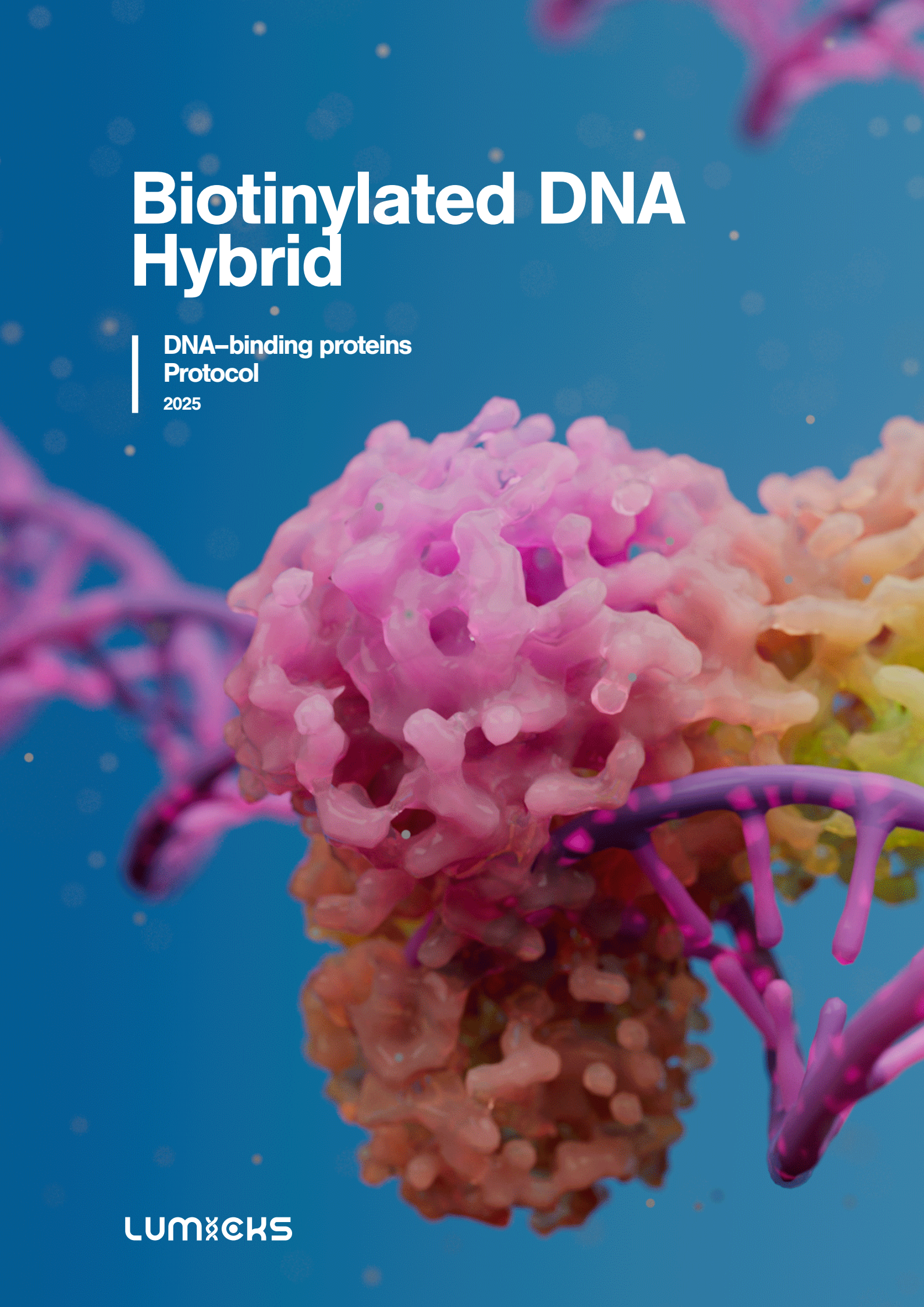
Biotinylated DNA Hybrid

DNA–binding proteins Protocol

2025



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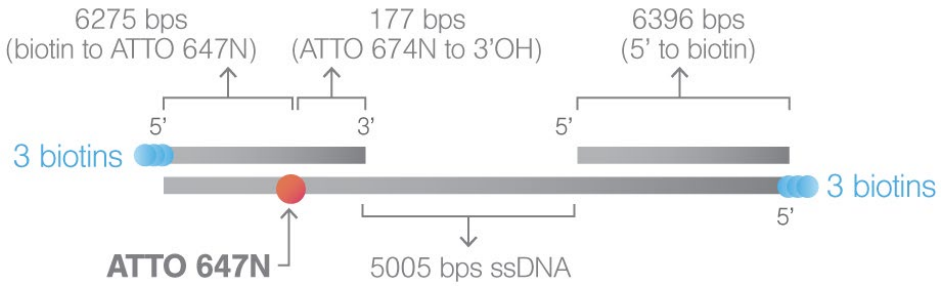
Biotinylated DNA Hybrid

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 1.5 mM sodium azide and 0.5 mM EDTA) to use in channel 1 and 2. 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. Use a low-salt buffer in Ch 3, we recommend a 40 mM Tris buffer at pH 7.5 with 5-10 mM NaCl.

Channel 1

- Dilute streptavidin coated beads in running buffer 1x according to the beads’ size and concentration (e.g. 1:100 for streptavidin beads (4.0 – 4.9 µm, 0.5% w/v) or 1:1000 for streptavidin beads (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

- Load 500 µl of the low-salt buffer in Ch 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 horizontally in the brightfield image.
- 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 for double-stranded DNA with a contour lenght of 17.853 bp and has an overstretching plateau at 60 pN. Double tethers show the onset of the force-respons at shorter distances and will have an overstretching plateau for higher forces. Double-tethers can be broken by increasing the distance between the beads, however, it can also happen that the tethers (partially) convert to hybrids instead of resulting in a single regular tethers. If multiple tethers are caught frequently, the DNA concentration in the syringe can be lowered.
- 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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