MCLAB
Molecular Cloning Laboratories

Manual

Version 2.2

Product name: Topomize DNA Library Prep Kit, V3

Cat #: TOPO-200

The Kit Includes:

End Repair Mix 1	(Yellow)
End Repair Mix 2	(Yellow)
Topomized Adapters V3	(Blue)
Topomization Mix	(Blue)
2x MCAmp Mix	(Red)
Index Primer (N5xx) (8X)	(Orange)
Index Primer (N7xx) (12X)	(Purple)
MCMag Beads (5 mL)	
Resuspension Buffer	(Clear)
V3 Sequencing Primers (200μM) (Read 1/P7 Index Read/Read 2)	(Green)

Required Materials Not Included:

Sheared DNA sample
Ultrasonicator for DNA shearing
80% Ethanol (freshly prepared)
Nuclease-free water
Pipets and tips 10μL, 100μL, 1000μL
PCR microtubes
Microcentrifuge tubes, 1.5mL
Microtube and microcentrifuge tube racks
Magnetic rack/stand
Thermocycler
Centrifuges
Rotator for bead mixing (optional)
Bioanalyzer (optional)
Horizontal electrophoresis system (optional)
Real-time PCR system (optional)
Fluorescence spectroscopy (optional)

Recommended Storage Condition:

