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How do you Analyze a Mutation of the Gene Consisting of One Hundred Exons?

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Today, responsible genes of about half of the estimated 7,000 Mendelian inherited diseases were identified, and genetic diagnosis in clinical practice is expanding for appropriate medical care and genetic counseling of the patient. At present, gold standard method of genetic diagnosis is still whole coding exon sequencing by Sanger method. This strategy is going well when analyze a small sized gene or analyze limited sequences of mutational hot spots of a large gene. However, this approach is expensive and time consuming, when the gene of interest has a large number of exons and you need to examine all of them. In the practice of clinical genetics, many syndromes are easily diagnosed by phenotypic appearances, clinical data and imaging studies. But disease causative genes of these syndromes are frequently large and contain no mutational hot spots, which prevents easy DNA analysis. For example, tuberous sclerosis complex is caused by either *TSC1* (including 21 coding exons) or *TSC2* (including 41 coding exons) mutation, and osteogenesis imperfecta is caused by either *COL1A1* (including 51 coding exons) or *COL1A2* (including 52 coding exons) mutation. What is the best way to analyze this class of DNA sequence for clinical use?

Recently, Next Generation Sequencing (NGS) has been used in various fields of mutation screening. This massive sequencing technology is suitable for exome analysis or whole genome sequencing to explore unknown responsible gene for a genetic disease, or screening a list of genes causing similar phenotype at once. But NGS is too excessive for screening a few disease causative genes for molecular diagnosis. Additionally, mutations detected by NGS must be validated by Sanger sequencing because of its low accuracy. The running cost remains expensive even if multiple samples are processed simultaneously. So, NGS is positioned as one of the expensive DNA screening methods at present.

To fill this gap of whole coding exon sequencing by Sanger method and NGS, for 100 exons scale of mutation screening, we recently developed CHIPS (CEL nuclease mediated heteroduplex incision with polyacrylamide gel electrophoresis and silver staining) technology. CHIPS is a simple and effective screening method for unknown DNA variations based on the enzyme mismatch cleavage and finely optimized at every step to achieve maximum sensitivity and simplicity [1]. By mixing the sample DNA and control DNA, CHIPS can apply to not only autosomal dominant diseases, but also autosomal recessive and X-linked diseases. It achieves virtually 100 percent sensitivity of mutation detection, using only commercially available reagents and very basic apparatus. At the same time, CHIPS offers inexpensive easy mutation screening by cutting out 90 ~ 95% cost and effort of the direct sequencing. Historically, Single-Strand Conformation Polymorphism (SSCP) and Heteroduplex Analysis (HA) were most frequently used PCR based gel shift assays for this kind of mutation screening purpose, but these methods could not achieves enough sensitivity to clinical use. Soon after, several modified methods were appeared, such as Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE). Although these methods showed higher sensitivity, they require special equipment for running or making the gel. Thus, these modified methods have not become as popular as the original HA. In another trend, high throughput large machinery such as Denaturing High-Performance Liquid Chromatography (DHPLC) and MALDI-Time of Flight (TOF) mass spectrometry were induced to this area. Though promising high sensitivity, these methods require totally new instruments and are consequently not becoming popular because of the high initial cost for introducing these machines to a laboratory. CHIPS solved all of these problems at once. It can provide rapid, efficient, and inexpensive mutation screening, everywhere in the world [2].

Since the introduction of CHIPS technology from 2011, our hospital satisfies the needs of quick molecular screening and genuine genetic counseling in the Hokuriku district that is a small countryside region of Japan with three million inhabitants and thirty thousand annual births. We can now analyze more than 130 genes and more than 100 orphan disorders, and the list is still growing [3]. If you are considering a 100 exons scale of gene mutation screening for a clinical or a research purpose, I will recommend CHIPS without hesitation. As mentioned above, this method achieves maximum sensitivity and simplicity without using any special instruments; rather, it uses only conventional equipment and commercially available reagents. This is the greatest advantage of this old fashioned system. Because of its low technology, any researchers and clinicians, including those who are working in a disadvantaged scientific environment, can begin to use this system immediately. As history has proven, it is not necessarily the latest technology is truly useful.

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