

Review

The applications of high-fidelity DNA polymerase in the individualized medicine

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Individualized medicine requires not only the personalized treatment but also the individual genetic diagnosis. Technological progress may solve some restraints of existing molecular diagnostic assays, which will pave the ways to the clinical application of human genomic information as well as routine applications of mutation detection in addition to the traditional deoxyribonucleic acid (DNA) sequencing analysis. Therefore, the development of mutation detection technologies is still a contemporary urgent issue, particularly in individualized medicine. A simple, highly sensitive and accurate mutation detection technology is one of the area's extremely competitive fields in the post-genome era. The mutation sensitive molecular on/off switch mediated by high-fidelity DNA polymerase, a highly reliable, simple, cheap, practical and suitable technology for high-throughput analysis, meets some of the new requirements. The mutation sensitive molecular switch is extensively suitable for automation and high throughput screening and promising in clinical genetic detection and mutation screening for many types of known single genetic diseases. When the mutation sensitive molecular switch is combined with DNA pooling, it can be used for etiology correlation analysis of multi-gene genetic diseases and epidemiological investigations of rare genetic mutations and polymorphisms. Furthermore, the mutation sensitive molecular switch mediated by high-fidelity DNA polymerases can also be combined with DNA microarray which may make individual genome sequencing quick, cheap and suitable for mutation load analysis.

Key words: High-fidelity DNA polymerase, individualized medicine, mutation, molecular switch

INTRODUCTION

According to the online Mendelian inheritance in man (OMIM) and Human Gene Mutation Database (HGMD), about 1 000 kinds of human genetic diseases have a clear genetic cause. Methods available for the diagnosis of genetic mutation include denaturing high performance liquid chromatography (HPLC), high resolution melting (HRM), single strand conformation polymorphism (SSCP), DNA quantification analysis, gene chips and restriction endonuclease digestion and so on, in addition to DNA

sequence analysis (Table 1).

The completion of the Human Genome Sequencing Project and the full sequencing of other species genomes marked that human kind has entered into the post-genome era. Since then, the individualized medicine will replace the traditional healthcare model in a number of years. A report published in Nature Genetics listed ten key technologies benefiting to the healthcare including genetic diagnostics (Daar et al., 2002). It is not surprising that the molecular diagnostic technology for the individualized medicine is at the top of the list considering continuously increasing requirements of personalized treatment. The individualized medicine includes not only the traditional diagnosis of individual genetic mutations

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Table 1: Main representative available methods for the diagnosis of gene mutations

Method	Advantages	Disadvantages
DNA sequence analysis	specific and sensitive	special equipment, expensive and time-consuming
gene chips	efficient and high-throughput	special equipment, expensive and time-consuming
Restriction endonuclease digestion	simple and economic	poor sensitivity, time-consuming and non-high-throughput
DHPLC	rapid, specific and sensitive	special equipment and costliness
HRM	rapid, specific and sensitive	special equipment, professional and technical personnel and costliness
SSCP	Simple and economic	time-consuming, non-high-throughput, poor sensitivity and specificity

but also the timely diagnosis of both inherited and non-inherited mutations for different individuals, which can offer a therapeutic prediction, a marriage consultation and future risk assessment of their offspring according to genetic information. With the rapid development of biotechnology, it is possible to spend only about 5 000 renminbi in sequencing a mammalian genome or obtaining the genome map of a certain organization and its methylation pattern in the next 10 years. Thus, the information related to genetic mutations will be the content of medical history, no longer one object of routine diagnosis.

Mutation detection Strategy Using high-fidelity DNA polymerase

The individualized medicine mainly requires the gene diagnosis technology for somatic mutation, epigenetic mutation, pathogens drug-resistant mutation and a variety of micro-residue mutations. Although, at least more than 20 different methods have been developed for the diagnosis of gene mutations, none of them is sufficiently accurate or efficient to be competent for these mutations screening. A high rate of false positives remains one of the major obstacles to the effective application of available high throughput mutation assays, preventing them from being more widely used in clinical applications. As efficient and high-throughput compatible mutation assays are the bottlenecks in current mutation-related studies, the current researchers have focused on the high specific and sensitive mutation detection techniques.

