Nucleosome microscale assembly protocol

DNA–binding proteins
Protocol
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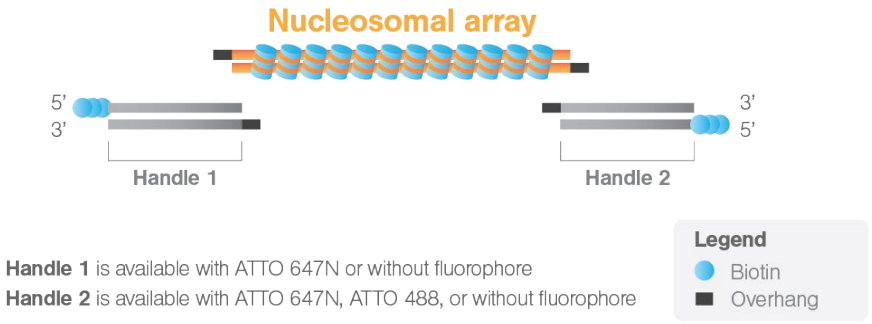
Using the DNA tethering kit, nucleosomes can be tethered in optical tweezers to study how DNA binding proteins and nucleosomes interact and regulate each other. Here we describe a procedure to reconstitute a nucleosomal array *in vitro* and to ligate the construct to biotinylated DNA handles for tethering in the C-Trap.

Using an adapted version of a microscale nucleosome assembly protocol[1], human histone octamers were loaded on a 2.1 kb DNA sequence (exact size 2,097 bp) containing 12 repeats of clone-601 DNA (172 bp). This sequence was obtained by digestion of pUC-LUMICKS-601 plasmid with BsaI enzyme, which produce overhangs compatible with the 6.3 kb handles provided with the Nucleosome tethering kit (protocol link coming soon). The nucleosomal array was then ligated to the 6.3 kb handles and tethered in the C-Trap to determine the presence of nucleosomes by mechanical unwrapping.

Important note: Reconstituted nucleosomal arrays are in high concentration of NaCl. Therefore, Salt-T4® DNA Ligase from New England BioLabs (supplied by the user; catalog #M0467) was used to efficiently ligate nucleosomes to the 6.3 kb handles.



Handle 1 is available with ATTO 647N or without fluorophore
Handle 2 is available with ATTO 647N, ATTO 488, or without fluorophore

Material

- 2.1 kb DNA with 12 repeats of clone-601 DNA (140 ng/µl), produced using the guidelines to make a sequence of interest described in the Nucleosome tethering kit protocol. **Important note**: purified 2.1 kb DNA was in 10 mM Tris pH8, 2 M NaCl to ensure the same concentration of NaCl as the histone octamer.
- Salt-T4® DNA Ligase (New England BioLabs, catalog #M0467)
- Human Histone Octamer stock (EpiCypher catalog, #16-0001) is 900 ng/µl in 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 2 M NaCl, 2 mM DTT, 20% glycerol.
- Amicon Ultra-0.5 mL Centrifugal Filters 30 kDa cut-off (Millipore UFC503008).

Protocol

Microscale nucleosome reconstitution was performed using a molar ratio of 1.7 to 1 of Histone Octamer to DNA repeats. Initial volumes and concentrations were chosen to ensure a final minimal concentration of ~10 ng/µl of DNA loaded with histone octamer.

The concentration of NaCl was reduced from 2 M to 200 mM following the steps below:

