Title: Illumina Library Preparation using the Nextera XT DNA Library Prep Kit		
Date last updated: 20th June 2024	Total Pages: 5	Written by: Jeanie Wu

Illumina Library Preparation Using the Nextera XT DNA Library Prep Kit

1. PURPOSE

To prepare dual-indexed paired-end amplicon libraries from cDNA using Illumina's workflow.

2. MATERIALS & EQUIPMENTS

Samples

- Dengue or Zika cDNA that was PCR amplified

Reagents

- Illumina Nextera XT DNA Library Preparation Kit (Illumina, FC-131-1096)
- Illumina Nextera XT Index Kit (Illumina, FC-131-1001)
- Qubit 1X dsDNA HS Assay kit (ThermoFisher, cat #Q33231)
- Qubit Assay Tubes (Invitrogen, Q32856)
- Mag-Bind TotalPure NGS magnetic beads (SciMed Asia Pte Ltd, M1378-01)
- Absolute (100%) Ethanol
    a. Freshly prepared 80% Ethanol in nuclease – free water from 100% Ethanol
- Nuclease – free ultrapure water

Consumables

- 0.2ml PCR tubes
- 1.5ml Eppendorf DNA Lo-Bind tubes
- Qubit 0.5ml assay tubes (ThermoFisher Scientific, Q32856)

Equipments

- Thermal cycler
- Ilumina MiSeq Sequencer
- Qubit 4 Fluorometer (ThermoFisher, Q33238)
- DynaMag-2 Magnetic Stand (Invitrogen, 12321D)

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3. PROCEDURE

Step 1

Tagment Genomic DNA:

1. Prepare 1ng DNA in 5ul water (PCR tubes or strips)

Sample name	Conc. (ng/ul)	Vol. DNA (1 ng) (ul)	H_20 (ul)	Indexes

2. Thaw reagents ATM (Amplicon Tagment Mix), TD (Tagment DNA Buffer) and NT (Neutralize Tagment Buffer)
3. Add 10ul TD (Tagment DNA Buffer) to DNA and pipette mix thoroughly
4. Add 5ul ATM (Amplicon Tagment Mix) to DNA and pipette mix 10 times. Quick spin down
5. Place tubes in thermal cycler and run TAG program
    a. Lid 100°C, reaction volume 50ul
    b. 55°C, 5mins
    c. Hold 10°C
6. Add 5ul NT (Neutralize Tagment Buffer) to each tube
7. Pipette mix 10 times and quick spin down
8. Incubate at room temperature for 5mins

Step 2

Amplify Libraries:

1. Thaw NPM (Nextera PCR Master Mix) and Index Adapters (i7 and i5 tubes)
2. Add 5ul of i7 index adapter to each tube
3. Add 5ul of i5 index adapter to each tube

Title: Illumina Library Preparation using the Nextera XT DNA Library Prep Kit		
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Note: Replace each index adapter with new caps (provided in kit) after opening

4. Add 15ul NPM (Nextera PCR Master Mix) to each tube
5. Pipette mix 10 times and quick spin down
6. Place in thermal cycler and run NXT PCR program

Lid temp. = 100°C, Reaction volume = 50ul		
1	72°C	3 mins
2	95°C	30 secs
3 (12 cycles)	95°C	10 secs
	55°C	30 secs
	72°C	30 secs
4	72°C	5 mins
5	10°C	Hold indefinitely

Note: Safe stopping point. Store at 4°C for up to 2 days.

Step 3
Clean Up Libraries

Note: Thaw magnetic beads at room temperature for 30mins before starting. Resuspend frequently to ensure even distribution.

