**RNA-seq library preparation and sequencing**

Total RNA was extracted from \_\_\_\_\_\_\_ using \_\_\_\_\_\_\_\_ kit with on-column DNase treatment according to manufacturer's protocol. Final elution was made in \_\_\_\_µl of \_\_\_\_\_\_\_\_\_. The concentration and integrity of the total RNA was estimated by Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, California), and Agilent 2100 Bioanalyzer (Applied Biosystems, Carlsbad, CA), respectively.

Five hundred ng of total RNA was required for proceeding to downstream RNA-seq applications. First, ribosomal RNA (rRNA) was removed using Ribo-Zero™ Gold (Epidemiology) kit (Illumina, San Diego, CA) using manufacturer's recommended protocol. Immediately after the rRNA removal the RNA was fragmented and primed for the first strand synthesis using the NEBnext First Strand synthesis module (New England BioLabs Inc., Ipswich, MA). Directional second strand synthesis was performed using NEBNExt Ultra Directional second strand synthesis kit. Following this the samples were taken into standard library preparation protocol using NEBNext® DNA Library Prep Master Mix Set for Illumina® with slight modifications. Briefly, end-repair was done followed by polyA addition and custom adapter ligation*.* Post-ligated materials were individually barcoded with unique in-house Genomic Services Lab (GSL) primers and amplified through 12 cycles of PCR. Library quantity was assessed by Qubit 2.0 Fluorometer, and the library quality was estimated by utilizing a DNA High Sense chip on an Caliper Gx (Perkin Elmer). Accurate quantification of the final libraries for sequencing applications was determined using the qPCR-based KAPA Biosystems Library Quantification kit (Kapa Biosystems, Inc., Woburn, MA). Each library was diluted to a final concentration of 12.5nM and pooled equimolar prior to clustering. Paired-End (PE) sequencing was performed on an Illumina HiSeq2500 sequencer (Illumina, Inc.).

**Processing of RNA-seq Reads**

Approximately 25 million, 100bp, PE reads were generated from each sample. Further downstream analysis of the sequenced reads from each sample was performed as per our unique in-house pipeline. Briefly, quality control checks on raw sequence data from each sample will be performed using FastQC (Babraham Bioinformatics, London, UK). Raw reads were imported onto the commercial data analysis platform, Avadis NGS (Strand Scientifics, CA, USA) and mapped to the reference *Acinetobacter baumannii* ATCC17978. After quality inspection, the aligned reads were filtered on the basis of read quality metrics where reads with a base quality score less than 30, alignment score less than 95, and mapping quality less than 40 were removed. Remaining reads were then filtered on the basis of their read statistics, where missing mates, translocated, unaligned and flipped reads were removed. The reads list was then filtered to remove duplicates. Samples were grouped and quantification of transcript abundance was done on this final read list using Trimmed Means of M-values (TMM) (Robinson and Oshlack 2010) as the normalization method. Differential expression of genes was calculated on the basis of fold change (using default cut-off ≥ ±2.0) observed between defined conditions, and the *p-value* of the differentially expressed gene list was estimated by *z-score* calculations using determined by Benjamini Hochberg FDR correction of 0.05.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B … 57:289–300.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics (Oxford, England) 25:2078–9.

Robinson MD, Oshlack A. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome biology 11:R25.

Trapnell C, Pachter L, Salzberg SL. 2009. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics (Oxford, England) 25:1105–11.