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DNA TOP-PCR Kit
Protocol

Further information

- Technical assistance: www.topscibio.com

End Prep and adaptor ligation

1) Add the following components to a sterile nuclease-free PCR tube:

Fragmented DNA (2 ng)	6.6 μL
End Modification Buffer	1.0 μL
End Modification Enzyme	0.4 μL
Total	8.0 μL

2) Gently mix thoroughly and incubate at 20 °C for 30 min, following at 65 °C for 30 min and hold at 4°C.
3) Add the following components to the same PCR tube:

Adaptor	2 μL
Ligation Mix (mix well before adding)	10 μL
Total	20 μL

4) Gently mix thoroughly and incubate at 20 °C for 4 hr and can be stored at -20 °C.

TOP-PCR reaction

5) Add the following components to a new sterile nuclease-free PCR tube:

Nuclease-free water	15 μL
Ligation mixture from step 4 (mix well)	5 μL
T3 oligo	5 μL
2X PCR Mix	25 μL
Total	50 μL

[Note: the rest of ligation mixture can be stored at -20 °C as back-up]

6) Gently mix thoroughly and put in a PCR machine following the thermocycling condition:

Step	Temperature	Time	Cycles
Initial denaturation	98°C	30 sec	1
Denaturation Annealing Elongation	98°C 59°C 72°C	10 sec 30 sec 1 min	15-30
Final elongation	72°C	5 min	1
Hold	4°C	∞	1

7) Add 2 μL of **Exonuclease** and gently mix thoroughly. Incubate at 37°C for 10 min, following at 80°C for 1 min and hold at 4°C.
8) Cleanup of PCR Reaction. Add 30 μL of nuclease-free water or 0.1X TE buffer and store at -20 °C.