Preparing multiplexed WGS/MetaG libraries with the Illumina DNA Prep kit for the Illumina NextSeq or MiSeq

André M Comeau[1], Gina V Filloramo[1]

[1]Integrated Microbiome Resource (IMR), Dalhousie University

Integrated Microbiome Resource (IMR)

André M Comeau
Integrated Microbiome Resource (IMR), Dalhousie University

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We use this protocol and it's working

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ABSTRACT

The preparation of (meta)genomic libraries using the Illumina Nextera Flex (now "DNA Prep") kit with CD/UDI indices at the IMR.

Based upon Illumina protocol 10000000254 16 v10 (Aug.2021).

MATERIALS

The following materials list contains those consumables+quantities used specifically at the IMR to complete the present protocol for 192 metagenomes (2 plates) on the NextSeq2000.

Pre-Library Sample Quantification

Quant-iT 1X HS dsDNA Kit (1000 samples)
Optical plate
PCR microplates 96-well Bio-Rad
PCR microplates sealing film Bio-Rad
Tips ClipTip 20
Tips ClipTip 200
Tips ClipTip 300
Reservoir
UltraPure water

Illumina Library Preparation

Nextera Flex DNA Sample Prep Kit (96 samples)
IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)
IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes, 96 Samples)
PCR microplates 96-well Bio-Rad
PCR microplates sealing film Bio-Rad
Manual p1000 tips
Tips ClipTip 20
Tips ClipTip 200

PCR tube strips (Axygen, 8 x 0.2 mL)
15 mL Falcon tubes
50 mL Falcon tubes
Ethanol
Reservoir
UltraPure water

Post-Library Quantification and Pooling
Quant-iT 1X HS dsDNA Kit (1000 samples)
Optical plate
PCR microplates sealing film Bio-Rad
Tips ClipTip 20
Tips ClipTip 300
PCR tube strips (Axygen, 8 x 0.2 mL)
1.5 mL Eppendorf tubes
Reservoir

Loading and Sequencing
PhiX Control Kit v3 (at least for 5 stocks)
NextSeq 2000 P3 Kit 300 cycle
Quant-iT 1X HS dsDNA Kit (1000 samples)
Qubit Tubes
1.5 mL Eppendorf tubes
Various manual pipette tips

Prepare and Quantify gDNA

1 Using two 96-well PCR plates, prepare 30 µL of 1:10 dilution using nuclease-free water (NFW) for up to 192 gDNA samples.

2 Quantify 5 µL of the **1:10 diluted gDNA** using the Invitrogen Quant-iT dsDNA HS assay kit on the fluorescent 96-well plate reader.

Note

Sample concentrations are required for calculating equal molar DNA ratios for library preparation input. Our standard practice is to add the appropriate volume of each sample so that 5 ng of gDNA are added to each sample well.

Perform Illumina DNA Prep Kit Library Preparation

7m

3 Follow the library preparation instructions as described for the **Illumina DNA Prep** kit (https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/illumina_prep/illumina-dna-prep-reference-guide-1000000025416-10.pdf) with the below modifications.

> Note
>
> We were originally doing up to 96 samples (1 plate) on the NextSeq550 and so using the **Nextera DNA CD Indexes (96 Indexes)** for indexing. However, we have now increased to up to 192 samples (2 plates) on the NextSeq2000 and so are now using the **IDT for Illumina DNA/RNA UD Indexes Set A and B, Tagmentation (96+96 Indexes)** for indexing.

3.1 **Amplify Tagmented DNA:** use 12 PCR cycles (based on 5 ng of input DNA).

3.2 **Final library clean-up:** wash beads with 80% ethanol as described, remove excess ethanol and allow beads to dry on the magnet for 00:05:00. Remove the plates from the magnet and add 60 µL of Resuspension Buffer (RSB) to the beads. Pipette mix until thoroughly resuspended and incubate at room temperature for 00:02:00. Place the plates back on the magnet until clear and transfer 55 µL of the supernatant into new 96-well plates. 7m

Quantify and Pool Final Library

4 Quantify 5 µL of each **tagmented, amplified and purified** sample using the Invitrogen Quant-iT dsDNA HS assay kit on the fluorescent 96-well plate reader.

5 Pool **equal molar amounts** of up to 192 samples in a 1.5 mL microcentrifuge tube.

6 **Dilute** pooled library with RSB to the recommended loading concentration specific to the intended sequencer per Illumina's guidelines.