DNA structural mechanics kit (for training)

DNA–binding proteins Protocol

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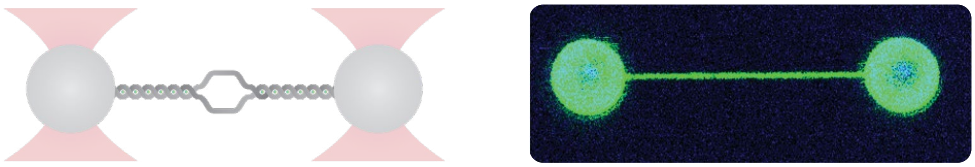
Fluorescent intercalators can bind between basepairs and are very suitable to visualize double-stranded DNA. The binding is force dependent and this training kit is therefore very suitable to show the force-fluorescence relation in the C-Trap and train new users.



Materials supplied in the kit

Components	Units	Volume	Storage temperature
Biotinylated double-stranded DNA (48,502 bp) with 2x ATTO 647N	1	20 µl	+4 ˚C
SYTOX Orange / YOYO-1 / DiTO-3	1	5 µl	-20 ˚C
Streptavidin-coated polystyrene beads (⌀ 4.0–4.9 µm)	1	200 µl	+4 ˚C

The concentrations of the dyes are: SYTOX Orange: 5mM, YOYO-1: 1 mM, DiTO-3: 5 mM.

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

Prepare the following conditions for the different channels of the microfluidics system and load them in their corresponding syringes.

Channel 1

- Dilute running buffer 10 times (creating PBS with 5 mM sodium azide and 0.5 mM EDTA) and use 1 ml for channel 1.
- Add 10 µl of streptavidin-coated polystyrene beads (4.0–4.9 µm, 0.5% (w/v)).

Channel 2

- Dilute running buffer 10 times (creating PBS with 5 mM sodium azide and 0.5 mM EDTA) and use 1 ml for channel 2.
- Add 1 µl biotinylated double-stranded DNA (48,502 bp) (20 ng/µl).

Channel 3

- Dilute running buffer 10 times (creating PBS with 5 mM sodium azide and 0.5 mM EDTA) and use 1 ml for channel 3.

Channel 4

- Dilute running buffer 10 times (creating PBS with 5 mM sodium azide and 0.5 mM EDTA) and use 1 ml for channel 4.
- Dilute the fluorescent intercalator (SYTOX Orange / YOYO-1 / DiTO-3) 1000 times and add 5 µl of this dilution to the buffer 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.
- The ATTO 647N fluorophores can be used to bring the DNA in focus. Activate the corresponding excitation and record either a sequence of 2D frames or a kymograph. In order to improve the quality of the images, the two traps might need to be adjusted on the z-axis to align the DNA with the imaging volume. You can adjust them by moving them in tandem on z. To start the measurement with the fluorescent intercalator, move to channel 4.
- Make sure the fluorescent intercalator solution has filled channel 4. After that, you it is not needed to apply flow to channel 4 anymore. For the optimized z-position of the two traps, either single intercalator binding events or full DNA coating will be observed depending on the concentration

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