MCLAB
Molecular Cloning Laboratories

Manual

Version3.2

Product name: Topomize Amplicon Library Prep Kit

Cat #: TOPOA -50A, TOPOA -50B

Recommended Storage Condition: -20°C

Includes: The Library Kit

Topomized Adapter, V3	(Blue)
Topomization Mix	(Blue)
MCMag Beads (5 ml)	
Resuspension Buffer	(Clear)
2x MCAmp Mix	(Red)
Index Primer (N5xx)	(Orange)
Index Primer (N7xx)	(Purple)
V3 Sequencing Primers (200μM)	(Green)

(Read 1/P7 Index Read/Read 2)

Required Materials Not Included:

Genomic DNA
Target region specific primers
80% Ethanol (freshly prepared)
Nuclease-free Water
Pipets and tips 10μl, 100μl, 1000μl
DNA LoBind Tubes (Eppendorf #022431021)
PCR microtubes
Microtube and eppendorf 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)

Introduction

MCLAB's Topomize Amplicon Library Prep Kit is an innovative, fast and high quality Amplicon library construction kit for next generation sequencing. Targeted sequencing of large cohorts of samples is a powerful tool for the discovery and detection of disease-causing variants associated with many inherited diseases and cancers. After PCR amplification of the targeted DNA regions to generate amplicon pools, MCLAB's Topomize Amplicon Library Prep Kit can be used to quickly add adapters with barcodes for deep sequencing. Instead of using traditional ligase-based methods, our kit uses topoisomerase-based technology to attach adapters with barcodes onto the amplicons. The Topomize Amplicon Library Kit has unparalleled efficiency and yield with no adapter dimers or chimeras. The kit is designed for 10ng to 1μg of each pooled amplicon DNA input, and is compatible with the Illumina platforms. The fast workflow allows the amplicon library to be ready in less than 40 minutes.

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

Protocol

Starting material: 10 ng -1 μg genomic DNA and target region primers
Warm up MCMag Beads to room temperature at least 30 minutes before use.

1 Pre-Kit Process

1.1 Design and order target region specific primers

- All based on your research project
- No linkers or extra nucleotides are needed
- Primers can be 100% matched to the target regions

1.2 Amplify Amplicon Library

- Recommended reagent: 2x BluntStar™ PCR master mix
- Set the annealing temperature based on your primer design (T_m)
- Set the time for extension based on the lengths of your amplicons
- Perform PCR on a thermocycler as a standard operation

The PCR product can be stored at -20°C for later use.

1.3 Polish Ends (Optional)

(For PCR using enzymes (e.g. pfu) that generate blunt end DNA, skip this step.)

- Recommended kit: Blunting Express Kit
- Purpose: Remove the additional 3' "A" bases to generate blunt end amplicons

1.4 Cleanup

- Recommended kit: MCMag PCR Purification Kit.
- Vortex MCMag Beads until well-dispersed.
- Add 1.8x volume 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 and allow the tube to sit on the magnetic stand for 30 seconds. 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). Try to avoid over-drying the beads. This may result in lower recovery of DNA target.
- Add 32 µl Resuspension Buffer to the dry beads. Remove the tube from the magnetic stand, and then mix thoroughly by pipetting. Incubate for 2 minutes at room temperature.
- Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
- Transfer 30 µl of the supernatant to a PCR microtube.

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

2 Kit Process

2.1 Topomization

- Add the following components to the tube containing the beads solution.

Beads solution from last step	30 µL
Topomized adapter	2 µL
Topomization Mix	8 µL
Total	40 µL

- Pipet up and down to mix well.
- Place in a thermocycler with the heated lid on, and run the following program:
  15 minutes at 16°C
  Hold at 4°C
- Proceed immediately to the next step.

2.2 Cleanup

- Vortex MCMag Beads until well-dispersed.
- Add 40µl 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 and allow the tube to sit on the magnetic stand for 30 seconds. 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). Try to avoid over-drying the beads. This may result in lower recovery of DNA target.
- Add 25µl Resuspension Buffer to the dry beads. Remove the tube from the magnetic stand, and then mix thoroughly by pipetting. Incubate for 2 minutes at room temperature.
- Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
- Transfer all the supernatant to a new tube.

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

3.1 PCR Amplification (Optional)

Topomized Amplicon Library	23 µL
2x MCAmp 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
10 ng	10-12
50 ng	8-10
100 ng	6-8
250 ng	4-6
500 ng	3-5
1 µg	2-4

2.4 Cleanup

PCR product	50 µL
MCMag Beads	50 µL
Total	100 µL

- Transfer 50 µl of the post-PCR sample into an eppendorf 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, 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 and allow the tube to sit on the magnetic stand for 30 seconds. Pipet the ethanol out and repeat this step one more time.
- Allow the beads to air-dry at room temperature for about 5 minutes. Try to avoid over-drying the beads. This may result in lower recovery of DNA target.
- Add 25 µl Resuspension Buffer to the dry 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.
- 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.

Workflow Process