Measuring Nucleosome Assembly Activity in vitro with the Nucleosome Assembly and Quantification (NAQ) Assay

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Abstract

Nucleosomes organize the eukaryotic genome into chromatin. In cells, nucleosome assembly relies on the activity of histone chaperones, proteins with high binding affinity to histones. At least a subset of histone chaperones promotes histone deposition *in vivo*. However, it has been challenging to characterize this activity, due to the lack of quantitative assays.

Here we developed a quantitative nucleosome assembly (NAQ) assay to measure the amount of nucleosome formation *in vitro*. This assay relies on a Micrococcal nuclease (MNase) digestion step that yields DNA fragments protected by the deposited histone proteins. A subsequent run on the Bioanalyzer machine allows the accurate quantification of the fragments (length and amount), relative to a loading control. This allows us to measure nucleosome formation by following the signature DNA length of ~150 bp. This assay finally enables the characterization of the nucleosome assembly activity of different histone chaperones, a step forward in the understanding of the functional roles of these proteins *in vivo*.

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Materials

RPMI 1640 Medium 11875093 by Thermo Fisher Scientific

Protocol

Step 1.
Micrococcal Nuclease digestion and DNA purification

Step 2.
Take 25 µl of the nucleosome assembly reaction and mix with 10 µl of 10x MNase buffer, 1 µl 100x BSA, 1 µl of MNase (stock at 25 U/µl) and 63 µl of water.

Step 3.
Incubate for 10 min in a thermoblock at 37 °C

Step 4.
Quench the reaction by adding 10 µl of 500 mM EDTA (final EDTA concentration 50 mM).

Step 5.
Optional: Treat the sample with 25 µg of Proteinase K (1.25 µl of a 20 mg/ml solution) for 20 min at 50

°C.

Step 6.
Add 550 µl of PB buffer from MinElute kit (QIAGEN) and 10 µl of 3 M Na acetate pH 5.0 solution.

Step 7.
Incubate for 10 min at room temperature.

Step 8.
Add 50 ng of loading control DNA (stock at 25 ng/µl).

Step 9.
Apply sample to the spin column.

Step 10.
Centrifuge for 1 min at 16,000 x g at room temperature.

Step 11.
Discard flow-through.

Step 12.
Wash membrane with 100 µl of PB buffer.

Step 13.
Centrifuge for 1 min at 16,000 x g at room temperature.

Step 14.
Discard flow-through.

Step 15.
Wash membrane with 700 µl of PE buffer.

Step 16.
Centrifuge for 1 min at 16,000 x g at room temperature.

Step 17.
Discard flow-through.

Step 18.
Centrifuge for 1 min at 16,000 x g at room temperature.

Step 19.
Discard flow-through.

Step 20.
Apply 10 µl of ddH2O to the spin column (make sure the tip of the pipette is in the center of the membrane).

Step 21.
Incubate for 10 min at room temperature.

Step 22.
Place spin column into a clean Eppendorf tube.

Step 23.
Centrifuge for 1 min at 16,000 x g at room temperature.