

RNA: An Expanding View of Function and Evolution

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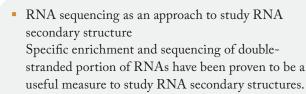
Supplement Aims and Scope

Next generation sequencing (NGS) technologies have enabled unprecedentedly deep characterization of transcriptomes. Compared to the microarray technology, NGS has been a much more favorable method for transcriptome profiling, as it doesn't require any pre-existing knowledge of the transcriptome of any given species. By sequencing transcriptomes to enough depth, several studies have reported a remarkably large number of novel RNA species and/or previously undetected splice forms. An inclusive identification of genes for organisms without genome information is also within reach. With intricate designs, it is even possible to specifically sequence the double-stranded portion of RNAs, such that the secondary structure of these RNAs could be inferred. Such comprehensive elucidation of transcriptomes has opened many new venues for studies on the function and evolution of the diverse RNA repertoire. The current supplement aims to serve as a portal to report original studies and summarize progress in this fast-moving area.

 Novel RNA species discovered by NGS and their unique evolutionary history Genome-wide studies utilizing NGS technologies have uncovered novel types of RNAs, such as lncRNA, circular RNA and spliRNA. Many of these novel RNA species have been reported to play important regulatory roles. With the continuous advancement of sequencing technologies and experimental designs, it is expected that even more RNA species will be discovered. Surprisingly, many lincRNAs, including several lincRNAs with known functions, have low interspecific sequence conservation. Several studies have suggested that structural conservation in these lincRNAs may have been retained, despite the apparent lack of sequence conservation. These recently identified non-coding RNAs represent an evolutionary history different from that of the protein coding genes, which remains to be explored.

- The function and evolution of alternative splicing Alternative splicing tremendously diversifies transcriptomes among organisms, even when their repertoires of protein coding genes are similar. The fast improvement on read length of NGS technologies will render a more thorough and unambiguous identification of alternative splice forms. Such resources could serve as the basis for exploring conservation and divergence of splicing events among organisms. They could also facilitate studies on the function of splicing events specific to certain organisms.
- RNA-seq of non-model organisms for phylogenomic studies

RNA-seq approaches enabled de novo identification of genes from organisms of no assembled genome sequences. Analyzing such extensive list of genes will result in better resolution of organism phylogeny. In addition, by comparing gene sequences among a wide range of organisms, many intriguing evolutionary questions may be addressed. For instance, how frequently genome duplication has happened in a certain taxon? How frequently horizontal gene transfer has happened between symbiotic organisms or parasites and hosts?



s Lord Kelvin put it, "nearly all the grandest discoveries of science have been but the rewards of accurate measurement and patient long-continued labor in the minute sifting of numerical results." The impacts of RNA-seq technology on current functional and evolutionary studies clearly reflect the importance of accuracy and scalability. Compared to traditional methods of gene expression quantification, such as microarray, RNA-seq does not require prior knowledge of the reference genome to design probes and shows much greater dynamic range, achieving more precise characterization of genes with extremely high/low expression. On the other hand, as with other high-throughput approaches, state-of-the-art data analysis is always a necessity to turn measurements into biological insights. Motivated by the striking advantages of RNA-seq and the challenges in data analysis, the goal of this supplement is to showcase how RNA-seq experiments could be designed to accurately measure transcriptomic dynamics and how elegant analysis of large-scale data could address specific biological questions.

Since its inception, RNA-seq has been widely adopted in various areas of studies. The unprecedented deep investigation of transcriptome enabled by RNA-seq has led to discoveries of novel RNA species and new ways of regulation.^{1,2} In this direction, Yin et al reported the detection of 30 conserved miRNA families and 113 novel miRNAs in Nicotiana benthamiana, most of which are likely to be involved in the immune response to Tobacco Mosaic Virus.³ The study by Taguchi found microRNA-target-specific histone modifications occur during mammalian spermatogenesis, inferring a new level of regulation.⁴ With the development of *de novo* assembly methods, RNA-seq has also catalyzed the blossom of studies on non-model organisms that currently do not have reference genome sequences.⁵ In this supplement, Zhou et al identified 59 differentially expressed genes under drought treatment in a non-model tropic plant, Bombax ceiba L., of which genes are involved in multiple functional pathways, indicating complicated genetic responses to drought stress.⁶ Wang et al conducted *de novo* transcriptome assembly for an endangered herb, Veratrilla baillonii, and discovered a large number of microsatellite markers and validated with experiments, providing valuable resources for the future population genetics studies and effective conservation management.7 Furthermore, RNA-seq provides previously inaccessible information for phylogenomic and evolutionary studies.^{8,9} Ma et al discovered 107 WRKY genes in desert poplar using a comparative genome approach, of which 10 genes were specific to Populus euphratica. By profiling



Comparison of RNA secondary structures among organisms will lead to identification of conserved structure motifs, and also may be used to infer their functions.

gene expression in both desert poplar and mesophytic poplar, they found this desert plant adapted to salt stress through *de novo* formation of new genes in WRKY gene family and acquisition of new functions for existing WRKY orthologs.¹⁰ Gao et al analyzed protein kinase superfamily and gene expression from 69 different conditions in Pacific oyster, and found strong positive correlation of sequence divergence and expression divergence within this gene family.¹¹

The sequencing capability behind RNA-seq is still progressing at an astonishing pace, with the cost of sequencing the same sample dropping exponentially. The scope of transcriptome characterization is continually expanding, especially fueled by large-scale tissue-specific and developmental-stagespecific studies.¹² We expect more such studies will emerge in the next few years to further push the limit. The accumulation of expression data, associated with fast increasing genotype information, will lead to a comprehensive identification of functional regulatory variants and deeper understanding of mechanisms of gene regulation. In this exciting era of highthroughput sequencing, we hope this supplement shares with you a sip of enthusiasm.

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