Manual

Version 4.1

Product name: MCNext™ UT DNA Sample Prep Kit

Cat #: MCUDS-4, MCUDS-24, MCUDS-96

Description:

This protocol explains how to prepare up to 96 pooled, indexed paired-end libraries from low input amplicons (>400bp), microbial genomes and plasmids for subsequent cluster generation and DNA sequencing using the Illumina NGS sequencing instruments.

The goal of this protocol is to fragment and add adapter sequences onto the template DNA with a single tube reaction to generate multiplexed single read or paired-end sequencing libraries.

The features of MCNext™ UT DNA Sample Preparation protocol include:

Fast Prep Workflow

- Complete protocol in 80 minutes
- Enzymatic fragmentation without sonication
- One-step fragmentation/tagging to save time

Low Input and High Throughput

- Only 1 ng input DNA needed
- Pre-mixed enzymes and nucleotides for multiplexed processing
- Plate-based processing compatible for up to 96 samples per kit

Higher Coverage Rate and Full Compatibility

- The Dialatum™ transposome increases the sequencing coverage
- Works on all Illumina NGS platforms

Best Value on Market

- Complete kit with all reagents included
- All 96 Illumina indices included
- Standardized library quantification prior to sample pooling and sequencing

4 Sample Kit Contents

Component	Abbreviation	Cap Color	Amount
5 x Tagmentation Buffer	TB	Blue	20 µl
Tagmentation Enzyme	TE	Red	10 µl
Neutralization Buffer	NB	Purple	15 µl

2x Amplification Master Mix	AMM	Clear	110 µl
Resuspension Buffer	RSB	Clear	200 µl
Index 1-1 Primer	N701	Orange	2 µl
Index 1-2 Primer	N702	Orange	2 µl
Index 2-1 Primer	N501	Yellow	2 µl
Index 2-2 Primer	N502	Yellow	2 µl

24 Sample Kit Contents

Component	Abbreviation	Cap Color	Amount
5 x Tagmentation Buffer	TB	Blue	110 µl
Tagmentation Enzyme	TE	Red	55 µl
Neutralization Buffer	NB	Purple	85 µl
2x Amplification Master Mix	AMM	Clear	660 µl
Resuspension Buffer	RSB	Clear	1.2 ml
Index 1-1 Primer	N701	Orange	4 µl
Index 1-2 Primer	N702	Orange	4 µl
Index 1-3 Primer	N703	Orange	4 µl
Index 1-4 Primer	N704	Orange	4 µl
Index 1-5 Primer	N705	Orange	4 µl
Index 1-6 Primer	N706	Orange	4 µl
Index 2-1 Primer	N501	Yellow	6 µl
Index 2-2 Primer	N502	Yellow	6 µl
Index 2-3 Primer	N503	Yellow	6 µl
Index 2-4 Primer	N504	Yellow	6 µl

96 Sample Kit Contents

Component	Abbreviation	Cap Color	Amount
5 x Tagmentation Buffer	TB	Blue	410 µl
Tagmentation Enzyme	TE	Red	210 µl
Neutralization Buffer	NB	Purple	300 µl
2x Amplification Master Mix	AMM	Clear	1.2 ml x 2
Resuspension Buffer	RSB	Clear	1.2 ml x 4
Index 1-1 Primer	N701	Orange	16 µl
Index 1-2 Primer	N702	Orange	16 µl
Index 1-3 Primer	N703	Orange	16 µl
Index 1-4 Primer	N704	Orange	16 µl
Index 1-5 Primer	N705	Orange	16 µl
Index 1-6 Primer	N706	Orange	16 µl
Index 1-7 Primer	N707	Orange	16 µl

Index 1-8 Primer	N708	Orange	16 µl
Index 1-9 Primer	N709	Orange	16 µl
Index 1-10 Primer	N710	Orange	16 µl
Index 1-11 Primer	N711	Orange	16 µl
Index 1-12 Primer	N712	Orange	16 µl
Index 2-1 Primer	N501	Yellow	16 µl
Index 2-2 Primer	N502	Yellow	16 µl
Index 2-3 Primer	N503	Yellow	16 µl
Index 2-4 Primer	N504	Yellow	16 µl
Index 2-5 Primer	N505	Yellow	16 µl
Index 2-6 Primer	N506	Yellow	16 µl
Index 2-7 Primer	N507	Yellow	16 µl
Index 2-8 Primer	N508	Yellow	16 µl

Storage condition: -20°C, except Neutralization Buffer @ Room Temperature

User needs to provide the following consumables and lab instruments in order to proceed and complete the sample preparation instructed by the following protocols.

