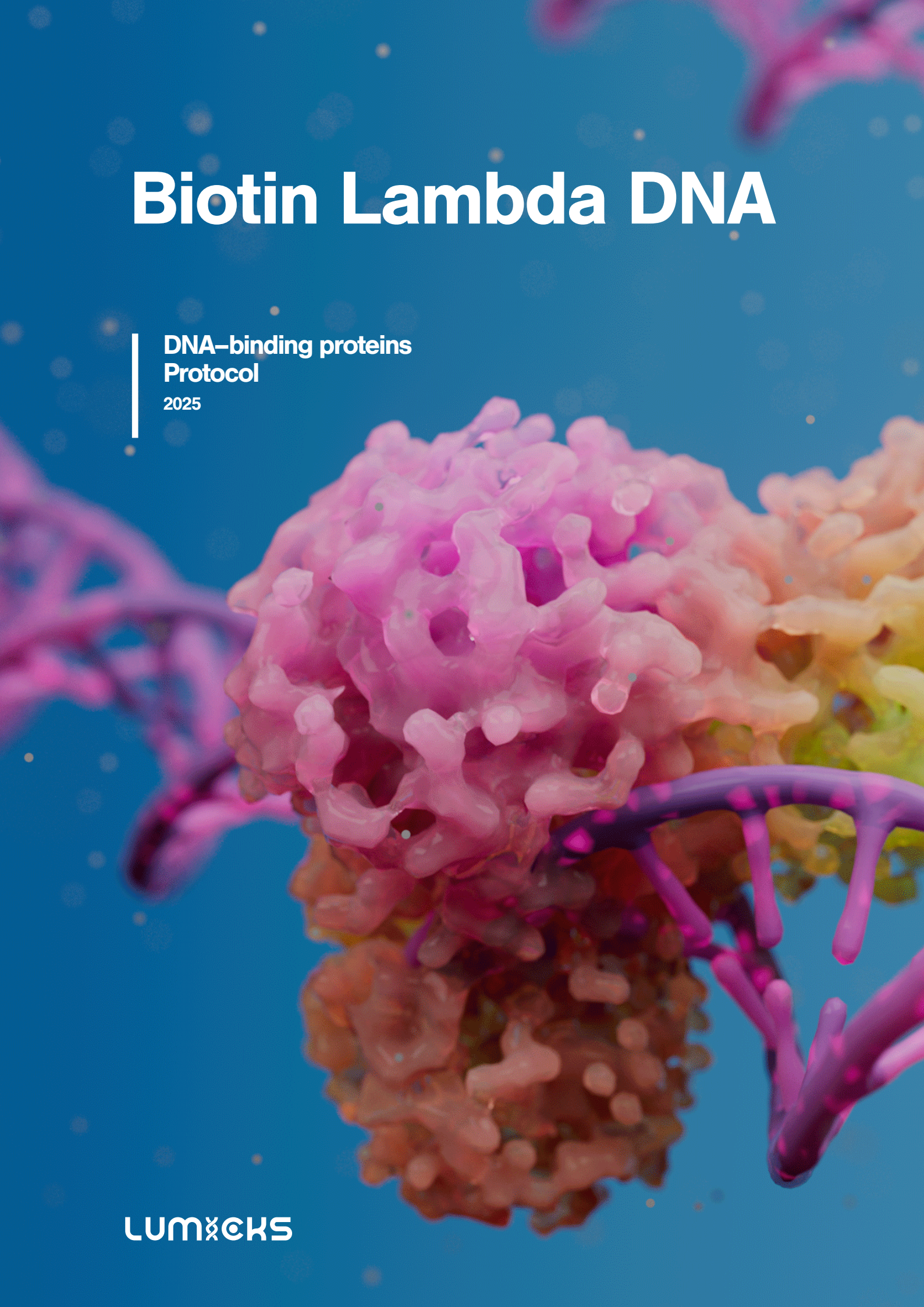
Biotin Lambda DNA

DNA–binding proteins Protocol

2025



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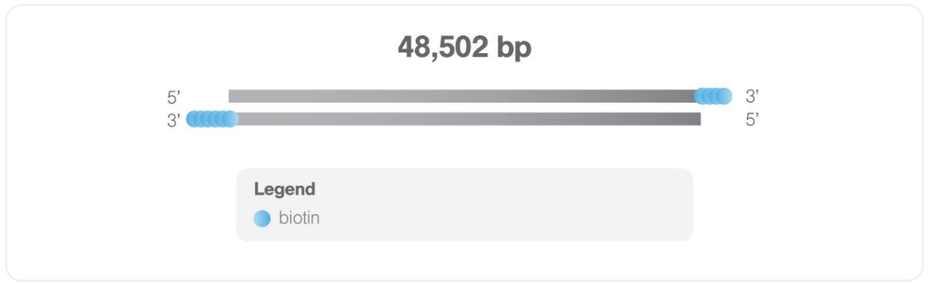
Biotinylated λ DNA

The length of the biotinylated double-stranded λ-DNA (48,502 bp) makes it ideal for assessing different types of DNA binding molecules.



Materials supplied in the kit

The λ-DNA can be used with streptavidin coated beads. We recommend using the ⌀ 4.0–4.9 µm beads, available in our store.

Components	Units	Volume	Storage temperature
Biotinylated λ DNA	1	20 µl	+4 ˚C

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

For overall detailed instructions, consult the C-Trap manual or watch the tutorial video at the following **link**.

Prepare the following conditions for the different channels of the microfluidics system and load them in their corresponding syringes. Prepare 3 ml of running buffer 1x by diluting 300 µl running buffer 10x in 2.7 ml Milli-Q (creating PBS with 1.5 mM sodium azide and 0.5 mM EDTA).

Channel 1

- Add 10 µl of streptavidin-coated polystyrene beads (4.0–4.9 µm, 0.5% (w/v)) to 1 ml of running buffer 1x and use for channel 1.

Channel 2

- Add 1 µl biotinylated λ DNA (20 ng/µl) to 1 ml of running buffer 1x and use for channel 2.

Channel 3

- Use 1 ml of running buffer 1x and use for channel 3.

Catching beads and DNA tethering

- 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 align the beads horizontally in the bright-field image and 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. 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.
- Move the single DNA tether to channel 3 and turn off the flow in all channels. Here, a force distance curve can be recorded to validate that there is only a single DNA tether.

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