Short Communication

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Current Status of Gene Expression Analysis

Hiroshi Yasue[1*], koji Doi[1] and Abdelfatteh El Omri[2]

[1]Department of Genetics, Tsukuba Gene Technology Laboratories, Ibaraki, Japan
[2]Department of Laboratory Medicine and Pathology, Hamad Medical Corporation, Doha, Qatar

***Corresponding author:** Yasue H, Department of Genetics, Tsukuba Gene Technology Laboratories, Ibaraki, Japan

hyasue@tsukuba-genetech.com

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Description

The change of biological state such as that of phenotypes in organisms is definitely based on the change of gene expression. The gene expression is the only path from genes to phenotypes. Therefore, in order to understand the biological process change in different organisms, gene expression analysis has been done using various methods.

Alwine et al. from Stanford University described for the first time the use of Northern Blot technique [1]. Few years later (from 1980), this method was frequently used. Northern Blot is based on RNA electrophoresis in agarose gel and the transfer of the RNA in the gel to nitrocellulose membrane, followed by hybridization with a labeled probe specific to a given gene sequence [2]. However, several days are necessary to obtain results of the Northern blot analysis for a single gene.

In 1984, the discovery of Polymerase chain reaction (PCR) by Kary Mullis revolutionize science and let to the invention of Real-time PCR [3]. This new technique has been applied through reverse transcription of mRNA, showing exact copy number of mRNA in a sample, though the number of genes for analysis in one sample tube is limited up to four. Almost in parallel with usage of the real-time PCR, microarray analysis has come to be used for the expression analysis of huge number of genes at one time. Microarray technique was introduced late 1977, then was robotized by 1980-1990 to generate reproducible and repeatable results, and it is only by 1995 that the technique was miniaturized and the first publication using the word “microarray” was published [4]. Microarray is based on the hybridization of probes fixed on glass, each of which is specific to the corresponding genes. These analyses are based on nucleic acids hybridization, so that copy number controls are necessary for the calculation of number of the targeted mRNA in the samples. In other words, the direct comparison of real-time PCR values/signal intensity of microarray analysis between samples doesn’t provide correct results. The comparison should be done through the copy number controls. In addition, these provide less information about slice variants because of the following reasons; the primer pairs for the real-time PCR/probe sequences for the microarray are usually designed for limited exons of the target genes. In addition, mRNA molecules are not analyzed as continuous RNA strand, so that, it is not possible to constitute full-length transcripts, which are particularly important information for the change of gene expressions.

By the mid-2000s, RNA sequencing and its analysis tools such as GeneStar have been available [5-7]. The gene expression studies using the RNA sequencing are increasingly published. As is seen in **Figure 1**, the chronological transition of gene expression analysis tools is represented by the number of publications over time. Publication on Northern blot analysis started around 1982, and showed its peak usage in 1998, and then started to decrease until now. The Microarray and real time-PCR usage peaked around 2011 and 2015, respectively. Since the past few years, RNA sequencing is increasingly used until now, though 2020 showed a slight decrease in its usage. This can be partially attributed to coronavirus pandemic that led to shut-down of universities and research institutes all over the world, and the perturbation of the global supply-chain logistics of genomic reagents and supplies.

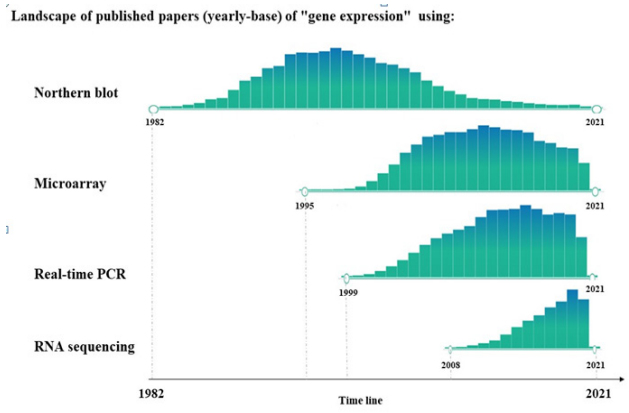

Figure 1: Chronological transitions of gene expression analysis methods..

RNA sequencing and the following in silico analysis can elucidate not only the amounts of gene expressions but also the change of gene splicing (change of exon usage). In addition, the digital data generated by RNA sequencing would be used for direct comparison between samples and even between experiments

performed in different occasions. As an example, **Figure 2** (not yet published data) illustrated splicing variants and expression level of exons of "Trafficking Protein Particle Complex 2 like TRAPPC2L" obtained from RNA sequencing. This gene showed no significant difference based on p-value ($0.01<p<0.05$) between samples, but when investigated at respective exons, it showed significant difference ($p<0.01$).

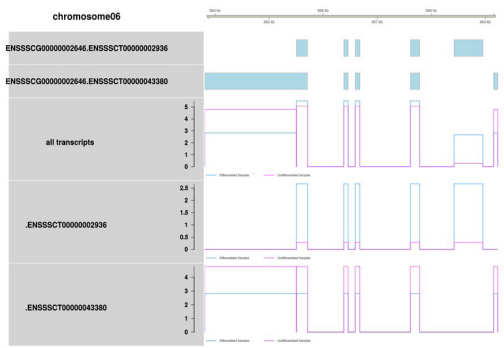

Figure 2: Trafficking protein particle complex 2 like trappc2l gene.

Discussion and Conclusion

This would provide deep insight for control of gene expression leading to phenotypic difference. This kind of observation and analysis cannot be obtained except by RNA sequencing, indicating that RNA sequencing is one of promising tools to understand the biological process of organisms deeply.

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