Nucleosome tethering kit

DNA–binding proteins Protocol

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LUMICKS

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1. Description

The nucleosome tethering kit enables the tethering of nucleosomal arrays in optical tweezers to study the interactions and regulation between DNA binding proteins and nucleosomes.

The kit includes biotinylated 6.3 kb DNA handles (exact size 6,298 bp) and the **pUC-LUMICKS-601** plasmid. The plasmid contains a 12-repeat array of a 172 bp clone-601 DNA sequence flanked by restriction sites for the type IIS restriction enzyme BsaI. Upon digestion with BsaI, the DNA repeat array can be purified from the plasmid, loaded with histones, and finally ligated to the 6.3 kb handles (Figure 1).

The ligation is designed so that DNA handle 1 will be ligated at the 5' end of the nucleosomal array, and DNA handle 2 will be ligated at the 3' end. Additionally, the kit offers different versions of handles with or without ATTO fluorophores. The options for handle 1 include with ATTO 647N or without a fluorophore, while for handle 2, the options are with ATTO 647N, ATTO 488, or without a fluorophore.

Once the nucleosomes are ligated to the two DNA handles, the biotin moieties of each DNA handle will allow tethering of the nucleosomes between two streptavidin-coated beads trapped in optical tweezers (Figure 1). In this configuration, if handles with fluorophores are chosen, the fluorophores will flank the nucleosomes (11 bps from the ligation sites) and can be used to position the SOI on the focal plane before incubation with fluorescent proteins. This enables the setup of optimal fluorescence imaging conditions before starting a DNA-protein interaction measurement, which allows capturing even the first interactions. By choosing handles labeled differently, an asymmetric configuration with the nucleosomal array can be achieved, and the 5'-3' directionality can be determined when tethered in the C-Trap. Additionally, in case both handles are labeled with ATTO fluorophores, the known distance between the two fluorophores can be used as a ruler on the tethered DNA to precisely determine the position on the DNA sequence of fluorescent proteins interacting with the sequence of interest.

In addition to the DNA handles and pUC-LUMICKS-601 plasmid, the kit includes a 3 kbp control DNA with overhangs (exact size 3,065 bp). As nucleosome preparations are typically performed in a buffer containing a relatively high concentration of salt (>0.1 M NaCl), we recommend using Salt-T4® DNA Ligase from New England BioLabs (supplied by the user; catalog #M0467) for the ligation of the nucleosomes to the DNA handles.

Figure 1.

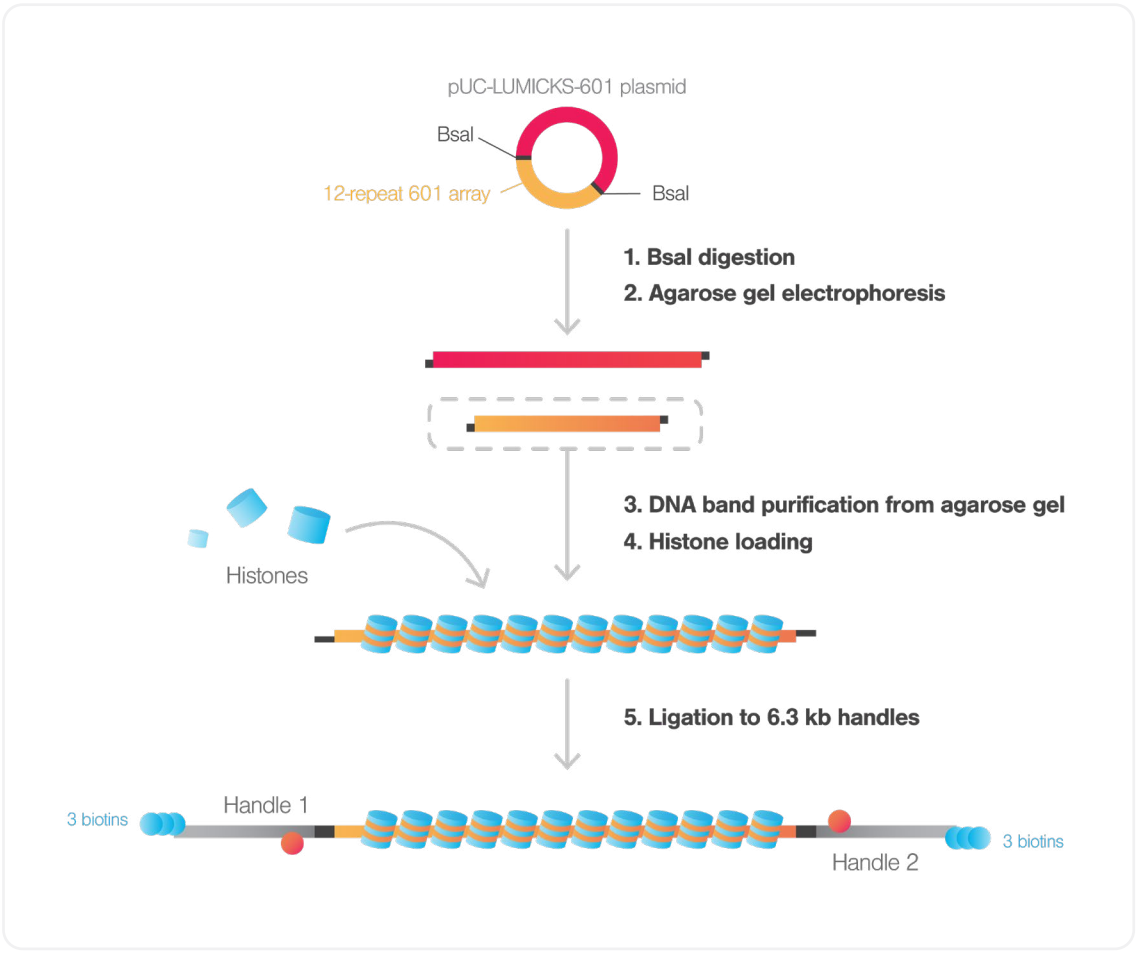

Figure 1 pUC-LUMICKS-601 plasmid is digested with BsaI and run on Agarose gel, where the band corresponding to the 12-repeat 601 array (yellow) is extracted and purified form gel. Purified 12-repeat 601 array DNA is used to reconstitute a nucleosome array (see section 3). Nucleosome array is finally ligated to the 6.3 kb handles provided with the kit (see section 4)

2. Materials supplied in the kit

Table 1.