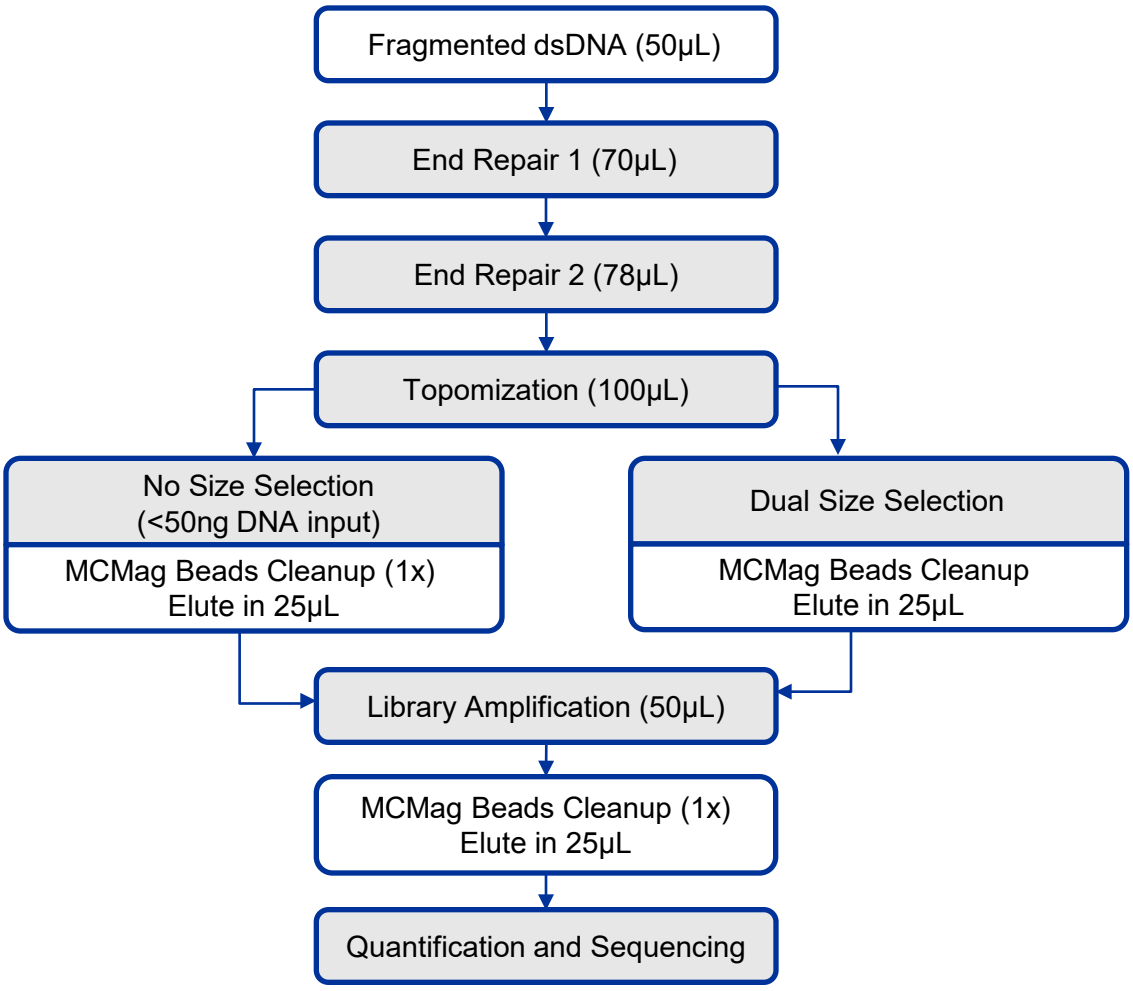
-20°C

Introduction:

MCLAB's Topomize DNA Library Prep Kit, V3 is an innovative, fast and high quality DNA library construction kit for next generation sequencing. Instead of using traditional ligase-based methods, our kit uses topoisomerase-based technology to attach adapters to fragmented DNA. The Topomize DNA Library Prep Kit has unparalleled efficiency and yield, with no adapter dimers or chimeras. The kit is designed for 10ng to 1μg of sheared genomic DNA input, and is compatible with the Illumina platforms. The streamlined workflow allows the DNA library to be ready in about 90 minutes.

The purpose of this protocol is to add adapter sequences onto the ends of DNA fragments to generate indexed libraries for single-read or paired-end sequencing on the Illumina platforms.

Workflow Process:



Protocol:

Starting material: 10 ng-1 µg fragmented DNA

Warm up MCMag Beads to room temperature at least 30 minutes prior to use.

1 End Repair Reaction 1

- Mix the following components in a nuclease-free PCR microtube.

End Repair Mix 1	20 µL
DNA (fragmented) 10ng-1µg	50 µL
Total	70 µL

- Mix gently by pipetting, followed by a quick spin to collect all liquid from the sides of the tube.
- Place in a thermocycler with the heated lid on, and run the following program:
  25 minutes @ 50°C
  10 minutes @ 75°C
  Hold at 4°C

2 End Repair Reaction 2

- Add End Repair Mix 2 directly to the previous reaction mixture.

End Repair Reaction 1	70 µL
End Repair Mix 2	8 µL
Total	78 µL

- Mix gently by pipetting, followed by a quick spin to collect all liquid from the sides of the tube.
- Place in a thermocycler with the heated lid on, and run the following program:
  5 minutes @ 72°C
  Hold at 4°C
- Proceed immediately to the next step.

3 Topomization Reaction

- Perform the following steps on ice.
- Add the following components directly to the previous reaction mixture.

End Repair Reaction Mixture	78 µL
Topomization Mix	20 µL
Topomized adapter	2 µL
Total	100 µL

- Mix gently by pipetting, followed by a quick spin to collect all liquid from the sides of the tube.
- Place in a thermocycler with the heated lid on, and run the following program:
  15 minutes @ 16°C
  Hold at 4°C
- Proceed immediately to the next step.

4 Size selection and cleanup

(For input less than 50 ng, size selection is not recommended. Use 1x beads to perform regular cleanup.)

- Transfer the reaction to a 1.5ml microcentrifuge tube.
- Vortex MCMag Beads until well-dispersed.
- Dilute MCMag Beads with PCR grade water to 160 µL per 100 µL of end-repaired sample with the following dilution formulas.

