Assays of nucleosome assembly and the inhibition of histone acetyltransferase activity. (2) Isolation and labeling of DNA fragments

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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.2007.334

Abstract

Introduction

\(see Note 1) There are several methods for assembling chromatin on DNA fragments or plasmids. Typical simple methods include \(1) the salt dialysis method, in which DNA and purified core histones are mixed and then dialyzed against a series of buffers, starting at 2 M NaCl and moving to lower concentrations of NaCl; and \(2) the histone octamer transfer method, in which histone octamers are redistributed from purified donor chromatin to specific and radiolabeled DNA fragments during incubation in buffer that contains 1 M NaCl, with subsequent reduction of the concentration of NaCl either by dialysis or by stepwise dilution[1]. The composition of the reconstituted chromatin is well defined and such chromatin is useful for the analysis of nucleosome positioning due to intrinsic sequence-dependent DNA structure and for assays of the binding of sequence-specific transcription factors or structural proteins to nucleosomal DNA. In vertebrates, core histones are strongly conserved and, to date, the limited interspecies variations that have been identified have not been found to be of any functional significance with respect to the analysis of chromatin. Therefore, cultured cells and chicken erythrocytes are convenient sources of core histones and chromatin. Moreover, contamination by proteases and nucleases of such preparations is relatively low. The above-described and other methods of salt-mediated assembly of nucleosomes fail to separate nucleosomes by the physiological linker distance of approximately 200 bp on general DNA fragments[1]. The multiple nucleosomes reconstituted by such methods are generally rather closely packed, with one nucleosome every 150 bp or so with little or no linker DNA. However, salt dialysis methods can be used to produce physiologically spaced oligonucleosomes when DNA templates with tandem repeats of strong nucleosome-positioning sequences are used. For example, a template containing ~200-bp tandem repeats of a DNA fragment that includes a Lytechinus gene for 5S rRNA has been used to assemble properly spaced oligonucleosomes[2]. Such oligonucleosomes have proved to be very useful for studies of higher-order chromatin structure and transcription[3]. Alternative methods for the assembly of chromatin make use of crude extracts of cell or of histone chaperones[1,4] at physiological ionic strength. We will not describe these methods in detail here. However, it is worth nothing that assembly of chromatin using cell extracts derived from Xenopus eggs[5] or Drosophila embryos[6] does produce regularly spaced chromatin in vitro. However, the composition of the reconstituted nucleosomes is complicated. \(see Note 2) A 197-bp fragment of pB100-Uless/strider is long enough to accommodate mononucleosomes and contains two tandem repeats of the 5S nucleosome-positioning element from Xenopus. Templates to be used for nucleosome reconstitution are typically radiolabeled to allow easy monitoring of the extent of reconstitution on gels, isolation of particles from sucrose gradients, and for subsequent footprinting analysis. **DRE and CRE Elements** For our model experiments, we isolated \[^{32}P]-radiolabeled 147-bp-long DNA fragments that contained either wild-type or mutated DRE[7] or CRE[8,9] sequences in triplicate by electrophoresis on a 10% polyacrylamide gel in 1x TBE buffer as described above. 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

Plasmid DNA \(pB100-Uless/strider, which contains the 197-bp fragment of 5S rDNA from _Xenopus borealis_) Oligodeoxynucleotides synthesized “in house” and corresponding to the differentiation response element \(DRE) and a mutated DRE[7], and to the cyclic-AMP response element \(CRE) and a mutated CRE[8,9]. CRE sequence: 5’-AGCTCCGATCCCG-3’, corresponding to the CRE that extends from nucleotide \(nt) –177 to nt –162 in the promoter of the mouse gene for fibronectin[9]. DRE sequence: 5’-TTACCTTCCCGAGCCT-3’, that corresponding to the DRE that extends from nt –194 to nt –175 in the promoter of the mouse _c-jun_ gene[7]. Mutated CRE and mutated DRE were 5’-AGCTCCGATCCCG-3’ and 5’-TTACCTTCCGAGCCTT-3’, respectively. _Ec_oR 1 \(New England Biolabs Inc., Beverly, MA, USA; cat. no. R0101) Calf intestinal alkaline phosphatase \(Toyobo Co., Osaka, Japan; cat. no. CAP-101) T4 polynucleotide kinase \(Toyobo Co.; cat. no. PNK-111) \[γ-^{32}P]ATP \(6000 Ci/mmol; GE Healthcare Biosciences Co.) Sephadex G-50 spin column \(GE Healcare Biosciences Co.; cat. no. 17-0041-01) 6% \(w/v) Polyacrylamide gel \(acrylamide:bisacrylamide, 19:1) prepared with 1x TBE \(10 x 10 x 0.1 cm3) 1x TBE: 89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA \(pH 8.3) Vertical gel-electrophoretic apparatus for fractionation of DNA and power supply Equipment for autoradiography Extraction buffer: 10 mM Tris-HCl \(pH 8.0), 1 mM EDTA, 0.1% sodium sulfate \(SDS; Sigma-Aldrich Corp., cat. no. L3771) Micropore separator \(Amicon, Millipore Corp., Bedford, MA, U.S.A; 0.22 mm pore size) Tris-EDTA \(TE) buffer: 10 mM Tris-Cl \(pH 8.0), 1 mM EDTA

Procedure

1. Digest ~20 mg of pB100-Uless/strider plasmid DNA with EcoR 1 and treat the digest with calf intestinal alkaline phosphatase \(Takara Biotech. Co.) at 37 °C for 60 min. Purify the DNA by ethanol precipitation and remove excess salt by gently rinsing the pellet with ice-cold 70% ethanol. 2. Radiolabel the 5'-end of the site of restriction cleavage using T4 polynucleotide kinase and \[γ-32P]ATP by standard methods. Remove unincorporated radiolabeled ATP with a Sephadex G-50 microcolumn, with subsequent ethanol precipitation as above. 3. Digest radiolabeled DNA with EcoR I, to liberate the 197-bp end-labeled DNA fragment. 4. Isolate the end-labeled DNA fragment on a preparative 5% nondenaturing polyacrylamide gel in 1x TBE. 5. After autoradiography of the wet gel, excise the radiolabeled band and crush the gel slice in an Eppendorf tube with a siliconized glass rod or pestle. 6. Add ~300 mL of elution buffer to the crushed gel, resuspend the fragments of gel in TE buffer and incubate the mixture at 37 °C to elute the end-labeled DNA fragment. 7. Filter the mixture with a MicropureTM separator \(Sartorius AG, Göttingen, Germany) to remove gel pieces. After extraction of DNA with a mixture of phenol, chloroform and isoamyl alcohol, precipitate the radiolabeled DNA fragment in ethanol. Resuspend the fragment in TE buffer.

Critical Steps

1. It is important to note that salt-mediated nuclesome-assembly methods fail to space nucleosomes with the physiological nucleosomal spacing of approximately 200 bp on general DNA fragments. Multiple nucleosomes reconstituted by these methods are generally “closely packed”, with one nucleosome every ~150 bp and little or no intervening linker DNA. However, salt-dialysis methods can be used to produce physiologically spaced oligonucleosomes if the

DNA template contains tandem repeats of strong nucleosome-positioning sequences. 2. For mononucleosome reconstitution, DNA of 200 to 270 bp is probably most suitable[1]. The 3'-end of the DNA is labeled using Klenow DNA polymerase and \[α-^{32}P]-NTPs.

References

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