Components	Units	Volume	Storage temperature
Biotinylated DNA Handle 1 (6,298 bp)	1	20 µl	-20 ˚C
Biotinylated DNA Handle 2 (6,298 bp)	1	20 µl	-20 ˚C
pUC-LUMICKS-601 plasmid (50 ng/ul)	1	15 µl	-20 ˚C
Control DNA (3,065 bp; 50 ng/µl)	1	6 µl	-20 ˚C

2.1 Additional Materials (supplied by the user)

- TypeIIS restriction enzyme BsaI (see protocol below).
- Salt-T4® DNA Ligase (New England BioLabs M0467)

To perform the microscale nucleosome tethering protocol

- Human Histone Octamer (EpiCypher catalog #16-0001)
- Amicon Ultra-0.5 mL Centrifugal Filters 30 kDa cutoff (Millipore UFC503008)

3. Digestion with BsaI and nucleosome preparation

By digestion with BsaI, the 12-repeat array is released as a 2097 bp fragment from the pUC-LUMICKS-601, while the remaining plasmid backbone will result in a 2680 bp fragment. It is necessary to purify the 2097 bp fragment for the loading of histones. One possibility is by electrophoresis on 0.6% agarose gel and purification of the band that corresponds to 2097 bp using a DNA purification kit of choice.

The purified DNA array can then be used to reconstitute nucleosomes using a method of choice. We have successfully utilized an adapted version of a microscale nucleosome reconstitution protocol (1) with the purified 12-repeat DNA array and Human Histone Octamer (EpiCypher #16-0001). A detailed protocol can be found at the following **link**.

4. Ligation of nucleosomes to DNA handles

After nucleosomes are formed, the BsaI overhangs can be used for the ligation to the 6.3 kb DNA handles provided with the kit. An example of a ligation reaction is provided here, which involves nucleosomal arrays and the 6.3 kb handles, as well as a control reaction with 3 kb control DNA included in the kit. Since nucleosome preparations typically have a high salt concentration, the Salt-T4® DNA Ligase (New England BioLabs M0467), which is not included in the kit, is used for the reaction.

1. Before use, spin down the kit vials briefly to prevent loss of reagents through the tube caps.
2. Prepare the following reaction mixtures, by first mixing 10x buffer with Milli-Q, then adding the DNA handles and Nucleosomes.
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.
5. Incubate for 2 hours at room temperature.

	Nucleosome Ligation	**Control Ligation**
Salt-T4® DNA Ligase 10x buffer	2 µl	2 µl
Biotinylated DNA Handle 1	5 µl	5 µl
Biotinylated DNA Handle 2	5 µl	5 µl
Nucleosomes or control DNA	0.05 pmol	2 µl (0.05 pmol)
Milli-Q	Up to 20 µl	6 µl
Salt-T4® DNA Ligase	1 µl	1 µl

5. 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.

6. Nucleosomes tethering in the C-Trap

6.1 Experimental set-up for the microfluidics system with laminar flow cell

Prepare the following solutions with buffer of choice for the different channels of LUMICKS' u-Flux™ microfluidics system and load them in their corresponding syringes.

Monitoring of nucleosome unwrapping as a function of forces applied in the C-Trap has been previously described (2).

Channel 1

- Add 1 µl of streptavidin-coated polystyrene beads (1.5–1.9 µm, 1% (w/v)) to 1 ml of buffer and load in the syringe of channel 1.

Channel 2

- Dilute the DNA construct formed by ligation of SOI to the 6 kbp to a concentration of 0.10 ng/µl in 500 µl buffer and add it to the syringe of channel 2.

Channel 3

- Load 1 ml of buffer in the syringe of channel 3.

Channel 4 (and 5)

Every channel where proteins of interest are flowed in or DNA-protein interactions are measured should be passivated, preventing proteins from non-specifically interacting with the surfaces of the flow cell.

6.2 Nucleosome tethering

- Apply flow to channels 1, 2, and 3 with a pressure of 0.3–0.4 bar.
- Catch a single streptavidin-coated bead in each of the traps in channel 1, align the traps on the y-axis, and move them to channel 2.
- Keep the flow of channels 1-3 open and move Trap 1 to a distance of approximately half the contour length of your construct from Trap 2. Then, increase the distance again and determine if there is a tether formed by observing the force response.
- Once tethers are formed, characterization of a 12-nucleosome array can be performed as previously described (2).
- For fluorescence microscopy, activate the corresponding excitation laser and begin recording either a sequence of 2D frames or a kymograph. To enhance the image quality, adjustments may be needed for the two traps along the z-axis to align the DNA with the imaging volume. This can be accomplished by moving the traps synchronously in the z-direction and utilizing the fluorophores of the DNA handles as a reference to determine the optimal height.

References

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2. Spakman, D., King, G.A., Peterman, E.J.G. et al. Constructing arrays of nucleosome positioning sequences using Gibson Assembly for single-molecule studies. Sci Rep 10, 9903 (2020).

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