DNA structural mechanics kit (for training)

DNA–binding proteins Protocol

2025

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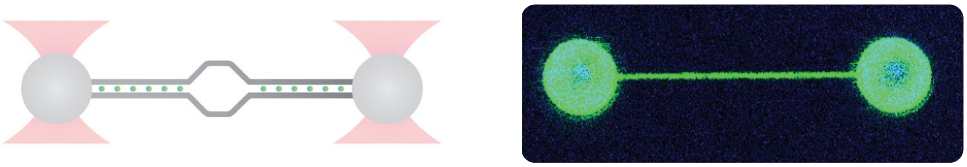
Fluorescent intercalators can bind between basepairs and are very suitable to visualize double-stranded DNA. The binding is force dependent and this training kit is therefore very suitable to show the force-fluorescence relation in the C-Trap and train new users.



Materials supplied in the kit

Components	Units	Volume	Storage temperature
Biotinylated λ DNA 2x ATTO 647N	1	20 µl	+4 ˚C
SYTOX Orange / YOYO-1	1	5 µl	-20 ˚C
Streptavidin beads (ø4.0–4.9 µm)	1	200 µl	+4 ˚C

The concentrations of the dyes are: SYTOX Orange: 5mM, YOYO-1.

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

For overall detailed instructions, consult the C-Trap manual or watch the tutorial video at the following **link**. The instruction video includes a tutorial with SYTOX Orange as well.

Prepare the following conditions for the different channels of the microfluidics system and load them in their corresponding syringes. Prepare 5 ml of running buffer 1x by diluting 500 µl running buffer 10x in 2.7 ml Milli-Q (creating PBS with 1.5 mM sodium azide and 0.5 mM EDTA).

Channel 1

- Add 10 µl of streptavidin beads (ø4.0–4.9 µm, 0.5% (w/v)) to 1 ml of running buffer 1x and use for channel 1.

Channel 2

- Add 1 µl biotinylated λ DNA 2x ATTO 647N stock to 1 ml of running buffer 1x and use for channel 2.

Channel 3

- Use 1 ml of running buffer 1x and use for channel 3.

Channel 4.

- Dilute the fluorescent intercalator (SYTOX Orange / YOYO-1) 1000 times and add 5 µl of this dilution to 1 ml running buffer 1x prepated for channel 4.

Catching beads, DNA tethering, and visualization

- 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 and while being aligned on the y-axis move these to channel 2.
- Keep the flow of channels 1-3 open and oscillate the trap on the right back and forth and determine if there is a tether formed by observing the force response. When a tether has formed, stop the flow for all channels and move to channel 3. You will be able to discriminate between single or multiple tethers by looking how the real-time force-distance data (displayed on the F,d Bluelake tab) matches the worm-like chain model for lambda DNA printed on the same tab. The presence of multiple tethers between the beads will appear as a force rise at a shorter distance than the distance predicted by the worm-like chain model. In addition, the DNA overstretching plateau will appear at higher forces proportionally to the number of tethers between the beads (i.e. around 65 pN for one molecule, 120 pN for two, and so forth).
- Aim for forming a single tether between the beads. In case of multiple tethers, they can be broken in channel 3 by increasing the force until a single tether remains. In case multiple tethers are regularly being formed the DNA concentration can be lowered.
- A force distance curve can be recorded to validate that there is only a single DNA tether. When the presence of a single tethered molecule is validated, it can be further moved to channel 4.
- Before going into channel 4, the confocal image settings can be finetuned using the two ATTO 647N fluorophores. Position a 2D scan region to include both beads. Take a single 2D scan with the red laser (638 nm) ON and confirm that both beads are within the field of view. Confirm that the ATTO 647N fluorophores of the DNA are in focus by moving “Trap1+Trap2” along the z-axis.
- Make sure the fluorescent intercalator solution has filled channel 4. After that, it is not needed to apply flow to channel 4 anymore. Activate the corresponding laser.
- With the finetuned image settings, either single intercalator binding events or full DNA coating will be observed depending on the concentration and force. Intercalators can only bind with sufficient tension on the DNA template. This tension can be exerted either by using the optical traps or by applying extra flow. Increasing the tension to the overstretching plateau will allow you to visualize melting bubbles of single-stranded DNA as black areas along the DNA.

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