Medical College of Wisconsin

Linda T. and John A. Mellowes Center for Genomic Sciences and Precision Medicine

Transcriptome Sequencing (RNA-Seq)

Test Description

Transcriptomic Sequencing (RNA-Seq) is a next-generation sequencing method used to compare the transcriptomes of sample cohorts and reveal shifts in gene expression. RNA-Seq can provide scientists an understanding of cellular and molecular mechanisms that underlie disease initiation, progression, response to environmental or therapeutic interventions and substantially more conditions. By identifying changes in gene transcript levels, transcript sequence, and splicing patterns that ultimately may alter protein expression patterns, investigators can associate pathway activation to phenotypic differences across samples.

Sample Types and Requirements

Cells, Blood, FFPE, Fresh Frozen Tissue for RNA extraction: Prior consultation with the lab is requested
Isolated Total RNA: from 100ng to 1ug (10pg to 100ng for low input) with quality and quantity of RNA verified at the Mellowes Center prior to initiation of library preparation

Recommended Sequencing Depth

50 million total reads, paired end, 2x100 base pair sequencing

Submission Requirements

Sample Intake Form and iLabs request. Contact lab for drop off or shipping requirements.

Turnaround Time

4-6 weeks for fastq files only
1-2 additional weeks for bioinformatics analysis

Deliverables

RNA quality control (PDF)
RNA-seq report (html with linked documents, Excel of DE)
BAM, fastq.gz files
All annotated NGS data files will be delivered via Mellowes Center portal

Test Methodology

Isolated RNA is fluorescently quantified using the BioTek Synergy LX or Qubit and quality assessed using the Agilent Fragment Analyzer. Libraries are then prepared according to the RNA quantity and quality with Illumina TruSeq mRNA Stranded or Takara SMART-SEQ Ultra Low v4 utilized for poly A enrichment and Takara SMART-SEQ Stranded for rRNA depletion. The quality and quantity of the cDNA library is checked by fragment analysis and qPCR respectively. Libraries are then pooled and distribution confirmed on the Illumina MiSeq before sequencing is completed on the Illumina NovaSeq.

Bioinformatic Core Analysis

RNAseq report includes:

- Quality control and sequencing metrics
- Gene and exon expression counts
- Differential expression (DE) of gene transcripts including pairwise and GLM statistics
- Principle component analysis (PCA), heat maps, volcano plots, and Venn diagrams of DE
- Pathway analysis (Qiagen, IPA)
- Additional analysis can identify gene sequence changes (single nucleotide, insertions, deletions, splice variants, fusions), isoform expression, transcript fusions, and gene regulatory networks.

Contact & Submission

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