MCMag™ Library Purification Beads	by MCLAB (Cat# NLP-100, NLP-200)
Agilent™ 2100 Bioanalyzer	by Agilent Technologies
Agilent™ High Sensitivity DNA Chip	by Agilent Technologies
Nuclease-free PCR tubes	
Nuclease-free 1.5 ml centrifuge tubes	
Nuclease-free dH2O	
Pipette tips	
Ethanol (100%)	
Microcentrifuge	
Thermocycler	
Magnetic stand	
Single channel pipettes	
Multichannel pipettes (optional)	
Tube rotator/plate shaker (optional)	

DNA Input Recommendations

Input DNA Quantitation

The MCNext™ UT DNA Sample Preparation protocol uses an enzymatic DNA fragmentation step and thus can be more sensitive to DNA input compared to mechanical fragmentation methods. The DNA amount optimized by this protocol is 1 ng to ensure a successful sequencing library preparation, and the concentration of DNA will be no less than 0.2 ng/µl.

The ultimate success of library prep strongly depends on using an accurately quantified amount of input DNA library. Therefore, the correct quantitation of the DNA library is essential. To obtain an accurate quantification of the DNA library, it is recommended to quantify the starting DNA library using a fluorometric based method specific for duplex DNA, such as the Qubit dsDNA BR Assay system. We recommend using 2 µl of each DNA sample with 198 µl of the Qubit working solution for sample quantification. Methods that measure total nucleic acid content (e.g. nanodrop or other UV absorbance methods) should be avoided because common contaminants such as ssDNA, RNA, and oligos are not substrates for the MCNext™ UT DNA Sample Preparation assay.

Library Pooling Considerations

The MCNext DNA Sample Preparation Kit enables preparation of up to 24 or 96 libraries with unique dual indexes, referred to as index 1 (i7) and index 2 (i5). Index 1 and 2 sequences are added via PCR primers during the limited-cycle amplification. In the case where less than the full set of 24/96 libraries will be pooled and sequenced, it is extremely important that libraries with the proper index combinations are contained in the multiplex pool. For further information and library pooling instructions please refer to the Dual Indexing Principle and Low Plexity Index Pooling Guidelines published by Illumina.

I. Tagmentation of DNA

During this step DNA is tagmentated (tagged and fragmented) by the Dialatum™ transposome (***US patent pending***). The Dialatum™ transposome simultaneously fragmentates DNA in the same fashion as the conventional transposome such as Illumina's Nextera transposome does.

Estimated Time

- Hands-on: 3 minutes
- Total duration: 25 minutes

Consumables this kit provides

- TB (5x Tagmentation Buffer)
- TE (Tagmention Enzyme)

Consumables user needs to prepare

- 200 µl PCR tube
- DNA Template (> 0.2 ng/µl) 1 ng

Preparation

1. Remove the TB, TE, and DNA sample from -20°C storage and thaw on ice. (Incubate TB at room temperature meanwhile for the next step, ensure there are no particles or precipitate visible in the solution).

2. After thawing, mix by gently inverting the tubes 3–5 times, followed by a brief spin in a microcentrifuge.

3. Turn on a thermocycler, set up the following program: 55°C for 5 minutes, hold at 10°C. Run and pause the program once lid is heated.

Procedure

The following reactions will be assembled at room temperature.

1. Transfer 4 µl of TB to a 200 µl PCR tube.

2. Add 1 ng DNA into the PCR tube. Adjust the final DNA volume to 14 µl using nuclease-free dH_2O.

3. Add 2 µl of TE to the PCR tube containing DNA and TB.

4. Gently pipette up and down 5 times to mix. Close the tube cap and centrifuge briefly.

5. Place the PCR tube in the thermocycler and run the following program:
- 55°C for 5 minutes
- Hold at 10°C

6. Once the sample reaches 10°C, immediately add 3 µl NB to the tagmentation tube. Pipette to mix.

7. Centrifuge at 280 × g at 20°C for 1 minute.

8. Incubate at 37°C for 15 minutes. The PCR tube contains 23 µl tagmented and neutralized gDNA, all of which can go to step II.

II. Library Amplification

In this step, the tagmented DNA (sequencing library) is amplified via a limited-cycle PCR program and extra index 1 and index 2 are added for sequencing, as well as common adapters (P5 and P7) required for cluster generation and sequencing.

