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Protocol status: Working
We use this protocol and it's working

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Bulk RNAseq - University of Minnesota TMCs

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Cellular Senescence Network (SenNet) Method Development Community

UMN SenNet

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ABSTRACT

Bulk RNA sequencing is the method of choice for transcriptomic analysis of pooled cell populations, tissue sections, or biopsies. It measures the average expression level of individual genes across hundreds to millions of input cells and is useful to get a global idea of gene expression differences between samples.

The following protocol was used after RNA isolation, purification and concentration for Bulk RNAseq analysis. Library preparation and sequencing was completed in collaboration with the University of Minnesota Genomics Center.

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Library Preparation

1 **Sample Quality Assessment:** Total eukaryotic RNA isolates are quantified using a fluorimetric RiboGreen assay. Total RNA integrity is assessed using capillary electrophoresis (e.g. Agilent BioAnalyzer 2100), generating an RNA Integrity Number (RIN). For samples to pass the initial QC step, they need to quantify higher than 500 ng and have a RIN of 8 or greater. Total RNA samples are then converted to Illumina sequencing libraries.

Library Creation: Total RNA samples are converted to Illumina sequencing libraries using Illumina's TruSeq RNA Sample Preparation Kit (Cat. # RS-122-2001 or RS-122-2002) or Stranded mRNA Sample Preparation Kit (Cat. # RS-122-2101). (Please see www.illumina.com for a detailed list of kit contents and methods). In summary, the mRNA from a normalized input mass of total RNA is isolated using oligo-dT coated magnetic beads, fragmented and then reverse transcribed into cDNA. The cDNA is blunt-ended, A-tailed and indexed by ligating molecularly barcoded adaptors. Libraries are amplified using 15 cycles of PCR. Final library size distribution is validated using capillary electrophoresis and quantified using fluorimetry (PicoGreen) and via Q-PCR. Indexed libraries are then normalized, pooled and size-selected to 320 bp (tight) using the PippinHT instrument.

RNA Sequencing

2 **Cluster generation and sequencing:** Pooled libraries are denatured and diluted to the appropriate clustering concentration. Denatured and diluted libraries are loaded onto the sequencing* cartridge and clustering occurs onboard the instrument. Once clustering is complete, sequencing immediately commences using Illumina's 2-color SBS chemistry. Upon completion of read 1, an index read 1 of varying length is performed depending on the library kit used. If libraries are dual indexed, a second index read is performed. Finally, the library fragments are re-synthesized in reverse orientation and sequenced from the opposite end of the read 1 fragment to produce the paired end read 2.

Note

Sequencers used at UMN Genomics Center:
- Illumina NextSeq 2000
- Illumina NovaSeq 6000
- Illumina NovaSeq X Plus

Primary Analysis and De-multiplexing

3 Base call (.bcl) files for each cycle of sequencing are generated by Illumina Real Time Analysis (RTA) software. The base call files and run folders are streamed to servers maintained at the Minnesota Supercomputing Institute. Primary analysis and de-multiplexing are performed using Illumina's bcl2fastq v2.20. The end result of the bcl2fastq workflow is de-multiplexed FASTQ files.
https://support.illumina.com/downloads/bcl2fastq-conversion-software-v2-20.html