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Metagenomic Library Prep from fecal sample lysate

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

dual index sequences here
Nextera 10bp dual index sequences

Abstract

Metagenomic library prep from fecal sample lysate using Illumina DNA prep kit (1/2 reactions).

Attachments

XLSX

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18KB

Guidelines

Abbreviations
BLT: Bead-Linked Transposomes
TB1: Tagmentation Buffer 1
MM: Master Mix
TWB: Tagmentation Wash Buffer
TSB: Tagmentation Stop Buffer
EPM: Enhanced PCR Mix
SPB: Sample Purification Beads
RSB: Resuspension Buffer

Materials

- PCR plates and covers
- Nuclease-Free water
- Illumina® DNA Prep (M) Tagmentation (24 Samples IPB) **Illumina, Inc. Catalog #**20060060
  - Bead-Linked Transposomes (BLT)
  - Tagmentation Buffer 1 (TB1)
  - Tagmentation Wash Buffer (TWB)
  - Tagmentation Stop Buffer (TSB)
  - Enhanced PCR Mix (EPM)
  - Sample Purification Beads (SPB)
  - Resuspension Buffer (RSB)
- Freshly prepared 80% ethanol **Fisher Scientific**
- Index Adaptors (96)
- Magnetic stand

Troubleshooting

Preparation

1 Add 18 µL of nuclease-free water and 2 µL of sample to a PCR plate

Tagmentation Reaction

15m

2 Take out TB1 and keep on On ice. Turn on thermocycler to 37 °C

3 Multiply by number of samples, for a 96 well plate use x100 and pipet mix together to create MM. Vortex BLT vigorously for 10 seconds to resuspend

	Reagent	x1	x100
	BLT	5 ul	500ul
	TB1	5 ul	500ul

4 Vortex and add 10 µL of reaction MM to each well

Note

We found this easiest using a repeater with a 0.5 mL tip for 50 aliquots of 10 µL

5 Pipette mix slowly 5 times using multichannel (should have 30 µL of volume in each well

6 Cover and seal plate. Then run "TAG" on thermocycler

15m

- Choose the preheat lid option and set to 100 °C
- Set the reaction volume to 30 µL
- 55 °C for 00:15:00
- Hold at 10 °C

7 During TAG incubation take out TSB and place at 37 °C and vortex before addition to samples

8 Add 10 µL of TSB to each sample well using repeater with 0.5 mL tip to stop enzymatic reaction

> Note
>
> Be careful because buffer is foamy and be sure to pipette mix after addition

9 Cover with thick plastic and run “PTC” protocol on thermocycler 15m

- Choose the preheat lid option and set to 100 °C
- Set the reaction volume to 40 µL
- 55 °C for 00:15:00
- Hold at 10 °C

10 Place on magnetic stand until beads pellet and discard supernatant

> Note
>
> Take out primer plates to thaw at 4 °C

11 1. Wash 2x with 100 µL of TWB. Place on magnetic stand until beads pellet + remove and discard supernatant after each wash. After both washes add in another 100 µL of TWB to plate while on magnetic stand to prevent beads from drying while you prep PCR mix (don’t pipette mix)

Make PCR Master Mix 1h 10m 15s

12 Take out EPR and thaw on ice

13 1. Make master mix, multiply by number of samples (use x100 for a 96 well plate) (use a 5 ml tube for a full plate of MM)

	Reagent	x1	x100
	EPR	11 ul	1,056 ul
	Nuclease free water	11 ul	1,056 ul

14 Place on magnetic stand until beads pellet + remove and discard supernatant (TWB)

15 Add 20 µL of MM using repeater and 5 mL attachment

16 Add in 2.5 µL of i5 and i7 primers

17 Pipette mix, cover with thick plastic and run “BLT” 12 cycle on thermocycler (01:00:00) 1h 10m 15s

- Choose the preheat lid option and set to 100 °C
- 68 °C for 00:03:00
- 98 °C for 00:03:00
- 12 cycles of:
  - 98 °C for 00:00:45
  - 62 °C for 00:00:30
  - 68 °C for 00:02:00
- 68 °C for 00:01:00
- Hold at 10 °C

Note

1. Take out SPB and RSB while PCR is running to thaw
2. You may need to place the RSB on the thermocycler at 37 °C to get it to thaw

Bead Clean Up

10m

18 Grab a new PCR plate and add 60 µL of nuclease-free water using a multichannel pipette and 45 µL of SPB using a repeater with a .5 ml tip to each sample well

19 After PCR is complete spin down plate → place on magnetic stand → transfer 25 µL of supernatant to new PCR plate containing water and SPB. Discard old plate.

20 Pipette mix 10 times and incubate for 00:05:00 at Room temperature 5m
During this incubation take out a new PCR plate and add 15 µL of SPB to each well using a repeater

21 After the incubation place plate on magnetic stand until beads pellet

22 Transfer 125 µL to the new plate containing the 15 µL of SPB. Discard old plate.

23 Pipette mix 10 times and incubate for 00:05:00 at Room temperature 5m

24 Place on magnetic stand + remove and discard supernatant

Ethanol Washes

3m 30s

25 Keep plate on magnetic stand and prepare fresh 80% ethanol

26 While keeping the plate on the magnetic stand add 200 µL of 80% ethanol without mixing

27 Incubate 00:00:30 + remove and discard supernatant. Repeat ethanol wash and discard supernatant. 30s

Note

Use a 20 µL pipette to remove as much ethanol as possible

28 Air dry while on magnetic stand (00:03:00) 3m

Note

be sure to not let beads crack

Elution 2m

29 Remove beads from stand and add 32 µL of RSB to beads

30 Pipette mix to resuspend and incubate at Room temperature for 00:02:00 2m

31 Place on magnetic stand and transfer supernatant to new and final sturdy PCR plate. Seal, label, and store!