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.

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RNA-Seq Workflow

RNA Sample Preparation
- As low as 10 picograms
- FFPE Compatible

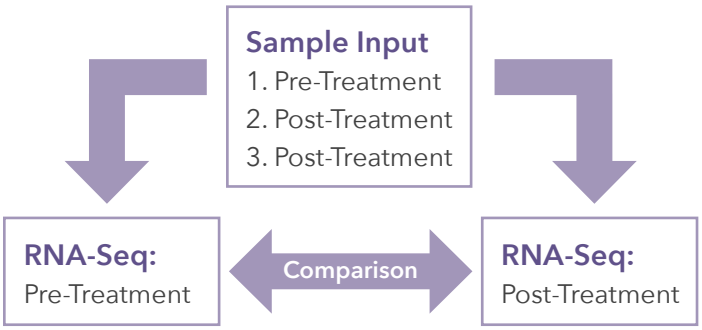
Library Preparation
- rRNA Depletion
- cDNA Synthesis
- Sample Indexing

Sequencing
- Cluster Generation
- Sequencing

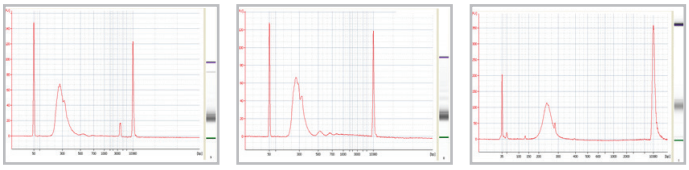
Data Analysis Annotation
- Alignment
  Isoforms/Variants
  Expression Profiling
  Pathway Analysis

Case Study and Platform Performance Assessment

Project Workflow



cDNA Library Preparation



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

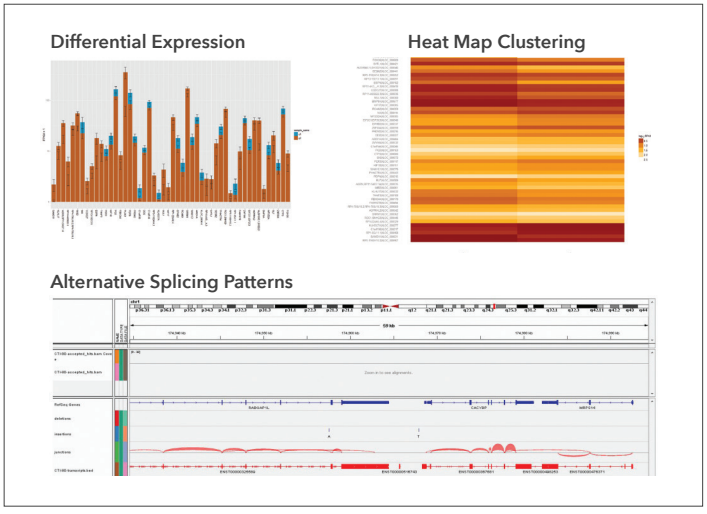
Sample	Sample Input	Yield (Mbases)	# Reads	Mean Quality Score (PF)
Sample Pre-Treatment	100ng, FFPE RNA	18,178	181,781,646	33.8
Sample Post-Treatment 1	87ng, FFPE RNA	24,428	244,279,388	33.81
Sample Post-Treatment 2	105ng, FFPE RNA	9,130	91,304,674	34.02

Comprehensive Reporting and Visualization Tools

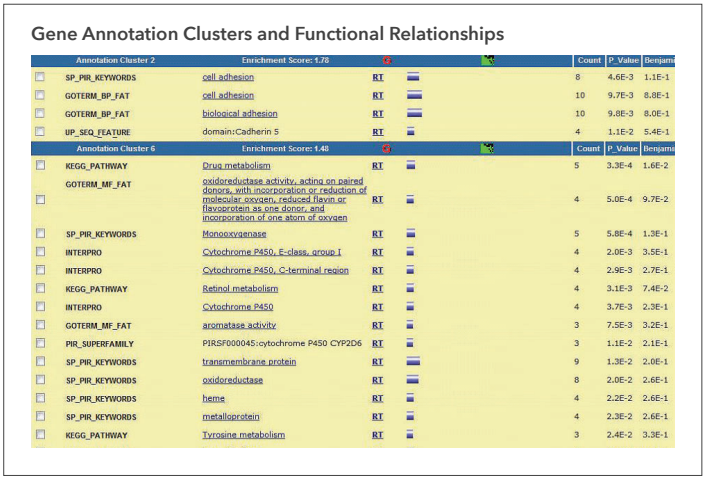
SNPs, Indels and Annotated Data Analysis

NeoGenomics ID	Transcript ID	Gene	Chr	Position	Reference Allele	Observed Allele	Quality Score	Coverage	Variant Allele Frequency (%)	Exon	WT Codon/ Mutant Codon	Observed AA Change	Mutation Type	Affect
Sample 1	ENST00000317869	HNRNPCL1	chr1	12907798	A	C	1014.22	217	29.95	2	ttT/ttG	F115L/ AA_length = 293	MISSENSE	MODERATE
Sample 2	ENST00000379370	AGRN	chr1	982994	T	C	5397.98	172	95.35	21	ttT/ttC	F1186/ AA_length = 2045	SILENT	LOW
Sample 3	ENST00000376572	KIAA2013	chr1	11983206	A	G	665.98	74	50	2	ttT/ttC	F458/ AA_length = 634	SILENT	LOW
Sample 4	ENST00000440484	CASP9	chr1	15844615	A	G	3021.64	237	46.41	2	ttT/ttC	F136/ AA_length = 253	SILENT	LOW
Sample 5	ENST00000361314	GPX7	chr1	53072454	T	C	7722.99	225	100	2	ttT/ttC	F79/ AA_length = 187	SILENT	LOW
Sample 6	ENST00000370399	TGFBR3	chr1	92177938	A	G	2854.55	180	51.11	14	ttT/ttC	F675/ AA_length = 850	SILENT	LOW
Sample 7	ENST00000526634	LGALS8	chr1	236700807	T	A	3329.2	97	100	3	tTt/tAt	F19Y/ AA_length = 317	MISSENSE	MODERATE
Sample 8	ENST00000367967	FCGR3A	chr1	161514542	A	C	1226.2	144	45.14	5	Ttt/Gtt	F176V/ AA_length = 254	MISSENSE	MODERATE
Sample 9	ENST00000392409	MUC4	chr3	195517553	A	C	6454.16	176	99.43	5	Ttt/Gtt	F274V/ AA_length = 1912	MISSENSE	MODERATE
Sample 10	ENST00000398107	ZNF2	chr2	95847040	TGCG	T	8931.23	206	92.72	4	ttgcgg/ ttg	LR114L/ AA_length = 384	MISSENSE	MODERATE

Differential Expression and Splice Isoform Variation



Ontology and Pathway Analysis



Figures generated with Partek™ and Integrated Genomics Viewer™ software

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.

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