- Dilute 2-fold 5 µl of the histone octamer stock (900 ng/µl) by adding 5 µl of 10 mM Tris-HCl, 2 M NaCl pH 7.6 obtaining a final volume of 10 µl and a concentration of 450 ng/µl.
- Mix 1.6 µl of diluted histones to 3.25 µl of 2.1 kb DNA. This results in a molar ratio of 1.7 to 1 of histone octamer per DNA repeat. *(DNA concentration is 94 ng/µl, salt concentration is 2 M. total volume 4.85 µl)*
- Incubate for 30 min at room temperature.
- Add 4.85 µl of 10 mM Tris-HCl, pH 7.6. Mix with pipette, avoiding bubble formation. *(DNA concentration is 47 ng/µl, salt concentration is 1 M, total volume is 9.7 µl).*
- Incubate for 1 hour at room temperature.
- Add 2.4 µl of 10 mM TrisHCl, pH 7.6. Mix with pipette, avoiding bubble formation. *(DNA concentration is 38 ng/µl, salt concentration is 0.8 M, total volume is 12.1 µl).*
- Incubate for 1 hour at room temperature.
- Add 2.4 µl of 10 mM TrisHCl, pH 7.6. Mix with pipette, avoiding bubble formation. *(DNA concentration is 31 ng/µl, salt concentration is 0.67 M, total volume is 14.5 µl).*
- Incubate for 1 hour at room temperature.
- Add 34 µl of 10 mM TrisHCl, pH 7.6. *(Final DNA concentration is 9.4 ng/µl, salt concentration is 0.2 M, total volume is 48.5 µl)*
- Incubate for 1 hour at room temperature.

DNA Ligase reaction of the nucleosomal array to 6.3 kb DNA handles

Here, reconstituted nucleosomal array (9.4 ng/µl in 0.2 M NaCl) is ligated to 6.3 kb handles as follow. The control reaction with the 3 kb control DNA (50 ng/µl, included in the DNA tethering kit) is also described

1. Quick spin down the kit vials before use to avoid losing any reagents through the tube caps.
2. Prepare the following reaction mixtures, by first mixingSalt-T4® DNA Ligase 10x buffer with Milli-Q, then adding the DNA handles and DNA.
3. Mix gently by pipetting up and down.
4. Finally add Salt-T4® DNA Ligase and mix again by gently pipetting up and down avoiding bubble formation.

Components	Nucleosome Ligation	Control Ligation
Salt-T4® DNA Ligase 10x buffer	2 µl	2 µl
DNA handle 1	5 µl	5 µl
DNA handle 2	5 µl	5 µl
DNA	8 µl	2 µl
Milli-Q	- µl	6 µl
Salt-T4® DNA Ligase	1 µl	1 µl

5. Incubate for 2 hours at room temperature.

Buffer exchange

Upon completion of the DNA Ligase reaction, perform a buffer exchange with Tris-HCl 40 mM, 140 mM NaCl, EDTA 0.1 mM, pH 8.

- Insert Amicon® Ultra-0.5 30 kDa cut-off device into one of the provided microcentrifuge tubes.
- To wash the filter device: add 500 µl Milli-Q water to the filter device, invert few times to clean, discard the Milli-Q.
- Repeat the previous step once.
- To equilibrate the filter device: add 500 µl of buffer and centrifuge at 11,500 RCF for 3 minutes.
- Discard remaining buffer both from the filter device and the microcentrifuge tube.
- Dilute DNA Ligase reaction up to 500 µl with buffer, add it to the filter device and centrifuge at 11,500 RCF for 5 minutes.

You should obtain ~50 µl of DNA Ligase reaction in the filter device after 5 minutes of centrifugation.

- Discard the flowthrough from the microcentrifuge tube.
- Add 450 µl of buffer to the filter device, centrifuge for 5 minutes at 11,500 RCF.
- As described by the manufacturer: to recover the concentrated solute, place the Amicon® Ultra filter device upside down in a clean provided microcentrifuge tube. Place in centrifuge, aligning open cap towards the center of the rotor; counterbalance with a similar device. Spin for 2 minutes at 1,000 RCF to transfer the concentrated sample from the device to the tube. The ultrafiltrate can be stored in the centrifuge tube.
- Measure concentration by nanodrop. You should obtain a concentration ranging from 10 ng/µl to 30 ng/µl.

References

[1] Pamela N. Dyer, Raji S. Edayathumanhalam, Cindy L. White, Yunhie Bao, Srinivas Chakravarthy, Uma M. Muthurajan and Karolin Luger. Reconstitution of Nucelosome Core Particles from Recombinant Histones and DNA. Methods in enzumology, 2004.

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