Insert size	Formula		10% excess for multiple samples	Your Calculation
350 bp	Beads	# of samples X 90 µL	# of samples X 99 µL	
	H_2O	# of samples X 70 µL	# of samples X 77 µL	
550 bp	Beads	# of samples X 70 µL	# of samples X 77 µL	
	H_2O	# of samples X 90 µL	# of samples X 99 µL	

- Vortex the diluted MCMag Beads until well-dispersed. Add 160 µL diluted MCMag Beads to each tube containing 100 µL of previous reaction from step 3, and mix thoroughly by pipetting up and
- Incubate at room temperature for 5 minutes, then place the tube on a magnetic stand and wait until the liquid is clear (~5 minutes).
- Transfer 250 µL of the supernatant to a new tube (**Caution: do not discard the supernatant**).
- Vortex the undiluted MCMag Beads until well-dispersed. Add 30 µL of the undiluted MCMag Beads to each tube, and then mix thoroughly by pipetting up and down.
- Incubate at room temperature for 5 minutes, then place the tube on a magnetic stand and wait until the liquid is clear (~5 minutes). Discard the supernatant.
- With the tube remaining on the magnetic stand, wash with 200 µL of freshly made 80% ethanol without disturbing the beads. Pipet the ethanol out and repeat this step one more time.
- Use a 20 µL pipette to remove the residual ethanol from each tube.
- Allow the beads to air-dry at room temperature (~5 minutes). Avoid over-drying the beads as this may result in lower recovery of target DNA.
- Add 25 µL Resuspension Buffer to the dried beads. Remove the tube from the magnetic stand, and then mix thoroughly by pipetting. Incubate for 2 minutes at room temperature.
- Place the sample onto a magnetic stand and wait until the liquid is clear (2–5 minutes).
- Carefully transfer 23 µL of the supernatant to a PCR microtube.

The sample can be stored at -20°C for up to 7 days.

5 PCR Amplification

5.1 Reaction

Saved Supernatant	23 µL
Library 2x Hotstart PCR Mix	25 µL
Index Primer (N5xx)	1 µL
Index Primer (N7xx)	1 µL
Total	50 µL

- Mix the above reagents gently in a PCR microtube by pipetting, followed by a quick spin to collect all liquid from the sides of the tube.
- Set up the following PCR program (with a heated lid):

	Denaturation	Amplification			Extension	Hold
Temperature	95°C	98°C	60°C	72°C	72°C	10°C
Time	10 min	10 sec	30 sec	30 sec	2 min	
		X (N) cycles				

Starting Material	(N) cycles
10 ng	10-12
50 ng	8-10
100 ng	6-8
250 ng	4-6
500 ng	3-5
1 µg	2-4

5.2 Cleanup

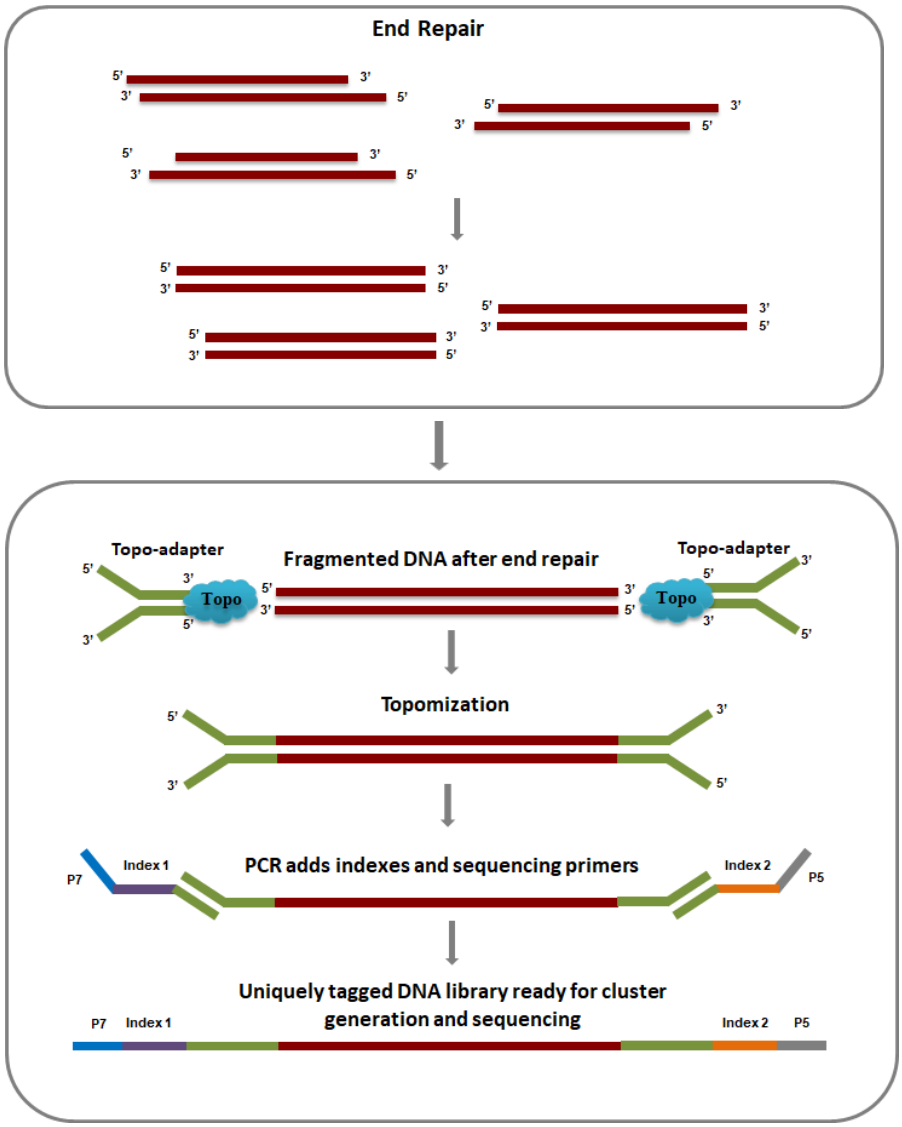
PCR product	50 µL
MCMag Beads	50 µL
Total	100 µL

