

5 kb Handle Kit for ssDNA or RNA – User Guide

1. Kit content and use

The kit includes two biotinylated 5 kb double-stranded DNA handles, Handle L and Handle R, each ending in a long single-stranded overhang, and a ligation STOP solution. Your single-stranded DNA or RNA oligonucleotide anneals to both overhangs and is ligated in place, giving a covalent tether from bead to bead, ready for force spectroscopy of hairpins, G-quadruplexes and other secondary structures. There are no purification steps before tethering in the C-Trap. The handles are sufficient for 5 ligation reactions (25 C-Trap runs).

SUPPLIED WITH THE KIT (5 RXN)	CONCENTRATION	VOLUME	STORAGE
5 kb Handle L-16 nt	30 ng/μl	30 μl	-20 °C
5 kb Handle R-15 nt	30 ng/μl	30 μl	-20 °C
Control hairpin (2 rxn)	12 nM	10 μl	-20 °C
STOP solution	5×	400 μl	Room temp.

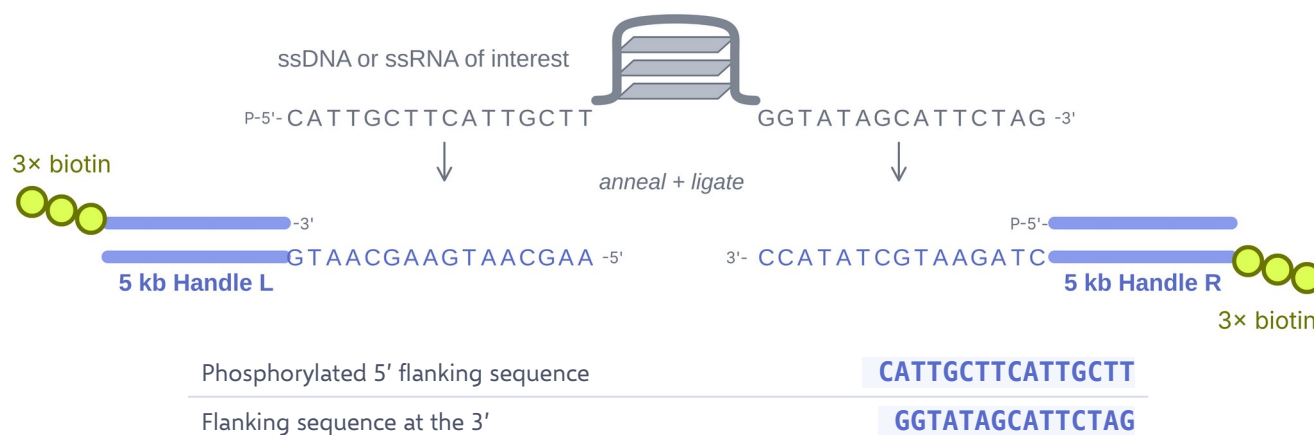
REQUIRED BUFFERS*	COMPOSITION	C-TRAP CHANNEL
Tethering	10 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1 mM EDTA (optional)	Channel 1–2 (beads, DNA)
Measurement	10 mM Tris-HCl pH 8.0, 50 mM NaCl, 10 mM MgCl ₂	Channel 3

* Not supplied; prepared by the user.

SUPPLIED BY THE USER Your 5'-phosphorylated ssDNA or ssRNA, 10× T4 DNA ligase buffer and T4 DNA ligase. Ligase tested: Thermo Scientific EL0011 (5 Weiss U/μl) and NEB M0202 (400 U/μl in NEB cohesive-end units, about 6 Weiss U/μl), both at 1 μl per 20 μl ligation.

Designing your construct

Each handle carries three biotins at its outer end for capture on streptavidin beads. Handle L (5,039 bp) ends in a 16 nt 5' overhang; Handle R (5,043 bp) ends in a 15 nt 3' overhang with its recessed strand 5'-phosphorylated. Order your ssDNA or RNA with a 5' phosphate and the two flanking sequences below, complementary to the handle overhangs.



2. Ligation

Use the handles and your ssDNA or RNA at a 1:1:1 molar ratio: 0.06 pmol of each per 20 µl ligation, a final concentration of 3 nM of each in the ligase reaction. For your ssDNA or RNA, add 5 µl of a 12 nM dilution.

