DNA sequencing of metransciptomics samples on the HiSeq 2500

1. Introduction

This protocol describes the procedure for preparing libraries form metatranscriptomic samples to be sequenced on the Illumina HiSeq 2500 platform. The library preparation follows the Ribo-Zero rRNA Removal Kit Reference Guide (Document # 15066012 v02, August 2016) and TruSeq Stranded mRNA Sample Preparation Guide (Document # 15031047 Rev. E, October 2013) with some protocol variations.

2. Sample QC

2.1. Verify the quality of the extracted RNA using the Bioanalyzer RNA 6000 Nano or RNA 6000 Pico Kit.

3. Library Preparation

Note: The Ribo-Zero rRNA Removal kits support rRNA depletion from 1-5µg of total RNA. Probes from the Bacteria and Pant Ribo-Zero kits can be combined (50:50 ratio) to simultaneously deplete both bacterial and plant rRNA. In this instance the inputs of RNA should be halved (i.e. 500 ng -2.5 ng of total RNA). The inputs for the sediment RNA sample maybe much lower than these recommended inputs.

3.1. Bring ≤1 µg of total RNA to a final volume of 28µl with RNase-free water.
3.2. Perform rRNA depletion following the Ribo-Zero Kit Reference guide
3.3. Perform clean-up of rRNA depleted supernatant using Agencourt RNAClean XP Kit according to the Ribo-Zero Kit Reference Guide. Elute samples in 8.5 µl of RNase-free water
3.4. Perform TruSeq Stranded mRNA-seq library preparation according the TruSeq Stranded mRNA Sample Preparation Guide, skipping the purification of poly(A) RNAs.
    Add 13 µl of *Fragment, Prime and Finish Mix* to 5 µl of rRNA depleted rRNA sample and follow the library prep protocol from the *Incubate RFP* step (page 20)
3.5. At the Enrich DNA Fragments step (page 38-41) perform 13-15 cycles of PCR
    For inputs of 1 µg use 13 cycles, for inputs < 200 ng use 15 cycles

4. Library QC

4.1. Asses the size of the RNA-seq libraries via electrophoresis using the Agilent TapeStation TapeScreen DNA 1000 Assay or similar (Agilent Bioanylzer, Perkin Elmer LabChip GXII)
4.2. Quantify libraries using qPCR (KAPA Library Quantification Kits for Illumina or similar)
4.3. Normalize the libraries and pool libraries for sequence following the library prep protocol (*Normalize and Pool Libraries*, page 44).

5. Sequencing

5.1. Denature the 2nM pool according to the Illumina's HiSeq and GAIIx Systems - Denature and Dilute Libraries Guide (Document # 15050107 v03, November 2016).
5.2. Following Illumina's cBot System Guide (Document # 15006165 v02 January 2016), perform clustering of HiSeq PE Cluster Kit v4 (PE-401-4001)
5.3. Following Illumina's HiSeq 2500 System Guide System Guide (Document # 15035786 v01 October 2015), prepare the HiSeq SBS v4 Reagents for a 2x100 bp PE or 2x125 bp PE sequencing run.
5.4. Start the HiSeq 2500 Rapid sequencing run according to the system guide referenced above.
5.5. After sequencing is complete, perform the basecalling and demultiplexing using bcl2fastq Conversion Software.