3'RNA-SEQ FOR ACCURATE GENE EXPRESSION ANALYSIS OF CHALLENGING TUMOR SAMPLES

A Powerful Workflow to Access Cancer Gene Expression Signatures

BACKGROUND

RNA-seq empowers researchers and clinicians to explore the **complete gene expression** profile of a tissue or a cell at a given time. It can provide valuable additional information to DNA sequencing, making the transcriptome profile dynamic and reflecting the state of the cells in real time.

RNA-seq specifically has applications in the field of oncology[1]. Given that transcriptional profile in cancer cells results from mutations and epigenetic alterations, the technology could be used as a surrogate to measure the global tumor genetic alteration pattern[2].

RNA applications for research and clinical purposes:

- Molecular characterization
- Biomarker discovery
- Diagnosis and prognosis
- Treatment orientation

Expression profiles can provide insights that enable the identification of **clinically relevant molecular[3] signatures** that could be used to determine tumor subtype, predict the possible outcomes of a disease[4] and guide treatment decisions[5]. They can also be useful to identify the tissue of origin in the case of cancer of unknown primary[6]. Moreover, expression profiles may help to decipher the immune and stromal composition of a tumor sample, which has important implications for immunotherapy eligibility decisions[7].

Yet, gene expression profiling using conventional RNA-seq protocol may be challenging for researchers and clinicians for whom samples of quality and in enough quantity may be complex to obtain.

CHALLENGES

Tumor samples are usually preserved as FFPE tissue. Their potentially low quality and quantity are a major hurdle that hampers the clinical use of RNA-seq. Since the degradation of the RNA starts from 5' end to 3' end, 3'RNA-seq may provide a solution to overcome this issue and offer an alternative to analyze the RNA expression in such challenging samples.

The method generates far less amount of data compared to conventional RNA-seq protocol, which enables researchers and clinicians to assess larger series of samples. Hence, 3'RNA-seq can be considered as a more cost-efficient protocol compared to conventional RNA-seq.

Technical features of IntegraGen's 3'RNA-seq:

- Minimum quantity to be provided for total RNA: 500 pg
- Suitable for FFPE samples
- Rapid turnaround time: 2 days (series of 96 samples)
- Molecular tagging: **UMI**
- Less quantity of data required for the gene expression analysis

SOLUTIONS

From **sample extraction to data analysis**, IntegraGen solutions are designed to **empower clinicians and researchers to explore and access relevant insights from their gene expression sequencing data.**

Benefits of 3' RNA-Seq:

- Low quality of required input material
- Possibility to work with FFPE samples
- No bias linked to the length of the mRNA
- Cost efficiency compared to RNA-seq protocol

3' RNA-seq Workflow

IntegraGen developed an **in-house 3'RNA-seq protocol** based on a fragmentation step followed by a capture of the 3'part of the RNA, to specifically capture mRNA. Unlike conventional protocols that encompass depletion steps, IntegraGen's 3'RNA-seq protocol avoids potential sample losses. It ensures better yield performance, starting with a small sample quantity[8].

3' RNA-seq data analysis

IntegraGen has developed Galileo, an easy-to-use and intuitive cloud-based app that allows a dynamic exploration and analysis of 3'RNA-seq data. This software also offers a rapid identification of relevant gene expression signatures, **oncogenes expression as well as differential expression**, and gives more autonomy to researchers and clinicians as it eases the visualization and the analysis of RNA-seq data.

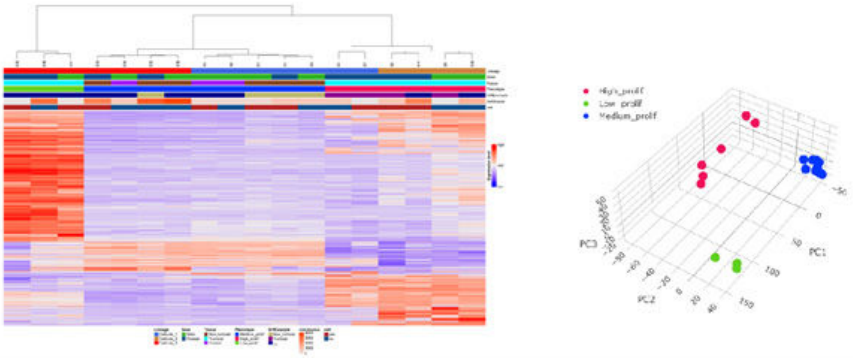

FIG. 1: GALILEO GRAPHIC REPRESENTATION EXAMPLES
(LEFT: HIERARCHICAL CLUSTERING; RIGHT: PRINCIPAL COMPONANT ANALYSIS (PCA))

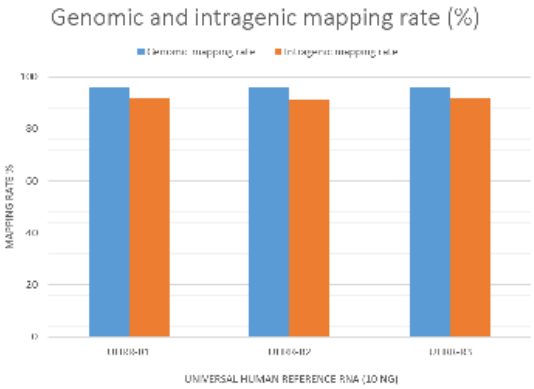

FIG. 2: SEQUENCING MAPPING RATE METRICS OF UNIVERSAL HUMAN REFERENCE RNA (UHRR) TRIPLICATE

CONCLUSION AND PERSPECTIVES IN GENE EXPRESSION ANALYSIS OF TUMOR SAMPLES

Analyzing gene expression with RNA-seq technology is an efficient way to obtain the complete transcriptome profile of a tumor sample compared to microarray technology. 3'RNA-seq protocols now enable the identification of gene expression signatures in challenging samples. This gives researchers and clinicians access to timely transcriptomic insights into gene expression and pathways activities that could be of importance in some cancer types.

Analysis of 3'RNA-seq data with Galileo:

- **Unsupervised analysis** (PCA, hierarchical clustering, t-SNE)
- **Differential expression, GSEA and ORA analyses**
- **Expression signature analysis**
- **Downloadable data tables and figures**

CONTACT US FOR A DEMO!
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