- Transfer the 50 µl post-PCR sample into a 1.5mL microcentrifuge tube and add 50 µL of MCMag Beads. Gently pipet up and down 10 times to mix thoroughly.
- Incubate at room temperature for 5 minutes. Then place the tube onto a magnetic stand for 5 minutes, or until the supernatant is clear, and discard the supernatant.
- With the tube remaining on the magnetic stand, wash with 200 µL of freshly made 80% ethanol without disturbing the beads. Pipet the ethanol out and repeat this step one more time.
- Use a 20 µL pipette to remove the residual ethanol from each tube.
- Allow the beads to air-dry at room temperature for about 5 minutes. Avoid over-drying the beads as this may result in lower recovery of target DNA.
- Add 27 µL Resuspension Buffer to the dried beads. Remove the tube from the magnetic stand, and then mix thoroughly by pipetting. Incubate for 2 minutes at room temperature.
- Place the sample onto a magnetic stand and wait until the liquid is clear (2–5 minutes).
- Carefully transfer 25 µL of the supernatant (purified DNA library) into a new tube, without disturbing the beads.

6 Validation and Quantification

- The purified sample can be checked on an agarose gel or Bioanalyzer. Dilute the sample at a ratio of 1:2 with water (e.g. 3 µL of sample with 6 µL water) for high sensitivity Bioanalyzer chips.
- The concentration of the purified library can be checked using qPCR, with the MCNext™ SYBR® Fast qPCR Library Quantification Kit.

7 Working Scheme



Index Information:

i7 Index Name	i7 Bases for Sample Sheet
N701	TAAGGCGA
N702	CGTACTAG
N703	AGGCAGAA
N704	TCCTGAGC
N705	GGACTCCT
N706	TAGGCATG
N707	CTCTCTAC
N708	CAGAGAGG
N709	GCTACGCT
N710	CGAGGCTG
N711	AAGAGGCA
N712	GTAGAGGA

i5 Index Name	i5 Bases for Sample Sheet HiSeq 2000/2500 and MiSeq	i5 Bases for Sample Sheet NextSeq and HiSeq 3000/4000
N502	CTCTCTAT	ATAGAGAG
N503	TATCCTCT	AGAGGATA
N504	AGAGTAGA	TCTACTCT
N505	GTAAGGAG	CTCCTTAC
N506	ACTGCATA	TATGCAGT
N507	AAGGAGTA	TACTCCTT
N508	CTAAGCCT	AGGCTTAG
N517	GCGTAAGA	TCTTACGC