Chapter 9
Personal Diagnostics Using DNA-Sequencing

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ABSTRACT

DNA sequencing is the process to identification of nucleotides order in genome which developed from very broad history, also it is derived from version of the Sanger biochemistry. SOLiD, 454 and Polonator sequencing based on emulsion PCR to amplify clonal sequencing with in-vitro construction of adaptor-flanked shotgun library, PCR amplified in the context of a water-in-oil emulsion. Solexa technology relies on bridge PCR to amplify clonal sequencing features. At the conclusion of the PCR, each clonal cluster contains ~1,000 copies of a single member of the template library. This chapter focused on next-generation sequencing technologies methods, capabilities and clinical applications of DNA sequencing technologies for researchers in molecular biology and physician scientists. This will also provide the power of these novel genomic tools and methods to use personal diagnostic at molecular level.

INTRODUCTION

Next generation sequencing (NGS) technologies have massively penetrated into biological research science in recent years by producing huge amount of data with low cost compare to Sanger sequencing technology. NGS technology enabled depth analysis in microbial research which associated in humans, plants, animals etc. DNA based studies of the human associated with diseases are of high value to address genetic diseases. Genomic analyses of individuals or population studies of whole genome provide insight into the composition and physiological potential of humans disease mechanisms. RNA based studies can extend such studies in order to elucidate the actual metabolic activities and transcriptional mechanisms of the cells under given conditions.

NGS applications can use various analysis based on DNA and RNA; they allow finding answers to questions that could not be addressed before, largely due to technical and financial limitations (Venter et al., 2001; Lander et al., 2001). The establishment of a reference human genome (Hg19) and large-scale human study in the 1000 Genome project are, in conjunction with the use of next generation techniques,

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triggering advances in many areas of basic and applied science. Apart from personal diagnosis, clinical applications of NGS include the sequencing of cell-free DNA fragments circulating in a patient's bloodstream. Similar to detection of a low-frequency variant, this experiment relied on deep sequencing (very high coverage) of cell-free DNA to detect changes in a small fraction of that DNA population which belonged to the organ donor. This result shows the potential of using NGS as a noninvasive method for detecting solid organ transplant rejection. Similarly, Palomaki and colleagues showed the potential of NGS as a noninvasive method for detecting Down syndrome and other fetal aneuploidies by sequencing the subpopulation of cell-free DNA in a pregnant mother's bloodstream belonging to her fetus. Results from this study showed the promise of an NGS plasma-based DNA test that can detect Down syndrome and other aneuploidies with high sensitivity and specificity. Together, sequencing of cell-free DNA by NGS in both of these examples offers enormous potential to reduce invasive medical procedures (Venter et al., 2001; Durbin et al., 2010).

Genome sequencing data is used in clinical practice of medicine at diagnostic level to identify disease. Continuous drop in sequencing cost, the actual translation of base pair reads to bedside clinical applications has finally begun. Personalized genome-based medicine would be the value of both whole-genome and targeted sequencing approaches in the diagnosis and treatment of diseases. DNA sequencing of cell-free DNA fragments circulating in a patient's bloodstream like DNA from a heart transplant donor's genome can be found in a recipients bloodstream when a transplant recipient is undergoing an acute cellular rejection, as validated by endomyocardial biopsy. Low-frequency variant detection by deep sequencing of cell-free DNA to detect changes in a small fraction of that DNA population which belonged to the organ donor. This result shows the potential of using NGS as a noninvasive method for detecting solid organ transplant rejection (Kapranov et al., 2012; Sucher et al., 2011; Clark et al., 2011).

BACKGROUND

NGS platforms produce a massive amount data (up to terabases) in parallel sequencing method. Often, NGS platforms are classified as second and third generation sequencing technologies. The methods which depends on a PCR step for signal intensification prior to sequencing as next generation sequencing instruments, opposed to single molecule sequencing. Next generation sequencing technology includes the 454 instruments from Roche, the different Illumina platforms and the Life Technologies instruments, i. e. the SOLiD (Sequencing by Oligonucleotide Ligation and Detection) and PacBio RS by Pacific Biosciences. The third generation sequencing instruments like Ion Torrent(PGM=Personal Genome Machine, IonProton) sequencers, MySeq & MsJunior. Next-Generation technology platforms differ in read length, throughput size, data metrics, read depth, etc (Venter et al., 2001; Mardis, 2008).

The sample preparation in NGS is clonal amplification of single strands of target DNA. Isolation of high quality DNA followed by DNA library preparation should be be performed before sequence. All next generation platforms will produce shorter reads comapare to sanger method, this limitation can overcome by development of paired-end/mate-paired sequencing. which can be performed using all three sequencing systems. Paired-end tags (PETs) are shorter sequences originating from the two ends of a target DNA. There are multiple ways of constructing a paired-end library. One is the clone based method, where the target sequence is ligated with adaptors containing MmeI restriction sites immediately next to the target sequence. Following amplification in E. coli, purification and MmeI digestion, the tag