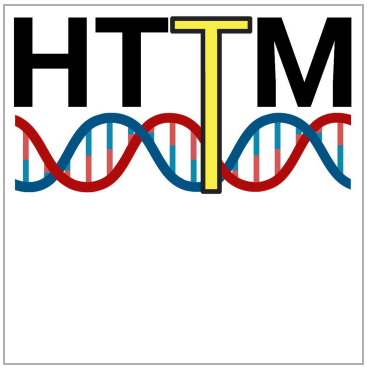

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We use this protocol and it's working

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HTTM : Illumina libraries V.1

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ABSTRACT

Part three of the HTTM protocol. A low-cost and high-throughput Tn-seq protocol. This part cover the preparation of Illumina sequencing libraries form genomic DNA.

MATERIALS

Preparation of Nextera adapters :

Nextera (NxT) adapters are prepared by hybridization of the following primers :

	A	B
	Nxt-XTv2-B-N701-T	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGT
	Nxt-XTv2-B-3R-ac3-phos5’	/5Phos/CTGTCTCTTATACACATCTCCGAGCCCACGAGAC/3InvdT/

- **Preparation of the 5X annealing buffer (5X Tris NaCl buffer : 50 mM Tris, pH 7.5-8, 250 mM NaCl) :**
- 500 µl Tris-HCl 1M pH 7.5
- 500 µl NaCl 5M
- 9 ml H2O mol.-grade

- **Preparation of the adapters (40 µM 50 µL) :**
- Resuspend both primers in water to obtain 100 µM stocks
- Mix 20 µl of each (Nxt-XTv2-B-N701-T and Nxt-XTv2-B-3R-ac3-phos5’)
- Add 10 µl of 5X annealing buffer
- Annealing reaction in a thermocycler (decrease temperature from 98 ℃ to 4 ℃ (-0.1 ℃/cycle(10s/cycle)))

Primers used for the first PCR :

	A	B

	A	B
	Nxt_A	AATGATACGGCGACCACCGAGATCTACAC
	Nxt_B	CAAGCAGAAGACGGCATACGAGAT

Primers template for barcoding PCR :

	A	B
	Nxt_i5_barcoding	AATGATACGGCGACCACCGAGATCTACAC [8 Nu Index] TCGTCGGCAGCGTCAGATGTGTA
	Nxt_i7_barcoding	CAAGCAGAAGACGGCATACGAGAT [8 Nu Index] GTCTCGTGGGCTCGGAGATGTGTATAAG

Kit used for library preparation :
NEBNext Ultra II DNA Library Prep Kit for Illumina
NEB CAT#: E7645S

PCR mix used :
Supermix 2X
Homemade

SPRI beads used:
Ampure XP DNA beads
Beckman Coulter CAT#: A63882

BEFORE START INSTRUCTIONS

- All steps and master mixes need to be kept on ice as much as possible. Thermocyclers need to be cooled at 4 ℃ before inserting sample plate.

Libraries

1h 34m

1 Transfer 2.5 µL of DNA from the DNA extraction plate to a new PCR plate.

2 Prepare a fragmentation master mix with :

	A	B
	NEB Ultra II FS buffer	77 µl
	NEB Ultra II FS enzyme	22 µl

	A	B
	Molecular grade water	11 µl

3 Add 1 µL of the fragmentation master mix to each well.

4 Incubate in a thermocycler with the following protocol : 45m

- 00:15:00 at 37 °C
- 00:30:00 at 65 °C

5 Add 1 µL of 4µM Nextera (NxT) adaptors to each well.

6 Prepare a ligation master mix with :

	A	B
	NEB Ultra II ligation master mix	377.4 µl
	NEB Ultra II ligation enhancer	12.1 µl

7 Add 3.5 µL of ligation master mix to each well.

8 Incubate in a thermocycler with the following protocol : 40m

- 00:30:00 at 20 °C
- 00:10:00 at 65 °C

9 Prepare a PCR master mix with :

	A	B
	NxT_A primer 20 µM	880 µl
	Nxt_B primer 20 µM	880 µl
	Molecular grade water	8360 µl
	PCR Supermix 2X	11000 µl

10 Add 192 µL of PCR master mix to each well.

11 Split the PCR reaction into 4 different plates (50µl per plate).

12 Incubate each plate in a thermocycler with the following cycles : 3m 15s

- 00:00:30 at 98 °C
- 00:00:15 at 98 °C
- 00:00:30 at 72 °C
- Repeat from step 2 for 20~25 cycles*
- 00:02:00 72 °C

13 Pool the 4 PCR replicates together in a PCR plate.

14 Transfer 2 µL of DNA from the pool plate to a new PCR plate.

15 Add 2 µL of each barcoding primer to the DNA :
- Nxt_i5_barcoding
- Nxt_i7_barcoding

16 Prepare a PCR master mix with :

	A	B
	Molecular grade water	2090 µl
	PCR supermix 2X	2750 µl

17 Add 44 µL of the PCR master mix to each well of the plate.

18 Incubate in a thermocycler using the following protocol : 3m 45s
- 00:00:30 at 98 °C
- 00:00:15 at 98 °C
- 00:01:00 at 72 °C (no anneal step)
- Repeat from step 2 for 5 cycles
- 00:02:00 at 72 °C

19 Pool together 2 µL of each sample.

20 Purify with Ampure XP SPRI beads using a 0.8 ratio. Resuspend with 50 µL of molecular grade water.

21 Proceed with QC and sequencing.