Nextera XT Metagenome Library Preparation

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1 Introduction

This protocol assumes that you have extracted DNA and quantified it using the Qubit. The kits and reagents used in this protocol are supplied by Illumina (Nextera DNA Sample Preparation Kit and Nextera Index Kit).

2 Protocol

2.1 Tagmentation of Input Genomic DNA

The function of this "tagmentation" step is to fragment the template DNA and tag the fragments with adapters.

Thaw	From
ATM	-20°C
TD	-20°C
NT	Room Temp

Using 8-strip PCR tubes, add the following to each tube:

1. 5 µl input DNA (1000 pg) per tube
2. 15 µl of master mix (containing 10 µl of TD reagent per 5 µl of ATM reagent). Use the repeat pipette for this step.

For step 2 above, prepare a master mix for large numbers of samples, as follows:

Premix for samples	8	24	48	72
TD (µl)	84	260	500	750
ATM (µl)	42	130	250	375

After combining master mix and DNA, mix well and spin briefly. Incubate at 55°C for 5 minutes, then hold at 10°C.

2.2 Neutralize NTA

Using the repeat pipette, add 5 µl of NT solution. Mix well, and spin briefly. Incubate at room temperature for 5 minutes, then hold at 10°C.

2.3 PCR Amplification

The purpose of this step is to add the Illumina sequencing adapters and indexes to genomic DNA fragments via PCR.

Thaw	From
NPM	-20°C
Index 1	-20°C
Index 2	-20°C

Using the repeat pipette, add 15 µl NPM solution, 5 µl of Index 1 and 5 µl of Index 2 to each tube. Mix well, and spin briefly. Then run the samples using the following PCR conditions:

Temperature (°C)	Time (mm:ss)
72°C	3:00
95°C	0:30
95°C	0:10
55°C	0:30
72°C	0:30
Repeat	for 11 cycles
72°C	5:00
10°C	hold

2.4 PCR Cleanup

Thaw	From
RSB	-20°C
AMPure beads	4°C
80% EtOh	(prepared freshly)

The purpose of this step is to clean up the amplified DNA and remove leftover PCR reagents such as free nucleotides, primers, and polymerase. We will be using Ampure magnetic beads that bind to DNA (Agricourt Ampure XP beads; product number A63880). Include the negative control in this purfication step. Steps in **BOLD** take place in the magnetic adaptor:

1. Using the multichannel pipette, add 30 µl AMPure beads (0.8:1) per tube.
2. Mix well and incubate at room temperature for 5 minutes
3. **Place on the magnetic stand for 2 minutes, then pipet and discard the supernatant.**
4. **Wash pellet twice with 200 µl 80% EtOh**
5. Dry completely for 15 minutes

6. Using a regular pipet, add 52.5 µl RSB (make sure solution is dissolved completely). Mix well, spin briefly and incubate at room temperature for 2 minutes.
7. **Place on magnetic adaptor for 2 minutes.**
8. **Transfer 50 µl supernatant to a new PCR tube.** At this stage, purified PCR products can be stored at -20°C.

2.5 Library Normalization

Thaw	From
LNA1	-20°C
LNB1	4°C
LNW1	4°C
LNS1	Room temperature
0.1N NaOH	(freshly prepared)

Calculate and prepare the required amount of LNAB solution in the following ratio:

LNAB for number of samples	24	48	72	96
LNA1 (µl)	896	1760	3520	4400
LNB1 (µl)	176	320	640	800

1. Using clean PCR tubes, add 45 µl of prepared LNAB solution to each tube.
2. Using a multichannel pipet, transfer 20 µl of the purified PCR Products (from previous PCR cleanup step) to the assigned tube.
3. Shake tubes at 1800 RPM for 30 seconds
4. LNW1 wash step.
   (a) Spin tubes briefly, place on magnetic adaptor for 2 minutes and then discard supernatant.
   (b) Using the multichannel pipet, wash the pellet with 45 µl LNW1 solution.
   (c) Shake tubes at 1800 RPM for 5 minutes.
   (d) Spin briefly. Place on magnetic adaptor for 2 minutes and then discard supernatant.
   (e) Repeat LNW1 wash steps. At the end of the second wash, dry samples for 5 minutes.
5. Add µl of 0.1N NaOH to each tube. Shake at 1800 RPM for 5 minutes. While waiting, Add 30 µl LNS1 solution to clean PCR tubes.
6. Place shaken samples on magnetic adaptor for 2 minutes. Afterwards, transfer 30 µl of the supernatant to the new PCR tubes containing LNS1 solution.
7. Spin at 1000g for 1 minute
8. At this stage, samples can be kept at -20°C. qPCR can also be used at this stage to check the quantity of the library.

Samples should now be submitted to the UC Davis core facility for pooling and loading onto the Illumina platform.