Nextera™ DNA Flex Library Preparation Kit for Metagenomics applications with Whole-Genome Sequencing

Demonstrated Protocol

Introduction

The following protocol demonstrates how to prepare crude lysate from bacterial culture and stool samples and proceed directly to Tagmentation as described in the Nextera DNA Flex Library Prep Reference Guide (document # 10000000025416).

DISCLAIMER

The information in this Illumina Demonstrated Protocol is being provided as a courtesy. In some cases, reagents are required to be purchased from non-authorized third-party suppliers. Illumina does not guarantee or promise technical support for the performance of our products used with any reagent purchased from a non-authorized third-party supplier.

Consumables

- BLT (Bead-Linked Transposomes)
- TB1 (Tagmentation Buffer)
- Nuclease-free water
- 96-well PCR plate
- Microseal 'B' adhesive seal
- 1.7 ml microcentrifuge tubes
- Qubit dsDNA Assay Kit (ThermoFischer)
- Extraction kit (at least one of the following):

Extraction Kit	Sample Type
PureLink Microbiome DNA Purification Kit (ThermoFischer)	Bacteria Cells Stool samples Collected in solid format Stool samples collected in DNA/RNA Shield™ - Fecal Collection Tube (Zymo)
UltraClean® Microbial DNA Isolation Kit (MOBIO)	Bacteria Cells
PowerSoil® DNA Isolation Kit (MOBIO)	Stool samples collected in solid format
PowerFecal® DNA Isolation Kit (MOBIO)	Stool samples collected in solid format
ChargeSwitch® gDNA Mini Bacteria Kit (ThermoFisher)	Bacteria cells
QIAamp® DNA Stool Mini Kit (Qiagen)	Stool samples collected in solid format

Equipment

Equipment	Supplier
Vortex-Genie 2	Sigma, catalog # Z258423
Vortex Adapter for 1.5–2.0 ml tubes (24)	QIAGEN, catalog # 13000-V1-24
Thermal Cycler with Heated Lid	-

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
BLT (Bead-Linked Transposomes)	2°C to 8°C	Bring to room temperature. Vortex to mix.
TB1 (Tagmentation Buffer)	-25°C to -15°C	Bring to room temperature. Vortex to mix.
At least one extraction kit	Follow storage directions from kit manufacturer	Follow instructions from kit manufacturer.

Item	Storage	Instructions
Qubit dsDNA Assay Kit (ThermoFischer)	Follow storage directions from kit manufacturer	Follow instructions from kit manufacturer.

Procedure

1 Use the selected extraction kit to obtain crude lysate. The stage to obtain crude lysate varies per extraction kit, as listed below:

Extraction Kit	Stage to Obtain Crude Lysate
PureLink Microbiome DNA Purification Kit (ThermoFischer)	1. Microbiome culture protocol: supernatant from step H, prior to adding S4 binding buffer. 2. Stool protocol: supernatant from step G, prior to adding S3 cleanup buffer.
UltraClean® Microbial DNA Isolation Kit (MOBIO)	Supernatant from step 11, prior to adding Solution MD3.
PowerSoil® DNA Isolation Kit (MOBIO)	Supernatant from step 10, prior to adding Solution C3.
PowerFecal® DNA Isolation Kit (MOBIO)	Supernatant from step 11, prior to adding Solution C3.
ChargeSwitch® gDNA Mini Bacteria Kit (ThermoFisher)	Supernatant from step 7, after completing incubation.
QIAamp® DNA Stool Mini Kit (Qiagen)	Lysate from step 12, 70°C for 10 minutes.

2 Quantitate the DNA concentration of the lysate using the Qubit dsDNA Assay Kit.

For bacteria culture, 15 ul crude lysate is recommended lysed by the extraction kits above as input for library preparation. Stool samples can be highly inhibitory to the library prep. High inhibition may manifest in these two formats: inability to pellet during post tagmentation clean up or very low library yield. The inhibitory effect is more profound with the QIAamp® DNA Stool mini kit and with samples collected using some collection tubes due to the inhibitory effect from the collection buffer. The table below lists the recommended amount of input. Decreasing the amount of lysate beyond the recommended amount may be necessary for some highly inhibitory samples.

Extraction Kit	Sample Collection	Input for Lysis	Recommended amount of Lysate for Library Prep
PureLink Microbiome DNA Purification Kit (ThermoFischer)	Solid stool	0.05 g solid stool	5 ul
PureLink Microbiome DNA Purification Kit (ThermoFischer)	Stool collected using Zymo collection tube	250 ul stool in collection tube	1 ul

Extraction Kit	Sample Collection	Input for Lysis	Recommended amount of Lysate for Library Prep
PowerSoil® DNA Isolation Kit (MOBIO)	Solid stool	0.05 g solid stool	1 ul
PowerFecal® DNA Isolation Kit (MOBIO)	Solid stool	0.05 g solid stool	5 ul
QIAamp® DNA Stool Mini Kit (Qiagen)	Solid stool	0.05 g solid stool	1 ul

3 Begin tagmentation. Modifications have been made to the tagmentation step of the original Nextera DNA Flex Library Protocol to alleviate the inhibitory effect from the crude lysate.

a Transfer either 1 ul or 5 ul of crude lysate from stool (see table above), or 15 ul lysate from bacteria culture into the wells of a 96-well PCR plate.
b Add nuclease-free water to the sample(s) to bring the total volume to 70 ul.
c Vortex BLT vigorously for 10 seconds, then visually check the beads for complete resuspension. Repeat as necessary.
d Prepare tagmentation master mix. For each reaction, use:

Reagent	Volume per reaction (ul)
BLT	10 ul
TB1	20 ul

e Vortex the tagmentation master mix thoroughly to make sure the BLT beads are evenly resuspended in the buffer.
f Using fresh tips, transfer the 30 ul of tagmentation master mix to each well containing a sample.
g Pipette the 100 ul reaction mix to resuspend.
h Seal the plate.
i Incubate the plate at 55°C for 15 minutes, followed by a 10°C hold. Use a thermal cycler with a heated lid set to 100°C.

Proceed to Post-Tagmentation Clean Up and all steps afterward following the protocols described in the Nextera DNA Flex Library Prep Reference Guide.

Revision History

Document	Date	Description of Change
Document # 1000000057812 v00	May 2018	Initial release.