Assays of nucleosome assembly and the inhibition of histone acetyltransferase activity. (6) Reconstitution of chromatin; (7) Analysis of nucleoproteins on an agarose gel; and (8) Purification of reconstituted chromatin on a sucrose gradient

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Introduction

Mononucleosomes can be reconstituted on 197-bp or 147-bp radiolabeled DNA fragments (see Notes 1 and 2) either by salt dialysis using purified core histones or by histone octamer transfer from donor H1-depleted chromatin. Usually, reconstitution yields naked DNA and DNA with mono-, di-, and trinucleosome cores.

The efficiency of reconstitution and the number of nucleosome cores on a labeled DNA fragment can be analyzed by agarose gel electrophoresis.

Reconstituted oligonucleosomes can be separated by sucrose gradient centrifugation on the basis of the number of histone octamers bound to a DNA fragment. We use 5% to 20% (w/v) linear sucrose gradients to isolate dinucleosomes from unreconstituted DNA, free histones, mono- and trinucleosomes.

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_assembly.php

Procedure

Salt Dialysis Using Purified Core Histones

1. Mix 5 µg of DNA (50-500 ng of radiolabeled DNA and unlabeled carrier DNA) and 5 µg of core histones in 75 to 100 µL of TEMP buffer prepared with 2M NaCl on ice, at a histone-to-DNA ratio (w/w) of 1:1 (see Notes 3 and 4).
2. Transfer the mixture to a dialysis bag and dialyze at 4 °C against 1 L of TEMP buffer that contains NaCl as follows: 2 M NaCl, overnight; 1.5 M NaCl, 1 h; 1 M NaCl, 4 h; 0.75 M NaCl, 4 h; and without NaCl, overnight.
3. Then dialyze the mixture against 1 L of TEMP buffer for another 4 h at 4 °C. Keep the reconstituted samples in siliconized tubes on ice until use (see Note 5).

Octamer Transfer from Donor Chromatin (see Note 6)

1. Mix 50-500 ng of radiolabeled DNA fragments with H1-depleted donor chromatin at a

chromatin DNA-to-fragment DNA ratio (w/w) of from 50:1 to 100:1 in 80 µL of TEMP buffer. Slowly adjust the concentration of NaCl to 1 M with 20 µL of 5 M NaCl.

2. Incubate the mixture at 37 °C for 20 min and then transfer it to a dialysis bag. Dialyze it at 4 °C against 1 L of TEMP buffer that contains 1 M NaCl for 4 h and then against 1 L of TEMP buffer that contains 0.75 M NaCl for 4 h.
3. Then dialyze the sample against TEMP buffer overnight at 4 °C.
4. Dialyze the sample for 4 h against 1 L of TEMP buffer at 4 h and then keep the reconstituted samples in siliconized tubes on ice until use (see Note 5).

1. Load samples in 3% (v/v) glycerol without dye onto a 0.7% agarose gel that is submerged in 2 L of 0.5x TBE.
2. Perform electrophoresis at room temperature, at 130 V, for 3 to 4 h (at less than 60 mA; see Note 7). After electrophoresis, put the gel on two sheets of 3MM paper (Whatman Co., Middlesex, UK) and start drying the gel without heating. Turn on the heater of the gel dryer after flattening the gel.
3. Subject the dried gel to autoradiography.

Purification of Reconstituted Chromatin on a Sucrose Gradient

1. Prepare 12-mL 5% to 20% (w/v) linear sucrose gradients using a gradient former or Gradient Master (see Note 8) at room temperature. Cool gradients at 4 °C and store for up to a few hours prior to use.
2. Load samples (less than 300 µL) on gradients and centrifuge at 36,000 rpm for 18 h at 4 °C in an SW41Ti rotor (Beckman).
3. Fractionate the gradients by pumping out from the bottom. The fraction collector should be programmed to collect 300-µL fractions. Analyze each fraction (10 µL of each) by electrophoresis on a 0.7% agarose gel.
4. Pool respective fractions that contain mono-, di-, and trinucleosomes separately, concentrate

proteins to greater than 5 µg/mL (as protein) using Centricon-30, and dialyze preparations against TEMP buffer at 4 °C to remove sucrose. Store samples in siliconized tubes on ice prior to use (see Note 5).

Binding of Linker Histones to Reconstituted Chromatin

Reconstituted short chromatin is a useful substrate for investigations of the binding of chromatin proteins, for example, linker histones and sequence-specific DNA-binding proteins, to nucleosomal DNA[2,3]. Native interactions between histone H1 and spaced dinucleosomes can be examined in vitro as follows.

1. Incubate dinucleosomes (20 ng of DNA) with various amounts of purified histone H1 in 10 µL of binding buffer at 25 °C for 20 min[4].
2. Analyze the binding of histone H1 by loading samples directly onto a 0.7% agarose gel and subsequent electrophoresis. The gel can be dried and subjected to autoradiography.

Critical Steps

1. It is possible to use biotinylated fragments of DNA that are generated by PCR with 5'-unlabeled and 5'-biotinylated primers[1]. Biotin-labeled dinucleosomes are detected with streptavidin-conjugated alkaline phosphatase and a chemiluminescent substrate, e.g., CPD-Star (Boehringer Ingelheim GmbH, Ingelheim, Germany), on a nylon membrane after gel electrophoresis. Biotinylated nucleosomes are not suitable for footprinting analysis.
2. When a high concentration of specific chromatin templates is necessary for a subsequent assay (e.g., a transcription assay in vitro), use unlabeled specific fragments of DNA as carrier DNA.
3. The nucleosome contains 200-bp DNA (MW 130,000) and a histone octamer (MW, 108,000), so that the ratio of histone to DNA (w/w) is almost equal to the molar ratio

of histone octamer to nucleosome DNA.

4. The efficiency of reconstitution is affected by the length and the structure of both the labeled and the carrier DNA. It is recommended that at least three different ratios of histone to DNA (w/w), for example, 0.6:1, 0.9:1 and 1.2:1, be tested in the first reconstitution to identify optimal conditions. A ratio that is too high (>2:1) yields aggregates. Also, it is not recommended that the volume be below 75 µL or that the DNA concentration be below 50 µg/mL.
5. Reconstituted chromatin should be stable for a few months under these conditions.
6. The chromatin reconstituted by this method contains a large amount of nonspecific donor chromatin.
7. Reconstituted nucleosomes are destabilized when the gel is heated during electrophoresis. Do not perform electrophoresis with a current in excess of 2.5 mA/cm.
8. Six gradients can be prepared rapidly and simultaneously using a “Gradient Master”.

References

1. Weissman, J. D., Brown, J. A., Howcroft, T. K., Hwang, J., Chawla, A., Roche, P. A., Schiltz, L., Nakatani, Y. & Singer, D. S. HIV-1 tat binds TAFII250 and represses TAFII250-dependent transcription of major histocompatibility class I genes. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 11601-11606 (1998).54.
2. Thoma, F. & Koller, T. Influence of histone H1 on chromatin structure. *Cell* **12**, 101-107 (1977).
3. Owen-Hughes, T. & Workman, J. L. Experimental analysis of chromatin function in transcription control. *Crit. Rev. Eukaryot. Gene Expr.* **4**, 403-411 (1994).
4. Hayes, J. J. & Wolffe, A. P. Preferential and asymmetric interaction of linker histones with 5S DNA in the nucleosome. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6415-6419 (1993).

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