RNA Sequencing

Service Highlights

**Comprehensive Genomic Information**
Discover gene expression changes, SNPs, Indels, splicing variation, novel transcripts, chromosomal rearrangements, gene fusion products, non-coding RNA and miRNAs

**No Probe Limitations or Bias**
Unbiased transcript information without probes. Sequence without a reference genome and identify yet-unknown exons and variants at base pair resolution

**Data Persistence**
Reanalyze previous data sets in the future for newly discovered RNA species without having to re-run samples

**Large Dynamic Range**
Fine tune RNA-seq data output based on project requirements and discover rare variants and isoforms down to single-transcript resolution

**Data Analysis and Visualization**
Comprehensive bioinformatics for project-specific data deliverables and analysis

**Sample Flexibility**
Ultra low sample requirements, FFPE compatible

**Introduction**
With RNA-seq, a leading next-generation sequencing (NGS) application, researchers can profile the entire human transcriptome and characterize gene expression levels in a specific sample or disease state while obtaining critical genomic information, not available through other gene expression methodologies. In addition to gene expression, RNA-Seq can also be used for SNP/variant discovery, the identification of novel transcripts as well as splice junction and gene fusion products. With NeoGenomics’ bioinformatics analysis pipeline, these types of analysis can be delivered in an intuitive format uniquely suited for direct visualization to highlight the most biologically significant features. Leveraging these services, researchers can monitor the genomic and gene expression makeup of an individual sample and can identify thousands of unique molecular drivers of a particular phenotype or disease state, all within a single assay.
NeoGenomics has performed an internal assessment of its RNA-Seq capabilities on low-quality/quantity FFPE tissue. Starting with ~100ng sample input from pre and post-treatment sample groups, quality library was successfully generated. Illumina HiSeq™ sequencing produced quality reads with sufficient read numbers to allow full transcriptome characterization. A comparison between both pre and post treatment samples revealed a number of biological features including:

1. SNPs, Indels and other variants important to the treatment results.
2. Cluster groups of genes with similar expression patterns between pre and post-treatment.
3. Cluster groups of genes involved in important pathways, cellular functions and disease states between pre and post-treatment.

### High-quality Sequence Data and Reads

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Input</th>
<th>Yield (Mbases)</th>
<th># Reads</th>
<th>Mean Quality Score (PF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Pre-Treatment</td>
<td>100ng, FFPE RNA</td>
<td>18,178</td>
<td>181,781,646</td>
<td>33.8</td>
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<tr>
<td>Sample Post-Treatment 1</td>
<td>87ng, FFPE RNA</td>
<td>24,428</td>
<td>244,279,388</td>
<td>33.81</td>
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<tr>
<td>Sample Post-Treatment 2</td>
<td>105ng, FFPE RNA</td>
<td>9,130</td>
<td>91,304,674</td>
<td>34.02</td>
</tr>
</tbody>
</table>
Comprehensive RNA analysis techniques such as whole transcriptome (RNA-seq) allow researchers to discover gene expression levels, alternative splicing patterns as well as a number of genomic variations on a global level. For each project, a report is generated summarizing the critical genomic features within the sample. Among standard deliverables, a summary report of SNPs, Indels and annotated data including amino acid changes and predicted functional effects is delivered. Differential gene and splice isoform expression can also be visualized with a variety of analysis tools such as differential expression plots and heat map cluster generation, which help investigators uncover meaningful biological information. Utilizing custom bioinformatics, the NeoGenomics team can also generate annotation clusters of differentially expressed genes involved in important cellular pathways, functional groups or based on disease relevance. These customized analysis deliverables can greatly expand the utility of RNA-Seq.
Quality Control
Leveraging a state-of-the-art bioinformatics analysis pipeline and quality assurance procedures, several levels of quality control from sample preparation throughout data reporting are implemented, to ensure high data quality and accuracy. Through extensive internal testing, sample, run and operator reproducibility has been assessed as well as platform error rates in low-quantity clinical FFPE samples giving unparalleled insight into the performance capabilities and limitations of the technologies.

Quality control assessments include:
• Basic Statistics Data
• Sequence Quality Scores
• Read Counts
• Read Length
• Data Output
• GC Content

Related Services
Whole Exome Sequencing
Exome capture and sequencing, providing deep-targeted sequencing on regions of the genome that code for protein
NeoGenomics Custom Targeted Sequencing Panels
Up to 500-gene whole-exome sequencing of any genes, exons or regions of interest

NeoGenomics RNA Seq sequencing is for research use only and are not intended for human therapeutic or diagnostic use.