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Introductory Chapter: Applications of RNA-Seq Diagnostics in Biology and Medicine

Irina Vlasova-St. Louis

1. Introduction

The advent of next generation sequencing along with the development of bioinformatics tools has opened avenues to explore this technology in numerous fields of biomedical research (**Figure 1**). This book evaluates and comprehensively summarizes the scientific findings which have been achieved through RNA-Sequencing technology (RNA-Seq). RNA-Seq allows accurate capture of all RNA molecule subtypes; in any sequenced organism or single-cell type, under various experimental conditions. Coding and noncoding RNA types of the gene can be analyzed as part of discovery research and diagnosis of diseases. RNA-Seq transcriptome profiling of both healthy and diseased tissues provides an understanding of the alterations, in cellular phenotypes, through the expression of RNA isoforms. Assessment of gene expression, by RNA-Seq, provides new insight into host response to pathogens, drugs, allergens, and other environmental triggers [1].

RNA-sequencing becomes even more powerful when combined with other assays. Merging genomic and transcriptomic profiling provides novel information about underlying causative DNA mutations and the cellular effects of genetic variants caused by single nucleotide polymorphism (SNPs), indels, etc. Combining RNA-Seq with, proteome evaluation, immunoprecipitation, and cross-linking techniques is a clever multi-omics strategy to assess transcriptional, posttranscriptional, and posttranslational levels of gene expression regulation. The optimization of
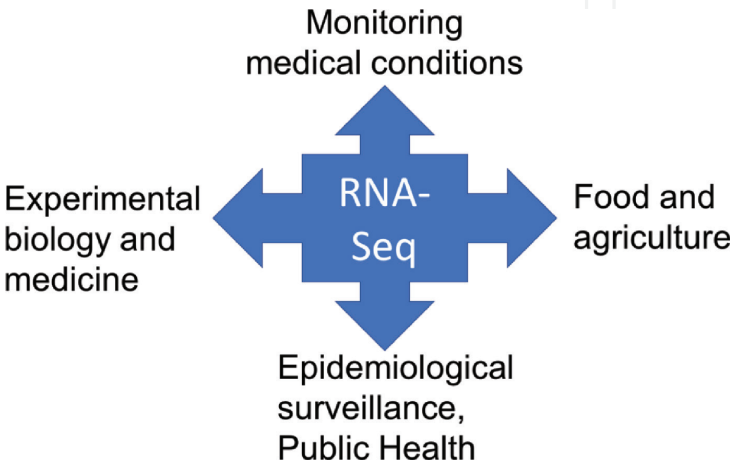

Figure 1.
Applications of RNA-Seq in biology and medicine. Applications of RNA-Seq discussed in this book.

RNA-Seq technology can provide countless opportunities in our pursuit of achieving the goals of systems biology and medicine, as described further in this chapter.

2. RNA sequencing in malignant and non-malignant disorders

RNA sequencing is a commercially available precision cancer diagnostic test [2]. No two tumors are alike. It is therefore imperative for cancer patients to have comprehensive testing of their tumors at the transcriptional and posttranscriptional levels [3]. Understanding the molecular features of cancer when the diagnosis is made allows oncologists to determine an optimal treatment path [4]. When making decisions about an individualized treatment many patients now understand the significance of having the most advanced molecular diagnostics available. Many companies (e.g., Caris Life Sciences) offer unique precision diagnostics services that are designed to maximize access to clinical trials, thus, offering patients a way toward novel treatments that are beyond the standard of care [5].

An increasing role of RNA sequencing in the detection of potentially malignant oral disorders, such as leukoplakia, lichen planus, or oral submucous fibrosis has been recently acknowledged. Transcriptome analysis of dysplastic tissues allows estimating of the rate of progression, of the pre-malignant conditions. Such estimation allows for individualized patient prognosis. The utilities for sequencing of oral, gut, and skin microbiota are becoming increasingly obvious. The steps in dual RNA sequencing are being continuously discussed, and RNA sequencing methodologies are being continuously improved [6].

3. Insights into normal microbiota and biofilms from metagenomics and transcriptomic approaches

The history of mapping the transcriptome via high throughput RNA sequencing methodologies is fascinating, with the earliest papers describing the term 'RNA-Seq' as pertaining to the yeast transcriptome [7].

RNA sequencing has become indispensable in metagenomics and meta- transcriptomics research, on human microbiota and biofilms. Our understanding of the magnitude and diversity of the species present within the gut, skin, and mucous microbiomes has increased dramatically in recent years. Meta- transcriptomic of 16s rRNA of human microbiome revealed exciting implications of altered biofilm maturation and dysbiosis, for various human diseases [8]. In this respect, a systems biology approach provides a larger picture of the molecules involved, in each system state or condition. Connecting RNA-Seq technologies with other multi- omics technologies allows for the identification, and selection, of key molecules and biomarkers of physiological as well as pathological processes [9]. The advancement of multi- omics technologies broaden the whole-system understanding of commensal and pathogenic microbiota [10]. It is very likely that we have still only scratched the surface of the plethora bacteria, fungi and viruses present within the human microbiome [11].

