Metatranscriptomics (MTX)

Preparation of RNA and RNA-sequencing. cDNA [CS1] libraries were prepared from total RNA using an adaptation of the RNAtag-Seq protocol (30). Briefly, RNA samples were fragmented, depleted of genomic DNA, and dephosphorylated prior to their ligation to barcoded adaptors. Barcoded RNAs were pooled, depleted of rRNA using RiboZero (Epicentre), used bacterial+host RZ, and converted to Illumina cDNA libraries in 2 steps. First, the RNA was reverse transcribed by SMARTScribe Reverse Transcriptase (Clonetech Laboratories) using a primer targeting the ligated adapter. During cDNA synthesis, a second DNA adapter was added to the 3' end of cDNAs through template switching [1,2]. Second, cDNAs were PCR amplified with primers targeting the ligated adapters and carrying the full sequence of the Illumina sequencing adaptors. Libraries were sequenced on HiSeq 2x101 to yield ~13 million PE reads.

Post-sequencing de-multiplexing and generation of BAM and Fastq files were generated using the Picard suite (https://broadinstitute.github.io/picard/command-line-overview.html).

30. Shishikin et. al Nature Methods 2015

48. **Mandlik A**, **Livny J**, **Robins W**. 2011. RNA-Seq-Based Monitoring of Infection-Linked Changes in Vibrio cholerae Gene Expression. Cell host … **10**:165–174.

49. **Anders S**, **Huber W**. 2010. Differential expression analysis for sequence count data. Genome Biol **11**:R106.

1. Zhu YY, Machleder EM, Chenchik A, Li R, Siebert PD (April 2001). "Reverse transcriptase template switching: a SMART approach for full-length cDNA library construction". *Biotechniques*. **30**(4): 892–7. PMID 11314272.

2. "Transcriptome analysis from single cells, enabled by SMARTer technology". Clontech.