DNA–protein interactions kit (for training)

DNA–binding proteins Protocol

2022

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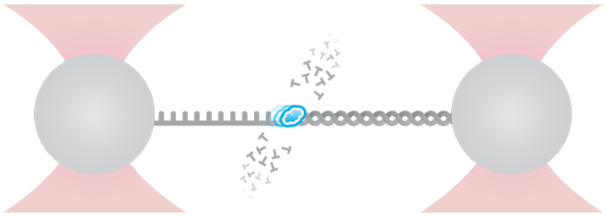
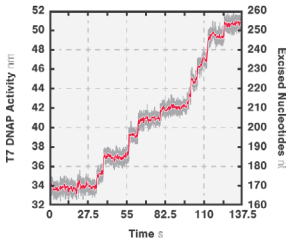
DNA–protein interactions kit (for training)

T7 DNA polymerase will either include (polymerization) or exclude (exonucleolysis) basepairs, depending on the force that you apply. This kit allows you to study these kinetics of enzymatic activities associated with DNA replication.





Materials supplied in the kit

Components	Units	Volume	Storage temperature
T7 DNA polymerase	1	20 µl	-20 ˚C
dNTP mix	1	300 µl	-20 ˚C
10x Polymerase buffer	1	500 µl	-20 ˚C
BSA passivation reagent	1	2 ml	-20 ˚C
Pluronic passivation reagent	1	2 ml	Room temperature
Biotinylated DNA (8,398 bp) with overhang	1	20 µl	+4 ˚C
Streptavidin-coated polystyrene beads (⌀1.5–1.9 µm)	1	25 µl	+4 ˚C

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. For the preparation of channel 1-3, a 40-60 mM Phosphate or Tris buffer at pH 7.5 containing 50-150 mM of common salts is recommended. Do not use chelating agents, such as EDTA, to avoid diffusion of chelating agents in the T7 DNA polymeras channel and consequent depletion of magnesion ions contained in the polymerase buffer.

Channel 1

- Add 1 µl of streptavidin-coated polystyrene beads (1.5–1.9 µm, 1% (w/v)) to 1 ml of buffer and load in the syringe of channel 1.

Channel 2

- Add 1 µl Biotinylated double-stranded DNA (8,398 bp) with overhang (20 ng/µl) to 1 ml of buffer and load in the syringe of channel 2.

Channel 3

- Load 1 ml of buffer in the syringes of channel 3.

Channel 4

- Use 500 µl of MiliQ to dilute passivation reagent BSA 10 times (obtaining 0.1 % BSA solution)
- Load in the syringe of Channel 4.
- Expose the syringe, tubing, and flow cell for at least 20 minutes to the first passivation reagent by flushing it at a pressure of 0.3–0.4 bar.
- Use 500 µl of MiliQ to dilute passivation reagent Pluronic 10 times (obtaining 0.5% Pluronic solution). Remove the remaining 0.1% BSA solution from the syringe of channel 4 and add 500 µl of 0.5% Pluronic solution.
- Expose the syringe, tubing, and flow cell for at least 20 minutes to the second passivation reagent by flushing it at a pressure of 0.3–0.4 bar.
- Dilute DNA polymerase buffer 10 times (obtaining polymerase buffer 1x) with MiliQ. Then, remove the remaining 0.5% Pluronic solution from the syringe of channel 4, add 500 µl of polymerase buffer 1x, and flow approximately 300 µl (0.3–0.4 bar) to fill channel 4.
- Remove the remaining polymerase buffer 1x from the syringe and add 468 µl (or 498 µl when only studying exonucleolysis) of polymerase buffer 1x.• Add 2 µl of T7 DNA polymerase and mix by pipetting. If both polymerization and exonucleolysis activities are to be studied, also add 30 µl dNTP (obtaining 600 µM of each dNTP) to the mix. Absence of dNTP will result in only exonucleolysis events.

Tip: For better passivation, the T7 DNA polymerase can be incubated overnight in channel 4. For the experiment, fresh T7 DNA polymerase should be prepared.

Catching beads, DNA tethering, and measuring enzymatic activity

- 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, 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 to a distance of 1-1.5 µm from Trap 2. Then, increase the distance again and determine if there is a tether formed by observing the force response.
- Once tethering is observed, move the traps to channel 3 and turn off the flow for all channels. You will be able to discriminate between single or multiple tethers by looking how force distance (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).
- 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.
- When the presence of a single tethered molecule is validated, it can be further moved to channel 4.
- Make sure the DNA polymerase solution has filled channel 4. After that, it is not needed to apply flow to channel 4 anymore.
- At this point, the force should be maintained constant by using the Force Clamp (FC) feature of Bluelake. In order to induce exonucleolysis activity, the force should be kept at 45 pN. At this high tension, T7 DNA polymerase will extend the length of the single DNA molecule by hydrolizing nucleotides. Once you have generated a section of ssDNA large enough for your tests, you can change the force to a lower range (10-25 pN) in order to study polymerization events.
- This will be observed as a shortening of the DNA tether due to nucleotide incorporation. The target force can be changed in order to study different distributions and frequency of polymerization, exonucleolysis, and pauses.

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