Estimated Time

- Hands-on: 5 minutes
- Total duration: 35 minutes

Consumables this kit provides

- AMM (Amplification Master Mix)
- Indices

Consumables user needs to prepare

- Nuclease-free PCR tube

Preparation

1. Remove AMM and the index primers from -20°C storage and thaw on ice.

2. Gently invert each tube 3–5 times to mix and briefly centrifuge the tubes in a microcentrifuge.

Procedure

The following reactions will be assembled on ice before thermocycler amplification.

1. Add 1 µl selected Index 1 Primer and 1 µl corresponding Index 2 Primer to neutralized tagmentation reaction mix.
2. Add 25 µl AMM to each PCR tube containing DNA.

3. Gently pipette up and down 3–5 times to thoroughly mix and briefly centrifuge.

4. Place the tube in the thermocycler and perform the following program with the heated lid:

1) 72°C 3 minutes
2) 98°C 3 minutes
3) 15 cycles of:
— 98°C 10 seconds
— 60°C 30 seconds
— 72°C 30 seconds
4) 72°C 2 minutes
5) Hold at 10°C

Please ensure that the thermocycler lid is heated during the incubation.
If you do not plan to immediately proceed to PCR Clean-Up step by completion of PCR, the plate can remain on the thermal cycler overnight, or store at 4°C up to 48 hours.

III. Clean-Up and Size Selection

This step uses the MCMag™ Library Purification Beads (Cat# NLP-100, NLP-200) to purify the library DNA, and provides a size selection.

Estimated Time

- Hands-on: 10 minutes
- Total duration: 20 minutes

Consumables user needs to prepare

- MCMag™ Library Purification Beads (Cat# NLP-100, NLP-200)
- 80% Ethanol
- Nuclease-free 1.5 ml centrifuge tube

Preparation

1. Set the MCMag™ Library Purification Beads at room temperature for over 30 minutes.

2. Prepare fresh 80% ethanol from absolute ethanol.

Procedure

The following reactions will be assembled at room temperature.

1. Briefly centrifuge the PCR tube containing the amplified library at room temperature.

2. Vortex the MCMag™ Beads for 30 seconds to evenly disperse the beads.

3. Add 60 µl (for < 250 bp read lengths) or 50 µl (for > 250 bp read lengths) of MCMag™ Beads to the tube with the library.

4. Gently pipette up and down 10 times, or shake the tube on a rotator/shaker at 1,800 rpm for 2 minutes.

5. Incubate the tube at room temperature for 5 minutes.

6. Place the tube on a magnetic stand for 2 minutes.

7. With the tube on the magnetic stand, remove and discard the supernatant.

If any beads have inadvertently aspirated into the tips, dispense the beads back to the tube and let the tube rest on the magnet for 2 minutes and confirm that the supernatant has been cleared.

8. With the tube on the magnetic stand, add 200 µl of freshly prepared 80% ethanol to wash the beads.

Note: Do not resuspend the beads at this time.

9. Incubate the tube on the magnetic stand for 30 seconds, then remove and discard the supernatant.

10. Repeat Step 8-9 again and use a fine pipette tip to remove excess ethanol.

11. Air-dry for 5 minutes while keeping the tube on the magnetic stand.

12. Remove the tube from the magnetic stand and add 27.5 µl of RSB to each tube.

13. Gently pipette up and down 10 times or shake the tube on a rotator/shaker at 1,800 rpm for 2 minutes.

14. Incubate at room temperature for 2 minutes.

15. Place the tube on the magnetic stand for 2 minutes.

16. Transfer 25 µl of the supernatant to a new nuclease-free centrifuge tube as the final library.

Validate Library

Library Quantitation

Accurate quantitation of the DNA library is a necessary step to ensure successful library sequencing. Qubit or Picogreen dsDNA-specific fluorescent dyes are recommended in the method to quantify the library. A qPCR kit with a set of DNA standards is an alternative method to quantify the library.

Quality Control

It is recommended to check the size distribution for some/all libraries by running 1 µl libraries on an Agilent 2100 Bioanalyzer using a High Sensitivity DNA chip. Typical libraries show a size distribution from 200 bp to 1500 bp. For larger libraries, the DNA concentration used for clustering may need to be adjusted. Libraries with an average size greater than 1000 bp may require clustering at several concentrations to achieve optimal density.