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HTTM : Illumina library preparation V.3

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Protocol status: Working
We use this protocol and it's working

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Abstract

Part of the HTTM protocol dedicated to the preparation of Illumina sequencing libraries.

Attachments

HDTM Protocol-3.pd...

214KB

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Materials

Preparation of Nextera Adaptaters :

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

	A	B
	Nxt-XTv2-B-N701-T	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGT
	Nxt-XTv2-B-3R-ac3-5phos	/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-N7XX-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 4C (-0.1C/cycle(10s/cycle)))

Primers used for the first PCR :

	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

Before start

All steps and master mixes need to be kept on ice as much as possible. Thermocyclers need to be cooled at 4C 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 for 96 samples with :

	A	B
	NEB Ultra II FS buffer	77 µl
	NEB Ultra II FS enzyme	22 µl
	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 for 96 samples 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 10 µM	883 µl
	NxT_B primer 10 µM	883 µl
	Molecular grade water	7507 µl
	PCR Mix 2X	11040 µl

10 Add 92 µL of PCR master mix to each well.

11 Split the PCR reaction into 2 different plates (50 µl 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 2 PCR replicates together in a.

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	2098 µl
	PCR mix 2X	2760 µl

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

18 Incubate in a thermocycler with 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 7 cycles
- 00:02:00 at 72 °C

19 Pool together 5 µL of each sample.

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

21 Proceed with QC and sequencing.