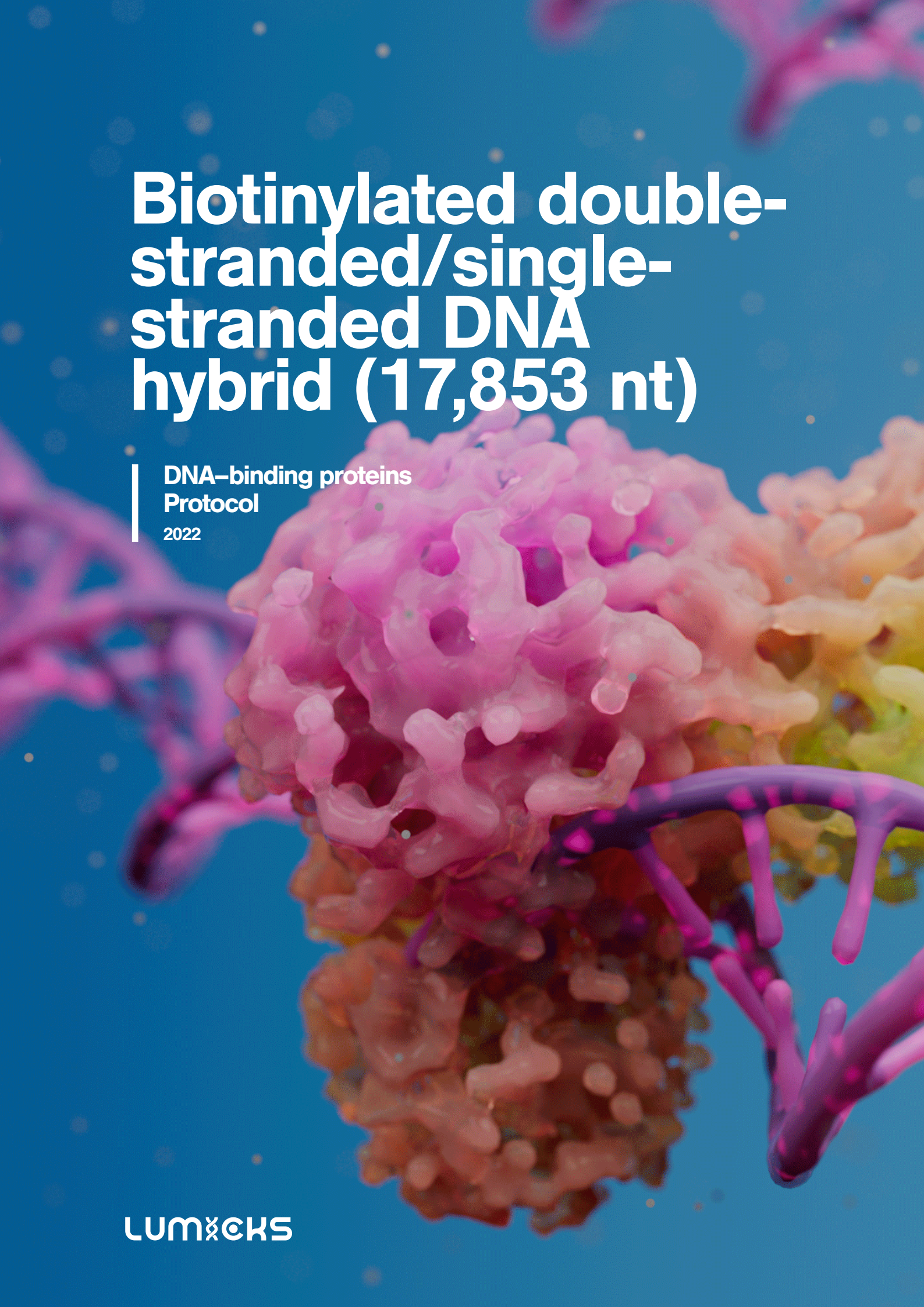
Biotinylated double-stranded/single-stranded DNA hybrid (17,853 nt)

DNA–binding proteins
Protocol
2022



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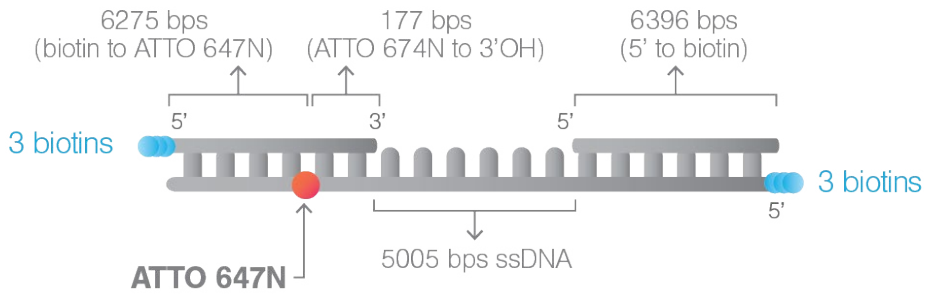
Biotinylated double-stranded/single-stranded DNA hybrid (17,853 nt)

The single-stranded/double-stranded hybrid DNA is shipped as double-stranded DNA that is biotinylated at both 5’ ends. The construct contains two nicks and after melting the DNA, a 5,005 nt single-stranded region remains in the centre of the construct.



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

- Dilute streptavidin coated beads in running buffer 1x according to the beads’ size and concentration (e.g. 1:100 for streptavidin coated polystyrene beads ⌀ 4.0 – 4.9 µm and 0.5% w/v or 1:1000 for polystyrene streptavidin beads of ⌀ 1.5 – 1.9 µm and 1% w/v) and load it into the syringe of channel 1.

Channel 2

- Add 1.5 µl of the biotinlyated DNA hybrid to 500 µl of buffer and load in the syringe of channel 2.

Channel 3

- Dilute running buffer 1x another 10 times and load it into the syringe of 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 beads in each of the traps in channel 1, move to channel 2 and align the beads on the y-axis.
- Keep the flow of channels 1-3 open and move Trap 1 between a distance of ~2.5 µm and 6 µm to determine if a tether has formed, by observing a force response. For a single tether, the measured Fd-curve follows the worm-like chain model and has an overstretching plateau at 60 pN. Double tethers show the onset of the force-response at shorter distances and will have an overstretching plateau for higher forces.
- Move the DNA tehter to channel 3, keep the flow on and overstretch the tether at a distance of ~9 µm for 2 seconds. This allows denaturing of the the DNA double-helix between the nicks and the generation of a DNA hybrid.
- When retracting the DNA back to the original position, you should see that the force-reponse of the Fd-curve now occurs at larger distances.
- Alternatively, for genereting the hybrid DNA, have a look at the automation script of harbor.

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