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Extended Abstract

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Transcriptome analysis to identify disease resistance genes through novel next generation sequencing applications for the predictive medicine

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Predictive medicine could be a comparatively new subspecialty in health care, however the construct isn't novel. Within the simplest terms, predictive medicine utilizes specific laboratory and genetic tests to see the chance if a individual can develop a disease. The use of biomarkers has been common within the field on medical specialty to predict repeat of cancer, however currently the aim is to extend the employment of comparable biomarkers to predict the lot of common clinical disorders in lifestyle. Breakthrough within the next generation sequencing (NGS) chances to research genetic variations in humans and their roles in health and disease. NGS offers regional genomic sequencing like whole exome sequencing of coding regions of all genes, as well as whole genome sequencing. RNASeq offers sequencing the complete transcriptomics and ChIP-Seq permits for sequencing the epigenetic architecture of the genome. Identifying genetic variations in each individual may be used to predict disease risk, with the potential to halt or retard the disease progression. NGS also can be used to predict the response to or adverse effects of medication or to calculate appropriate drug dosage. Such a personalized drugs also provides the chance to treat diseases supported the genetic makeup of the patient. Although before selling, the drug safety and efficacy thought to be approved by strict regulations, a drug might not show an expectable efficacy and safety in some people because of genetic variability among people in general. Unraveling the profile of human genomic makeup might give a sensible direction in prescribing medicine to every person; this is often attainable by NGS technologies. The drugs, therefore, would be prescribed in individualized manner for decreasing the adverse effects of the medicine, preventing their excessive consumption. With genomic information, more efficient, cheaper, and safer new medicine may be developed; therefore, Pharmacogenomics plays a central role in personalized medicine. Completion of the Human Genome Project in 2001 brought with it the realization that while understanding the genome is of great value, our understanding of biology is woefully incomplete without the knowledge of the functional elements of the genome. The functional element of the genome is the transcriptome, which is the set of RNA molecules such as mRNA, rRNA, tRNA, and various small RNAs. A large number of research projects are now focused on the transcriptome rather than on genome and proteome as only 1-2% of genes are coding and 80-90% of the transcribed genes are not translated to proteins. However, these are known to be involved in epigenetic regulation and gene expression regulation. Gene expression is a complex process regulated at multiple levels such as gene transcription, post-transcriptional modifications, and translation. Briefly, complexity at the transcription regulation arises from the presence of multiple Transcription Start Sites (TSSs), which can result in production of multiple transcripts from a single gene and alternate splicing as well as alternate polyadenylation of the primary RNA to produce several different forms of transcripts originating from the same gene. Because of different TSSs, eventually each mature transcript will code for different protein. Additionally, noncoding RNAs, which are not translated to proteins, play catalytic and structurally important roles. For example, tRNAs and rRNAs play a critical role in translation, small nuclear RNAs (snRNAs) participate in mRNA splicing, small nucleolar RNAs (snoRNAs) regulate rRNA splicing, guide RNAs (gRNAs) regulate RNA editing, and miRNA are involved in translational repression. Study of the transcriptome provides an understanding of the regulation of gene expression pattern, alternative splicing and transcript structure, dynamic regulation of transcripts in different tissues, and detailed information about the gene regulation in normal and diseased condition. Transcriptome profiling is typically performed using hybridization or sequencing-based methodologies. Hybridization-based methods involve binding of fluorescently labeled fragments to complementary probe sequences either in solution or on a solid surface, e.g., microarray. These approaches, however, suffer from limitations such as low resolution, low specificity, and low sensitivity. Later, Sanger sequencing-based approaches such as SAGE (Serial Analysis of Gene Expression), CAGE (Cap Analysis of Gene Expression), and MPSS (Massively Parallel Signature Sequencing) were developed, but these approaches have serious limitations such as consideration of partial transcripts structure for gene expression and inability to distinguish between isoforms. With the advent of Next Generation Sequencing (NGS), a technology that enables sequencing of millions of nucleotide fragments in parallel, RNA Sequencing (RNASeq) has emerged as a powerful method for studying the transcriptome. Though microarrays are high-throughput and economical, RNASeq offers numerous advantages over microarray. Some of the key benefits of using RNASeq over microarrays are:Genome-wide coverage of transcripts is offered by RNASeq. No prior knowledge of genome sequence is required in the case of RNASeq as opposed to microarray and hence RNASeq experiment can be performed in the absence of the reference genome. Improved sensitivity and specificity: RNASeq offers enhanced detection of transcripts and differentially expressed genes and isoforms. Moreover, RNASeq is known to be more accurate in terms of fold change detection for both high- and low-abundance genes. Detection of novel transcripts: Unlike microarray, RNASeq enables genome-wide unbiased study and is not dependent on transcript or region-specific probes and hence it investigates both known and novel transcripts. Detection of low-abundance transcripts if sequencing is done at high depth.

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