1. Thaw RSB (Resuspension Buffer)
2. Prepare fresh 80% EtOH (500ul per sample)
3. Transfer 50ul DNA from PCR tube to a new DNA LoBind 1.5ml tube

Title: Illumina Library Preparation using the Nextera XT DNA Library Prep Kit		
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4. If using small PCR amplicon sample input, add the magnetic beads volume according to input size

Input size (bp)	Beads Recommendation	Beads volume (ul)
300 - 500	1.8x Beads	90
>500	0.6x Beads	30

5. Pipette mix 10 times and quick spin down
6. Incubate at room temperature for 5mins
7. Place on magnetic stand ~ 2mins (wait till liquid is clear and colorless)
8. Remove and discard supernatant without disturbing beads
9. Wash 2 times with 200ul of freshly prepared 80% EtOH as follows:
    a. With tube on magnetic stand, add 200ul fresh 80% EtOH without mixing
    b. Incubate for 30s
    c. Remove and discard supernatant without disturbing beads
10. Use 20ul pipette to remove and discard residual supernatant
11. Air-dry on magnetic stand ~ 2 – 5mins (do not over-dry as it makes resuspension of beads difficult)
12. Remove from magnetic stand and resuspend beads with 52.5ul RSB (Resuspension Buffer)
13. Pipette mix 10 times and quick spin down
14. Incubate at room temperature for 2mins
15. Place on magnetic stand ~ 2mins (till liquid is clear and colorless)
16. Transfer 50ul supernatant to a new DNA LoBind 1.5ml tube

Note: Safe stopping point. Store at - 20°C for up to 7 days.

Step 4

Send for Sequencing

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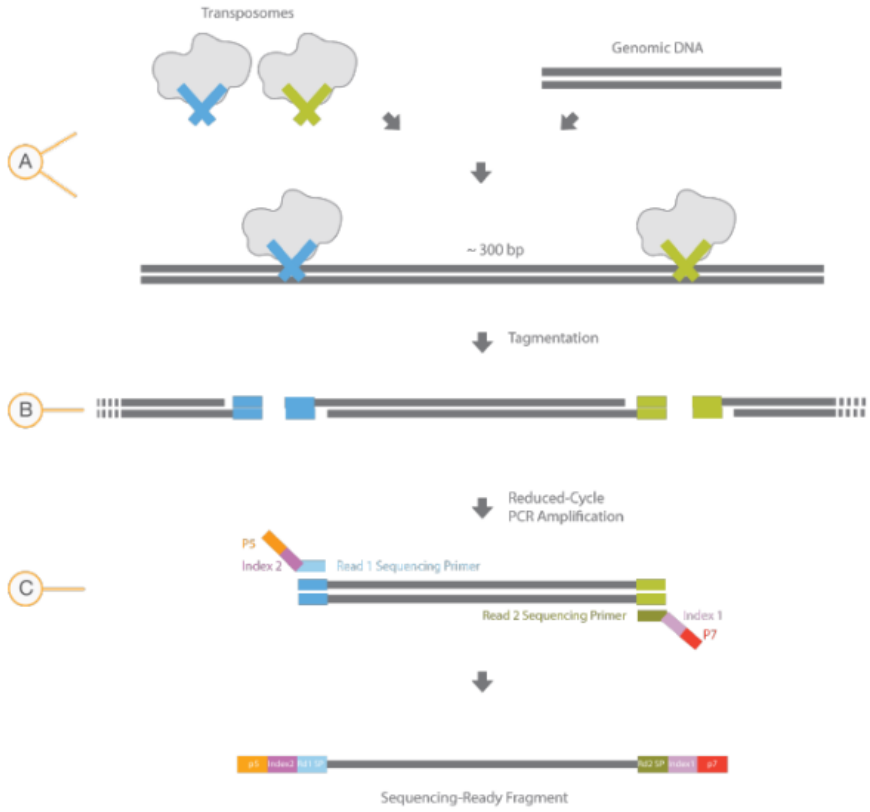
Process Map/ workflow chart



A. Nextera XT transposome with adapters combined with template DNA
B. Tagmentation to fragment and add adapters
C. Limited-cycle PCR to add index adapter sequences