Biotinylated and digoxigenin-labeled double-stranded DNA (48,524 bp)

DNA–binding proteins
Protocol
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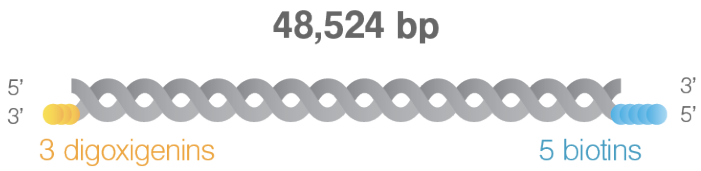
Biotinylated and digoxigenin-labeled double-stranded DNA (48,524 bp)

The length of the biotinylated and digoxigenin-labeled double-stranded λ-DNA (48,524 bp) makes it ideal for assessing different types of DNA binding molecules. The differently labeled ends of the DNA (3 digoxigenins on the 3' end and 5 biotins on the 5' end of the same strand) enables to determine the directionality of λ-DNA trapped in optical tweezers.



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. For optimal results, dilute running buffer 10 times with Milli-Q water to obtain running buffer 1x (PBS with 5 mM sodium azide and 0.5 mM EDTA). Alternatively, 40-60 mM Phosphate or Tris buffer at pH 7.5 containing 50-150 mM of common salts are recommended.

Channel 1

- Add 5 µl anti-digoxigenin coated beads (2.0-2.4 µm beads (0.5% (w/v)) into a new tube and vortex for 10 s.
- Add 1 µl of biotinylated and digoxigenin-labeled DNA (48,524 bp) to the beads. Mix immediately by gently pipetting 5 times. Avoid bubble formation. Incubate for 10 min at room temperature.
- Dilute the bead-tethered DNA with 500 µl of running buffer 1x and load it into the syringe of channel.

Channel 2

- Load running buffer 1x into syringe of channel 2.

Channel 3

- Dilute streptavidin coated beads in running buffer 1x according to the beads' size and concentration (e.g. 1:1000 for polystyrene streptavidin beads of ⌀ 1.5 – 1.9 µm and 1% w/v) and load it into the syringe of channel 3.

Catching beads and DNA tethering

Here, we describe an optimized workflow to make tethers with two type of beads (i.e. different coating and size).

Trap positioning and calibration:

- Open channels 1–3 and apply a pressure of 0.3-0.5 bar.
- Determine the positions of Trap 1 and Trap 2 by catching 1 bead per trap.
- Bring both trapped beads into channel 2 by moving the microstage and stop the flow.
- Visualize Trap 1 and align Trap 1 with the red cicrle, either by moving the “Trap 1 + 2” control or press “shift” and left-click on the bead in Trap 1 (Figure 1).

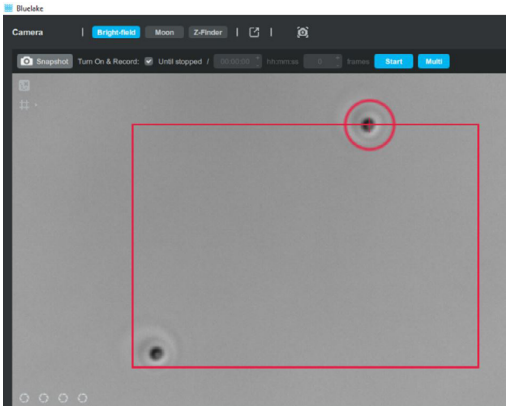
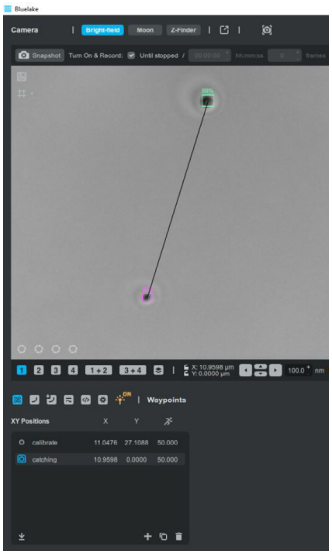


Figure 1

- Move Trap 1 vertically to the top edge of the rectangle and Trap 2 to the bottom part of the rectangle to maximize the y-axis distance between the two beads, as shown in Figure 2. You can now save the position of Trap 1 as “catching”.
- Define a template for each of the two beads on the brightfield camera window (Figure 2).
- Align Trap 1 at a 15 µm distance from Trap 2 at the same horizontal plane (Figure 3). You can save the Trap 1 position as “tethering”. The saved “catching” and “tethering” positions of Trap 1 allow you to automatize and speed up the tethering procedure.
- Perform trap calibration according to the different sizes of the two trapped beads.



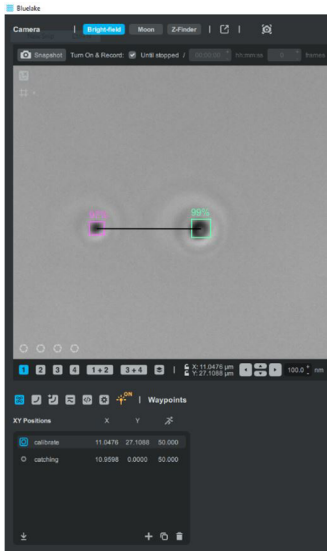


Figure 2 (left) and figure 3 (right).

Catching beads with the optical traps:

- Release the beads from the previous step by applying flow to channels 1, 2, and 3 with a pressure of 0.3–0.5 bar.
- Set the position of Trap 1 to “catching”.
- Fine-tune the position of the microstage to move Trap 2 to channel 1 and catch a single anti-digoxigenin bead.
- Move the microstage until Trap 1 localizes at the edge between channels 2 and 3.
- Fine-tune the position of the microstage until Trap 1 is in channel 3 and catches a single streptavidin-coated bead. Keep Trap 2 inside channel 2.
- Move the two traps to channel 2 by moving the microstage keeping the flow on.

Tethering of the DNA between two beads to form the dumbbell configuration:

- In channel 2, align Trap 1 with Trap 2 on the horizontal plane (same y-axis) at a distance of 15 µm apart (Figure 3).
- Move Trap 1 at 8-10 µm distance from Trap 2. Increase the distance again and determine if there is a tether formed by observing the force response. Repeat and tentatively reduce the initial distance between the two beads until a force response is observed. 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 fror a single tether between the beads. In case of multiple tethers, they can be broken in channel 2 by increasing the force until a single tether remains. In case multiple tethers are regularly being formed, the DNA concentration incubated with the anti-digoxigenin beads can be lowered.

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