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Finding Gene Mutations by the Enzyme: Chips for a Simple and Highly Sensitive Enzyme Mismatch Cleavage Method

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Determination of unknown DNA variations is one of the substantive matters in many fields of molecular biology. Sanger sequencing has been used to the routine for this purpose. However, when you need to examine a large sized DNA or abundant samples, this method is bothersome, expensive and time consuming. Recently, next generation sequencing (NGS) has been used in various purposes of mutation screening [1]. This massive sequencing technology is suitable for a scale of genome size screening, as well as to screen the list of genes that cause similar phenotypes, such as maturity onset diabetes of the young (MODY) [2]. If enough samples are gathered at once, NGS is a hopeful and fascinating strategy, because pooling of samples lowers the running cost per sample. But, if you are intended to examine 10~50 kb of DNA sequence by single experiment, you require an efficient and convenient screening method.

Historically, single-strand conformation polymorphism (SSCP) and heteroduplex analysis (HA) were the most commonly used methods for this purpose [3]. But, the sensitivity of these methods was not satisfactory for the rigorous experiment. Although many of the modified PCR based mutation screening methods have been produced, none of these become popular due to the low sensitivity and/or inconvenience. Two modified methods of HA, denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE), were developed to enhance the migration delay of heteroduplex DNA in the gel by changing the gel component or temperature [4]. Although these methods potentially have an advantage over HA, they require special equipment for running or making the gel. Thus, these modified methods have not become as popular as the original HA. Denaturing high-performance liquid chromatography (DHPLC) is a type of mobility shift assay that does not involve electrophoresis, but instead detects mutations based on the decreased retention time of the heteroduplex in an HPLC column. Although this new technology achieves high sensitivity, time-consuming optimization of the mutation detection conditions for each DNA sequence is required to obtain the maximum sensitivity [4].

An ideal mutation screening method would require only conventional equipment and reagents; a single protocol can be applied to any DNA sequences and mutation types; and would achieve high sensitivity, high throughput and high cost performance. Enzyme mismatch cleavage (EMC) potentially fulfills these criteria. After PCR amplification, heteroduplex DNA can be generated readily on the thermal cycler by continuing program of denaturing and gradual re-annealing of PCR products. Heteroduplex DNA is cleaved by the specific enzyme, and they are physically separated on the gel by their sizes. Therefore, there is no need to adjust the gel components or gel running conditions, like as SSCP and HA or various methods of modified them. Thus, if the sensitivity of the enzyme for mismatch cleaving is sufficiently high, EMC could become one of the ideal mutation screening methods. To confirm this point, we previously examined four commercially available enzymes, T4 endonuclease VII, endonuclease V, T7 endonuclease I and CEL nuclease (SURVEYOR Nuclease S). As a result, we found that the CEL nuclease demonstrated the highest sensitivity and was able to cleave any types of single base substitutions and deletion/insertion mutations. However, CEL nuclease showed substrate preference, as C/C was cleaved the most, T/T was cleaved least. When the mismatch site was located nearby the end of the PCR product, the difference in length of the cut band and the non-cutting band is little for discriminate. For this reason, i.e. low sensitivity and low spatial resolution, some cleaved bands were difficult to detect in agarose gel with ethidium bromide staining. Same result has been confirmed in studies of other laboratory [5]. We overcame the sensitivity problem by using the silver staining that was simplified as much as possible for increasing the working efficiency. It is known that silver staining of double-stranded DNA is at least 100 times as sensitive as fluorescence staining with ethidium bromide. In order to improve the spatial resolution, we used polyacrylamide gel which can easily discriminate the band size change as little as primer length. The combination of CEL nuclease, polyacrylamide gel electrophoresis and silver staining, also adjust the every step of the protocol, we finally achieved 100% sensitivity for mutation detection [6].

We named this method as CHIPS (CEL nuclease mediated heteroduplex incision with polyacrylamide gel electrophoresis and silver staining), and utilized for various tasks of molecular genetics in clinical and research fields. It is especially useful for the genetic testing in the practice of clinical genetics because of its high convenience and cost performance. By mixing the sample DNA and control DNA, CHIPS can apply to detect the mutation not only autosomal dominant diseases, but also autosomal recessive and X-linked diseases [7]. 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. In our clinic, we provide various genetic testing to our patients in $100 US per sample. We can now analyze more than 130 genes and more than 100 orphan disorders, and the list of test item is still growing [8].

CHIPS uses only commercially available reagents and very basic apparatus. This is the greatest advantage of this old fashioned system. Because of its superficial low technology, any researchers and clinicians, including those who are working in a disadvantaged scientific environment, can begin to use this system immediately. Also, the principle of CHIPS is possible application to high-tech equipment, such as WAVE HS system [9]. You can choose the platform to fit the

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experimental environment. If you are considering a middle scale gene mutation screening that containing 50 ~100 kb of DNA sequence, I will recommend CHIPS without hesitation.

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