pharmacia, Uppsala, Sweden) TEP buffer: 10 mM Tris-HCl \(pH 7.5), 1 mM EDTA, 0.1 mM PMSF Concentrator \[MWCO, 30,000; Amicon YM membrane, Millipore Corp.] Phosphate buffer: 50 mM sodium phosphate \(Na-phosphate; pH 7.0, 0.15 M NaCl, 0.1 mM PMSF Cation-exchange resin AG 50W-X2 \(Bio-Rad Laboratories Inc.) TEP buffer: 10 mM Tris-HCl \(pH 7.5), 1 mM EDTA, 0.1 mM PMSF

Procedure

1. Thaw the nuclear pellet rapidly at 37 °C with gentle agitation. 2. Dilute the nuclear pellet to 2 mg/mL DNA with nucleus-isolation buffer and incubate the solution at 35 °C for 5 min with constant gentle agitation. 3. Add Micrococcal nuclease \(25 U/mg of DNA) and digest for 10 min at 35 °C with constant gentle agitation to break up the chromatin in the nuclei. 4. Stop the digestion by adding 100 mM EGTA to a final concentration of 2.0 mM. Pellet the nuclei by centrifugation at 500 x g for 5 min at 4 °C and remove the supernatant. 5. To lyse the nuclei, resuspend the nuclear pellet in 10 mL of lysis buffer and mix vigorously on a vortex mixer. 6. Transfer the suspension of lysed nuclei to a dialysis bag and dialyze against 100 volumes of lysis buffer overnight at 4 °C. 7. Pellet the nuclear debris by centrifugation at 10,000 x g for 10 min at 4 °C and save the supernatant, which contains the solubilized chromatin \(the chromatin includes from 1 to 10 nucleosomes). Measure the absorbance of a small aliquot in water at 260 nm to determine the concentration of chromatin DNA. The yield should be about 50% of the original total DNA \(see Note 2). Add a cocktail of protease inhibitors to the solubilized chromatin. 8. Equilibrate hydroxyapatite in solution A and remove fine particles. Prepare a 20-mL hydroxyapatite column for FPLC. Equilibrate the column at 4 °C and wash it with 200 mL of 1% solution B \(10 mM K-phosphate) at a flow rate of 8 mL/min using the FPLC system. 9. Apply no more than 15 mg \(as DNA) of solubilized chromatin to the hydroxyapatite column at a flow rate of 4 mL/min in 1% buffer B. Wash the column with the same buffer for at least 20 min. 10. Elute material from the column under the following elution conditions: a linear gradient of 1% to 9% buffer B \(10 to 90 mM K-phosphate) over 5 min; a linear gradient of 9% to 35% buffer B \(90 to 350 mM K-phosphate) over 70 min \(Hl-depleted chromatin is eluted); and a linear gradient of 35% to 100% buffer B \(0.35 to 1.0 M K-phosphate) over 30 min \(H1 is eluted), at a flow rate of 4 mL/min. The fraction collector should be programmed to collect 8-mL fractions. 11. Analyze the proteins in each fraction by SDS-polyacrylamide gel electrophoresis \(18% polyacrylamide; see Note 3). Collect H1-depleted chromatin. 12. Dialyze the H1-depleted chromatin against TEP buffer overnight. Determine the concentration of DNA from the absorbance \(OD_{260nm} = 20 corresponds to 1 mg/mL DNA). Concentrate the chromatin to 0.3 mg/mL \(as DNA content) using a concentrator \(MWCO, 30,000) for chromatin reconstitution, if necessary. Freeze the chromatin sample rapidly and store at 80 °C \(see Note 4). 1. Dialyze the solubilized chromatin against K-phosphate buffer at 4 °C. 2. Add 4 mL of cation-exchange resin AG 50W-X2, equilibrated with K-phosphate buffer, to 20 mL of solubilized chromatin. Stir or shake gently at 4°C for 90 min. 3. Filter or decant the H1-depleted chromatin from the resin. Examine proteins in the preparation by SDS-PAGE \(18% polyacrylamide; see Note 3). 4. Dialyze overnight against TEP buffer. Determine the concentration of H1-depleted chromatin DNA from the absorbance \(OD_{260nm} =20 corresponds to 1 mg/mL DNA). Freeze the chromatin sample rapidly and store at 80 °C \(see Note 4).

Critical Steps

1. Sonication of isolated nuclei in ice-cold 0.2 mM EDTA \(pH 7.5), 0.25 mM PMSF, is also a possible method for breaking chromatin. However, insufficient sonication results in loss of most of the chromatin in the pellet of nuclear debris. 2. The yield depends on the conditions during the digestion of nuclei with the nuclease. The length of digested chromatin DNA should be checked by electrophoresis on a 1% agarose gel. The DNA should be approximately 146 bp to 2 kb in length. 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). 4. When purifying core histones from H1-depleted chromatin, skip this dialysis step.