4. RNA-Seq profiling of host-pathogen interactions

Extraordinary effort by the scientific community has led to the development of various RNA-Seq procedures, including single-cell RNA-Seq and dual host-pathogen RNA-Seq for biology and medicine [12, 13]. Selection of specific RNA

species for research can be carried out either by enriching transcripts expressing poly- adenylated tails (usually for mRNA profiling), or by removing the abundant ribosomal RNAs or globin RNAs [14]. However, these techniques still face many challenges when using RNA-Seq profiling in assessing host-pathogen interaction networks. Various RNA-Seq chemistries such as pyro- sequencing, sequencing by hybridization, and sequencing by synthesis of RNA molecules converted into cDNAs, brings forth a more functional and integrated view of expressed genes. Recently developed, and commercialized, nanopore-based sequencing allows direct RNA sequencing detection based on a unique fluctuation in ionic current while nucleotides pass through nano- channels [15]. These advances in medical sequencing methodologies enable deciphering the transcriptome architectures of human immunodeficiency virus (HIV), as well as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In turn, this led to the rapid establishment of viral sequencing-based genomic surveillance world-wide. Owing to RNA-Seq technologies we can now perform nearly real-time phylogenetic studies, and genomic epidemiology surveillance of novel SARS-CoV-2 variants.

Bioinformatics efforts significantly improve genomic annotations of novel RNA isoforms through examination of translated, untranslated, differentially spliced regions, or the allele-specific RNA expressions [16–18]. Bioinformatics characterization of pathological host immune gene expression allowed for discoveries of novel biomarkers of immune reconstitution inflammatory syndrome (IRIS), in the HIV-infected population [19–21]. Additionally, severe illnesses that begin with uncontrolled overexpression of proinflammatory cytokines (the so-called "cytokine storm") have been discovered through transcriptomics in viral (e.g., SARS-CoV) infections and many bacterial infections [22–25].

Transcriptional heterogeneity, within and across the complex human specimens like blood, is a major obstacle in understanding inflammation and multicellular immune response [26]. Single-cell RNA sequencing (scRNA-Seq) is a new technology that provides significantly greater benefits to researchers than bulk RNA sequencing. The reason is that individual cell gene expression is masked if the bulk tissue specimen is used for analysis [27]. Deconvolution analysis, for bulk RNA-seq signals is impossible as data for each single cell type does not exist. The advantage of single-cell sequencing is that it can overcome this issue because scRNA-Seq captures the transcriptome of individual cells; in a particular condition and/or at a particular point in time [28]. Future applications of scRNA-Seq will likely lead to major breakthroughs in systems biology of combinatorial evaluation of host gene expression and microbial transcriptome [27].

5. Recent advances of RNA sequencing in food and agriculture

The expression and biogenesis of various types of RNA molecules are analyzed by RNA-Seq in the fields of food and agriculture [29]. Differential gene expression (DGE) has been measured in plant and animal cells, throughout the cell cycle, stages of cellular development and differentiation, and in response to specific environmental factors. Also, RNA-Seq analysis has been successfully utilized to identify DEGs associated with the eggshell formation in birds, fat deposition in animals, or flavonoids, anthocyanin, etc. biosynthesis in plants [30–32]. Moreover, the role of micro-RNAs, in naturally occurring food-derived compounds, has been implicated in determining the human and microbial gene expression, which contributes to overall health and well-being of individuals [33].

Numerous databases collect RNA-Seq results of allele-specific RNA expressions, alternatively spliced or processed RNAs, and structural RNA variants that are

economically important breeding traits in plants and livestock [34]. Assessment and cataloging SNP diversity in coding and non-coding regions subsequently allows to alter the path of negative or positive selection in natural populations of RNA species [35]. For example, the remodeling of root-associated transcriptomes, through the alternative RNA polyadenylation (APA), was found to increase the resistance to diverse abiotic stresses (observed in bamboo, sorghum, and arabidopsis) [36–38]. In rice species, APA site usage manipulation led to phylogenetic divergence into subspecies with beneficial agronomic traits [39].

The next few years should prove to be an exciting growth period for single-cell technology, in biomedical research [40]. Currently, there are several novel methodologies are available for generating single-cell RNA-Seq [41]. The biases that exist, within single-cell RNA-Seq protocols, represent challenges that need to be met to ensure its upward trajectory. A new biological discovery phase has just begun, and single-cell RNA-Seq has proven to be an invaluable tool, capable of guiding us through this phase.

In conclusion, we hope readers enjoy learning more about the application of RNA-Seq technologies provided throughout this book.

Acknowledgements

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