Protocol for DNA sequencing of metagenomic samples on the HiSeq2500 Rapid Mode.

1.0 Introduction

This protocol describes the procedure for preparing libraries from metagenomic samples to be sequenced on the Illumina HiSeq2500 platform. The library preparation follows *Illuminas TruSeq Nano DNA Sample Preparation Guide (Part # 15041110 Rev. B, November 2013).*

2.0 Quality check of the input DNA

2.1 Verify the quality of the extracted genomic DNA using the Caliper LabChipGX (Perkin Elmer) or the Tapestation genomic chip (Agilent), and the nanodrop (Thermo Scientific) or Xpose (Agilent). The concentration should be verified with the Qubit or picogreen assay (Invitrogen).

3.0 Library preparation

3.1 Dilute 200ng of DNA in a volume of 52.5ul of RSB or EB.
3.2 Shear the DNA as per protocol following the 550bp Covaris settings.
3.3 Perform clean-up of the DNA using AMPure beads following the 550bp insert bead ratio. End-repair, A-tailing and ligation of adapters follow the HT Nano Protocol of the *TruSeq Nano DNA Sample Preparation Guide (Part # 15041110 Rev. B, November 2013)*. Use the DAP plate to index each individual library.
3.4 Follow the protocol to perform the PCR, using 8 PCR cycles.
3.5 Clean-up the PCR-amplified DNA following the 550bp insert ratio of AMPure beads.

4.0 Quality check of the libraries

4.1 Verify the size of the libraries on the LabChipGx HS chip, the Bioanalyzer HS or the Tapestation.
4.2 Measure the concentration of the libraries using Qubit HS or picogreen.
4.3 Dilute the libraries to 10nM stocks and pool an equal amount of each library. Bring the final 10 nM pool to 2nM.

5.0 Sequencing

5.1. Thaw Illuminas HiSeq 2500 Rapid v2 kits. Use one 200-cycle kit and two 50-cycle kits if performing one run, and three 200-cycle kits if performing two runs simultaneously. Follow *Illuminas HiSeq Rapid SBS Kit v2 Reference Guide (Part# 15058772, Rev. B, May 2015).*
5.2 Denature the 2nM pool according to the Illumina protocol *(Denaturing and Diluting Libraries for the HiSeq and GAIIx, Part# 15050107 Rev. C, November 2014).*
5.3 Start the HiSe2500 Rapid sequencing run according to the user guide *(HiSeq2500 System User Guide, Part# 15035786 Rev. D, November 2014)*.
5.4 After sequencing is complete, demultiplex the data using Casava 1.8.2.