Generating single-stranded DNA

DNA–binding proteins
Protocol
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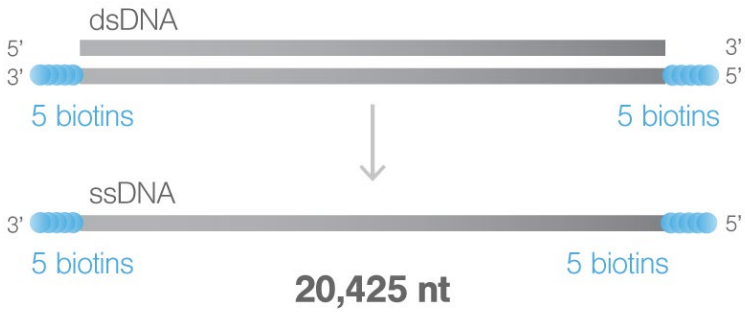
The single-stranded DNA is shipped as double-stranded DNA that is biotinylated at the 5’ and 5’ ends of the same strand. After melting the DNA one of the strands will fall off and a single stand remains.



Important points before generating single-stranded DNA

- When pipetting the DNA from the stock solution, do so very slowly to avoid centrifugal forces that might nick the DNA. Alternatively, cut the pipette-tip with clean scissors to enlarge it.
- A 25%-40% efficiency of single strand formation upon DNA melting is standard for this sample. In case your efficiency rate is much lower, please contact LUMICKS for advice.
- Local heating induced by the optical trap affects efficiency of single strand formation. Large beads and low laser power are recommended for generating single-stranded DNA. For example, for 4.0-4.9 μm beads, adjust the laser power of the trapping laser to achieve a trapping stiffness of 0.2-0.25 pN/nm.
- Single strand formation is highly dependent on the concentration of ions in the buffer. Here we described a procedure that uses 15 mM NaCl in the buffer to overstretch the DNA and obtain single stranded DNA. Higher concentration than 15 mM NaCl reduces efficiency of single stranded formation.
- Avoid using of divalent cations, such as magnesium, (even at concentration as low as 5 mM) in the buffer to obtain single stranded DNA.

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

Prepare the following solutions for the different channels of the microfluidics system and load them in their corresponding syringes. 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) to use in channel 1 and 2. Dilute running buffer 100 times to obtain low salt PBS (15 mM NaCl) to use in channel 3 for single stranded DNA formation. Alternatively, a 40-60 mM Phosphate or Tris buffer at pH 7.5 containing 50-150 mM NaCl can be used for channel 1 and 2, while a 5-10 mM Phosphate or Tris buffer at pH 7.5 containing 10-15 mM NaCl can be used for channel 3.

Channel 1

- Add 10 µl of streptavidin-coated polystyrene beads (⌀4.0–4.9 µm, 0.5% (w/v)) to 1 ml of buffer and load in the syringe of channel 1.

Channel 2

- Add 1 µl biotinylated single-stranded DNA (20,452 nt) (20 ng/µl) to 1 ml of buffer and load in the syringe of channel 2.
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Channel 3

- Add 1 ml of low salt buffer (100 times dilution of running buffer) to channel 3.

Catching beads and DNA tethering

- Apply flow to channels 1, 2, and 3 with a pressure of 0.3–0.5 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 as close as possible to Trap 2, allowing the beads to almost touch each other. Then move Trap 1 to the distance predicted by the worm-like chain model and determine if there is a tether formed by observing a force response. Repeat and tentatively reduce the initial distance between the two beads until a force response is observed. Important: the close distance between the two beads will hide the bead templates. You can use the brightfield camera or piezo tracking to maneuver Trap 1 when the beads are in close distance.

- You will be able to discriminate between single or multiple tethers by looking how forcedistance (Fd) curve matches the worm-like chain model.. 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).

- Move the single DNA tether to channel 3, keep the flow on and aim for forming a single tether between the beads. Keeping the flow on is especially helpful when channel 4 or 5 contain buffers with magnesium ions, to prevent diffusion of these ions into channel 3. 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.

- Then, extend the DNA tether at low speed (e.g. 0.3 µm/s) to the overstretching plateau and keep increasing the distance until the force signal ramps up again. Wait a few seconds at a force signal of 80-100 pN to allow denaturing of single the DNA double helix and formation of single stranded DNA.

- When retracting the DNA back to the original position, you should see that the curve follows the freely-jointed chain model (backward curve) instead of the standard worm-like chain model (forward curve) shown in Figure 1.

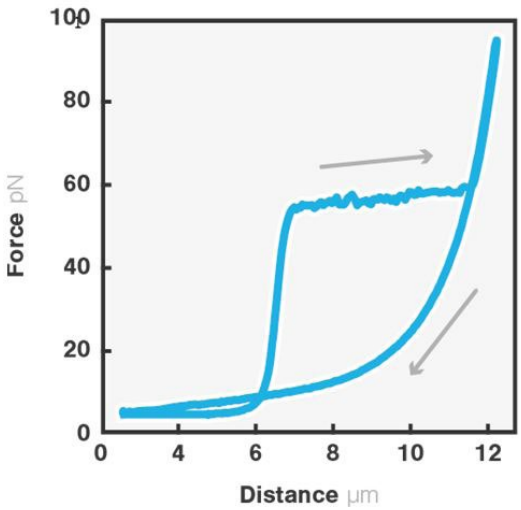

Figure 1

- For troubleshouting a low denaturing efficiency, have a look at the suggestions in this source[1].

References

[1] Belan O, Moore G, Kaczmarczyk A, Newton MD, Anand R, Boulton S, Rueda DS. Generation of versatile ss-ds-DNA hybrid substrates for single-molecule analysis. STAR Protocols 2021.

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