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Transcript Profiling towards Ameliorating Future

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SUMMARY

Transcription profiling is one of the most popular study types, also known as expression profiling'. It involves the quantification of gene expression of many genes in cells or tissue samples at the transcription (RNA) level. The quantification can be done by collecting biological samples and extracting RNA (in most cases, total RNA) following a treatment or at fixed time-points in a time-series, thereby creating 'snap-shots' of expression patterns. The functional genomics methodologies and strategies for understanding the function, directly or indirectly, of the genome. A major function of the genome is the expression (read: RNA synthesis) of the information that is encoded within it; transcription occurs as a consequence of the proper engagement of the regulatory (promoter) elements with RNA polymerase and all of the requisite ancillary factors. Whereas the genome is indicative of what a cell can potentially do, functional genomics is concerned with what a cell is actually doing at a particular moment and is, therefore, a logical extension of genome sequencing.

INTRODUCTION

Transcriptome analysis has been successfully applied, specifying valuable information on plant-pathogen interactions and system-wide variations in plant metabolism under pathogen infection as well as allows the identification of compounds that play a crucial role in plant innate immunity (Hagemeier J. et al. 2001).

Techniques to Study Gene Expression at m-RNA Level

DNA Microarrays (High throughput gene expression analysis)

Microarray analysis is a high-throughput method for the simultaneous assay of huge numbers of genes, Genomes, Transcriptomes, Proteomes, and Bioinformatics. A global method for the analysis of gene expression, microarrays (gene chips) consist of a solid support upon which hundreds of thousands of individual gene sequences, referred to as probes, have been applied. As a fluorescence-based assay, it offers a high level of sensitivity. Microarray applications include SNP profiling, detection of alternative RNA splicing patterns, direct comparison of genomes from different organisms, changes in gene expression patterns as a result of some type of experimental manipulation, and measurement of gene copy number variation. (Kar M.M. et al. 2012)

- A DNA microarray is a collection of microscopic DNA spotted to a solid surface (glass or silicon slides)
- Using a conventional hybridization process, the level of expression of genes is measured
- Microarrays are read using laser-based fluorescence scanners

Real-Time PCR

It is the premier method for quantitative measurement of DNA and RNA (RT-PCR). RT-PCR is a mainstream functional genomics tool for gene expression studies and is surely the most widely used, versatile, and arguably the most sensitive method, and the gold standard, for the assay of mRNA transcript abundance. Real-time PCR should be viewed as a complementary partner technique to RNA-Seq, rather than a competitor.

Northern Hybridization

A northern blot is a laboratory method used to detect specific RNA molecules among a mixture of RNA. Northern blotting can be used to analyse a sample of RNA from a particular tissue or cell type in order to measure the RNA expression of particular genes.

RNA-Sequencing

It is a powerful next-generation sequencing tool that has gained widespread acceptance. The massively parallel nature of RNA-Seq has revolutionized and invigorated mining of the transcriptome by virtue of the quantitative (transcript amount) and qualitative (transcript variety) data that it generates. Given the power of RNA-Seq, it is likely that many as-of-yet to be detected transcript species may soon be identified and added to the ranks

of annotated members of the transcriptome. While the expense associated with performing RNA-Seq has prevented some laboratories from taking full advantage of this technology, there has been a continuous decline in the per-reaction cost. This is likely to promote RNA-Seq to the functional genomics tool of choice because of the much greater dynamic range that it offers in comparison with microarrays and related methods. RNA-Seq can also be used to validate microarray data, and vice versa (Wenshu K. et al. 2018). Long RNAs are first converted into a library of cDNA fragments through either RNA fragmentation or DNA fragmentation.

- Sequencing adaptors (blue) are subsequently added to each cDNA fragment and a short sequence is obtained from each cDNA using high-throughput sequencing technology.
- The resulting sequence reads are aligned with the reference genome or transcriptome, and classified as three types: exonic reads, junction reads and poly (A) end-reads. These three types are used to generate a baseresolution expression profile for each gene
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CONCLUSION

Transcription profiling provide valuable information for understanding molecular mechanisms by which infected resistant and susceptible varieties respond to infection. Also, a combined transcriptomic and metabolomic analysis used to identify any differences in gene expression and secondary metabolite. It helps to better understand the host responses to infection by any bacteria, virus, etc. at the molecular level, to establish a model pathosystem for particular disease.

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