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Commentary

Targeted RNA-Sequencing for Measuring Gene Expression Profiling

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DESCRIPTION

Every living organism, DNA (Deoxyribonucleic Acid) contains all the data necessary to decide every cell's characteristics and duties. Through "gene expression," or the selective on/off switching of a particular group of genes, cells can flexibly access this blueprint and interpret particular commands [1]. The information contained in the chosen genes is transformed into RNA (Ribonucleic Acid) molecules, which can then either be used directly to regulate gene activation or translated into proteins [2]. Therefore, the set of RNAs that are transcribed at a specific time and under specific conditions represent the state of a cell at that moment and can disclose pathological processes underpinning illnesses.

In recent years, Next-Generation Sequencing (NGS)-based RNA-seq, a technique for RNA fingerprinting, has replaced microarrays for the analysis of gene expression. The RNA-seq sequencing framework makes it possible to characterize the sequences of all the RNAs contained in a material while also measuring their abundances [3]. An extremely precise technique for choosing and sequencing particular transcripts of interest are targeted RNA-sequencing (RNA-Seq). It provides data that is both quantifiable and intuitive. Both amplicon-based and enrichment-based methods can be used to perform targeted RNA-Seq, which allows for the study of gene expression in a narrowly defined collection of genes of interest [4]. Formalin-Fixed Paraffin-Embedded (FFPE) tissue is one sample type that enrichment tests can be used to identify, along with other known and unknown gene fusion partners.

Due to their high yield and affordable price tags, hybridization-based methods like microarrays were previously the most popular options for gene expression monitoring and DE analysis [5]. These technologies use a variety of sensors whose patterns correspond to specific areas of the genes that need to be tracked. The array is rinsed with the test material, allowing RNAs to freely hybridize to the sensors that have a complementary sequence. The RNAs are fluorescently labeled, allowing for picture capture of the entire array and the quantification of the expressed genes [6]. Despite being extensively used in quantitative transcriptomics, these methods have a number of drawbacks.

Although RNA-Seq is still in active research, it has already largely replaced microarrays in gene transcription level measurement and comparison because it provides a number of significant benefits over hybridization-based technologies, including:

- Reconstruction of known and novel transcripts at single-base level.
- Broad dynamic range, not limited by signal saturation.
- High levels of reproducibility.

The most potent characteristic of single-base resolution is undoubtedly its versatility, which enables the quantification and sequencing of every transcript present in a sample [7]. In contrast to microarrays, which can only measure portions of the transcripts that match the probes, RNA-seq uses the sequencing framework to get past the task of pure quantification, opening up new applications like the transcriptome profiling of non-model organisms, the discovery of novel transcripts, research into RNA editing, and measurement of allele-specific gene expression. Using a targeted RNA sequencing strategy is an option to sequencing the complete transcriptome. This serves as the foundation for Cellecta's DriverMap Targeted RNA Expression Assay [8]. The DriverMap method uses multiplex RT-PCR (Reverse Transcription Polymerase Chain Reaction) amplification to amplify a defined and conserved 80-250 base segment of each targeted gene's transcript rather than reverse-transcribe the entire transcriptome. Next-Generation Sequencing (NGS) is then used to quantitatively assess the abundance levels of each of these transcript amplicons.

Targeting, amplifying, and reading selective localized regions of interest in the expressed transcriptome have many benefits. From a methodological standpoint, the use of reverse transcriptase and PCR primers that specifically target each gene of interest eliminates the need for RNA processing to clear undesirable sequences like ribosomal RNA, beta-globins, or other non-coding RNA [9]. Only the transcript segments that fit the desired targets are expanded. The chemistry disregarded the rest. Therefore, since the test is PCR-based, only total RNA is

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necessary, and even that isn't much. For the majority of target transcripts, 10 pg of total RNA from single-cell lysate is sufficient for detection. The cDNA collection that needs to be sequenced is significantly simplified by the DriverMap RT-PCR tailored method [10]. The number of amplicons available for sequencing from a multiplex test that includes all 19,000 human protein-coding genes. Several thousand targeted amplicons can be safely scanned with much less NGS read depth than the entire transcriptome. As a consequence, the targeted method requires much less sequencing than other RNA-seq approaches and routinely and dependably identifies lower level expressed transcripts.

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