DNA sequencing of metagenomic samples on the HiSeq 2500

1 Introduction

This protocol describes the procedure for preparing libraries from metagenomic samples to be sequenced on the Illumina HiSeq 2500 platform. The library preparation follows Illumina's Nextera XT DNA Library Prep Reference Guide (Document # 15031942 v01, January 2016) except for the bead-based normalisation and pooling steps.

2 Sample QC

2.1 Verify the quality of the extracted genomic DNA using the NanoDrop (Thermo Scientific) or DropSense 16 (Trinean, formerly known as Xpose). The concentration should be verified with the Qubit or PicoGreen assay (Invitrogen).

3 Library preparation

3.1 Dilute 1ng of DNA to a concentration of 0.2ng/ul.

3.2 DNA tagmentation follows Illumina's Nextera XT DNA Library Prep Reference Guide (Document # 15031942 v01, January 2016).

3.3 Library amplification follows the protocol and includes 12 cycles of PCR to enrich for fragments with correctly added indexed adapters.

3.4 Clean up the amplified libraries using AMPure XP or AxyPrep Mag PCR clean-up beads according to the protocol.

4 Library QC

4.1 Verify the size of the libraries on the LabChip GXII (Perkin Elmer) or the TapeStation (Agilent).

4.2 Measure the concentration of the libraries using Qubit, PicoGreen, or qPCR.

4.3 Normalise and pool the libraries manually, do not do follow bead-based normalisation & pooling protocol detailed in the sections of the Prep Guide entitled "Normalize Libraries" and "Pool Libraries".

4.4 Dilute the final pool to a concentration of 2nM.

5 Sequencing

5.1 Following Illumina's HiSeq 2500 System Guide (Document # 15035786 v01, October 2015), thaw one HiSeq 2500 Rapid SBS 500-cycle v2 kit per flowcell.

5.2 Denature the 2nM pool according to the Illumina's HiSeq and GAIIx Systems - Denature and Dilute Libraries Guide (Document # 15050107 v03, November 2016).

5.3 Start the HiSeq 2500 Rapid sequencing run according to the system guide referenced above.

5.4 After sequencing is complete, perform the basecalling and demultiplexing using bcl2fastq Conversion Software.