

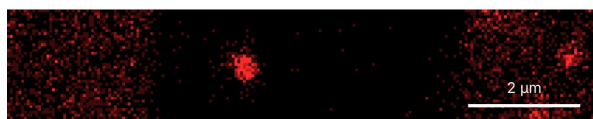
ssDNA Gap Construct – User Guide

1. Product description

The ssDNA Gap construct is a 17,853 bp DNA molecule carrying three biotins at each end and two nicks on the same strand, 5,005 nt apart. Used as supplied, it serves as a nicked double-stranded substrate; stretching the tethered molecule in low-salt buffer releases the 5,005 nt fragment between the nicks and generates a single-stranded gap flanked by two double-stranded arms of 6,452 bp and 6,396 bp. The construct is designed for single-molecule studies of DNA damage repair, homologous recombination and DNA end resection, DNA replication and helicase activity, and DNA damage checkpoint signalling, combining force spectroscopy with confocal fluorescence imaging.¹

Two configurations are available, defined by the position of a single Atto fluorophore that marks the polarity of the gap: **L**, labelled at the 3' ss/ds junction (177 bp from the junction, on the 6,452 bp arm), and **R**, labelled at the 5' ss/ds junction (121 bp from the junction, on the 6,396 bp arm). The configurations shown below are in stock for next-day shipment. **Atto488, Atto565 or Atto647N can be placed in either configuration on request.**

ssDNA Gap L – Atto647N



ssDNA Gap R – Atto565



Schematics of the gapped constructs (not to scale), each above a confocal scan of a single molecule held between two optically trapped 4.4 μm streptavidin beads; the fluorophore appears as a single spot between the beads. Scale bars, 2 μm.

VIAL CONTENT		CONCENTRATION	VOLUME	STORAGE
ssDNA Gap construct (≥ 20 C-Trap runs)		20 ng/μl	30 μl	4 °C
REQUIRED BUFFERS*	COMPOSITION		C-TRAP CHANNEL	
Tethering	10 mM Tris-HCl pH 8.0, 50 mM NaCl , 0.1 mM EDTA (optional)		1–2 (beads, DNA)	
Conversion	10 mM Tris-HCl pH 8.0, 5 mM NaCl , 0.1 mM EDTA (optional)		3 (gap conversion)	

* Not supplied; prepared by the user.

¹ **Keywords:** PARP1, APE1, XRCC1, Pol β, FEN1, LIG1/LIG3, RPA, RAD51, RAD52, BRCA2, RAD54, EXO1, DNA2, MRE11–RAD50–NBS1, RFC, PCNA, Pol δ, Pol ε, CMG, BLM, WRN, UvrD, PIF1, ATR–ATRIP, 9-1-1.

2. Tethering

Flow cell: Channel 1 beads · Channel 2 DNA · Channel 3 conversion buffer

1. **Spin the vial briefly before opening.**
2. Channel 1: streptavidin polystyrene beads (4–4.9 μm) in tethering buffer.
3. Channel 2: add **1.5 μl of ssDNA Gap construct to 500 μl of tethering buffer.**
4. Catch one bead in each trap.
5. In channel 2, fish for a tether: move the bead in trap 1 between 2 and 3.5 μm from the bead in trap 2, at 0.3–0.5 bar flow pressure.

3. Gap conversion

6. Move the tether to **channel 3** (conversion buffer).
7. Stretch the DNA: move the bead in trap 1 to **8.5 μm** from the bead in trap 2.
8. Wait **1–2 s**, then relax the DNA.

Expected result

Conversion efficiency >60 % of tethered molecules under optimal conditions.

Force–distance curve of a single ssDNA Gap molecule in conversion buffer (5 mM NaCl). During the first extension (blue), the molecule is stretched to a bead distance of 8.5 μm , at which the 5,005 nt fragment between the nicks is released. The retraction curve (orange) follows the model for the gapped molecule (12.8 kb dsDNA + 5,005 nt ssDNA), indicating formation of the ssDNA gap.