COMPONENT	20 µl
5 kb Handle L-16 nt	6.0 µl
5 kb Handle R-15 nt	6.0 µl
ssDNA or RNA, 12 nM (0.06 pmol)	5.0 µl
10× T4 DNA Ligase buffer	2.0 µl
T4 DNA Ligase	1.0 µl
Total	20.0 µl

1. **Spin the handle tubes briefly** before opening, to recover liquid from the caps.
2. Combine both handles, your ssDNA or RNA and the ligase buffer. Mix gently.
3. Add the ligase last. Mix by slow pipetting; do not vortex, avoid bubbles.
4. Incubate 1 h at room temperature.
5. Stop the reaction: add 80 µl of STOP solution (4 volumes) and mix gently. No heat inactivation is required.
6. Use 20 µl of the stopped reaction per C-Trap run (5 runs per ligation), directly or after storage at 4 °C. No buffer exchange is needed.

3. Tethering

Add 20 µl of the stopped reaction to 500 µl of tethering buffer*, then capture the construct between two streptavidin-coated polystyrene beads under flow. Small beads (1.75 µm) give the lowest noise (see the result below).

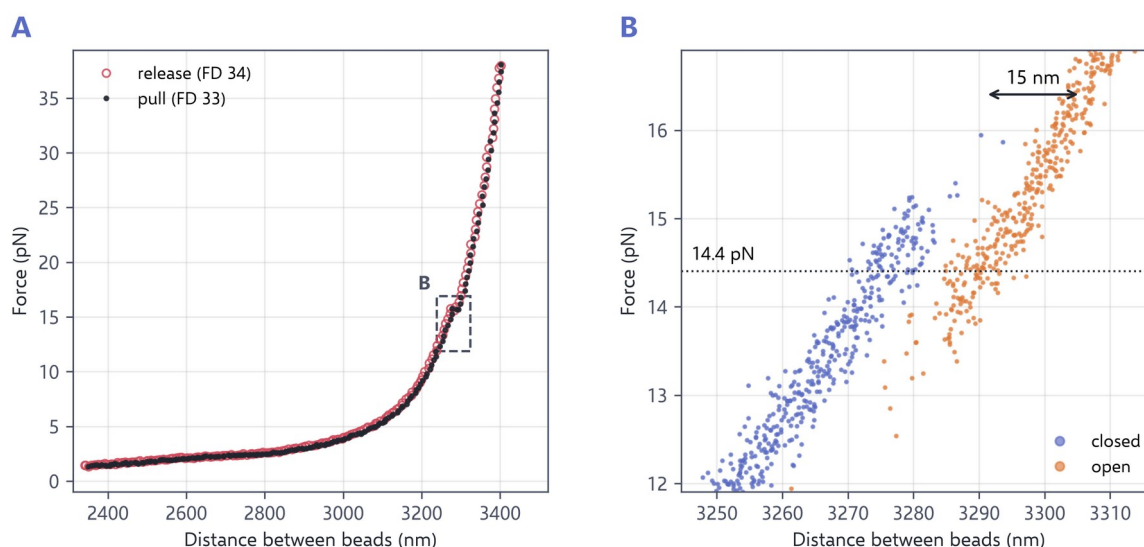
* On the C-Trap, use tethering buffer in channels 1 and 2 (beads and DNA) and measurement buffer in channel 3. Keep NaCl between 50 and 150 mM during tethering; once formed, tethers are stable in a wide range of salt and buffer conditions.

Expected result with the control hairpin

The kit includes a control hairpin for 2 ligation reactions, to test your ligase and set up the C-Trap: the 44 nt DNA hairpin 20R55/T4 (20 bp stem, TTTT loop; Woodside *et al.*, PNAS 2006), flanked by dT spacers and the two handle-complementary sequences. Ligate 5 µl in place of your ssDNA or RNA (section 2).

Control hairpin sequence:

5' -P-CATTGCTTCATTGCTTTTTTTGAGTCAACGCTCTGGATCCTGTTTTTCAGGATCCAGACGTTGACTCTTTTTGGTATAGCATTCTAG-3'



Force-distance curve of a single control hairpin tether (10.1 kb) held between two 1.75 µm streptavidin beads, 1 ms time resolution. (A) Pull and release curves overlap: the hairpin opens and closes in equilibrium. (B) Zoom around the opening force: the hairpin hops between the closed (blue) and open (orange) state, with a jump of ~15 nm at 14.4 pN.