The mutation detection method using high-fidelity DNA polymerase in combination with an inert primer was first reported by Bi and Stanbrook in 1998 (Bi and Stambrook, 1998), inert primers were then combined with low-fidelity

DNA polymerase for mutation assays by Sommer's laboratory researchers (Liu and Sommer, 2000). However, inert primers are too expensive to be widely used, and polymerases used in laboratory practice were exclusively low-fidelity DNA polymerases until 2003, when the pioneering work focusing on the development of SNP assays by Li (Zhang et al., 2003a,b; Zhang and Li, 2003). Thereafter, 3' terminal-modified allele-specific primers in conjunction with high-fidelity DNA polymerases have been extensively evaluated for their potential application in mutation detection (Chen et al., 2005; Cahill et al., 2003; Di Giusto and King, 2003; Gale and Tafoya, 2003; Hu and Li, 2007; Kumar et al., 2005; Nawrot et al., 2008; Brugère et al., 2008), and several groups have published results which confirmed the usefulness of the mutation assays mediated by high-fidelity DNA polymerase (Table 2). So far, as a result, there are the successful developments of three new types of mutation assays that are mediated by high-fidelity DNA polymerases: the inert primer extension (Chen et al., 2005), the 3' terminal labeled primer extension (Zhang and Li, 2001; Guo et al., 2003) and the single nucleotide operated on/off mutation sensitive molecular switch assay (Zhang et al., 2005; Guo et al., 2003; Li et al. 2005). The output from the inert primer extension assay results from matched primer off and mismatched primer on. The 3' terminal labeled primer extension works through a simple proofreading mechanism, by which the 3' terminal labels in the mismatched primers are excised, that's why the product of the perfect-match primer extension has labels, but the mismatched primers extension does not. The primers with the on/off switch assay perform in the pattern as matched primer on and mismatched primer off. From table 2, we can know the single nucleotide operated on/off mutation sensitive molecular switch assay has the most practical value.

Table 2: Several representative groups engaging in mutation assays mediated by exonuclease polymerases

Groups	Primers modification
Nanhua University, Hengyang, China	3[H]
Genome Therapeutics Corporation, Waltham, USA	Fluorescence
Nanhua University, Hengyang, China	
Nanhua University, Hengyang, China	Phosphorothioate
University of New South Wales, Sydney, Australia	
University of New Mexico School Health Sciences Center, Albuquerque, USA	
University of Illinois at Chicago, Chicago, USA	
GE Healthcare, Piscataway, USA	
Polish Academy of Sciences, Lodz, Poland	3' dehydroxylation
Nucleica S.A., Biopole Clermont-Limagne, Saint-Beauzire, France	
Nanhua University, Hengyang, China	

The Application of the On/off Switch on diagnosis

The on/off mutation sensitive molecular switch is achieved by high-fidelity DNA polymerase via 3'→5' exonuclease proofreading allele specific primers with a nuclease-resistant 3' terminal modification. And thus, the perfect-matched primers turn on DNA polymerization and the mismatched primers turn it off. The off-switch effect results from the exonuclease-resistant property of the phosphorothioate that blocks mismatch excision during proofreading, so high-fidelity DNA polymerase extends product only from the perfect-matched phosphorothioate-modified primer and no product is produced with the 3' terminal mismatched phosphorothioate-modified primer. Therefore, the on/off mutation sensitive molecular switch provides an unambiguous readout (yes or no) in single-nucleotide discrimination assay, and is also simple, economic, practical, high-throughput and high reliable comparing with other detection methods. Furthermore, it will become increasingly apparent and have the most practical value in individualized medicine in the next few years or even decades.

Before the universal application of sequencing a mammalian genome, the on/off mutation sensitive molecular switches mediated by high-fidelity DNA polymerase can be widely used in the diagnosis of known genetic diseases, new mutation, mosaic analysis and minimal residual mutant analysis including fusion genetic analysis, the diagnosis of infectious disease pathogens and somatic mutation. It can also be used to study the relationship between the drug effect and genetic influences in finding the target gene of drug susceptibility. When combined with DNA pooling, the on/off switch can be used for etiology correlation analysis of multi-gene genetic diseases and epidemiological investigations of

rare genetic mutations and polymorphisms. In addition, the on/off switch mediated by high-fidelity DNA polymerases can also be combined with DNA microarray, which makes sequencing a mammalian genome quick and cheap (Xiao et al., 2007). Currently, sequencing a mammalian genome costs tens of millions to hundreds of millions renminbi. As previously mentioned, once it will go down to about 5 000 renminbi for sequencing a mammalian genome, the information related to genetic mutations will be the content of medical history, no longer one object of routine diagnosis. Then, the somatic mutation, epigenetic mutation, pathogens drug-resistant mutation and a variety of micro-residue mutations test will become the focus of clinical genetic test. Because of its application in these areas, the on/off mutation sensitive molecular switches mediated by high-fidelity DNA polymerase, together with other available mutation detection methods with high sensitivity and high specificity, will become a powerful pool to promote the development of individualized medicine and greatly benefit to human health care business in the future.

Suggested strategies for somatic mutation load assay

It was almost impossible to analyze rare mutations or mutation loads before the development of highly sensitive genotyping methods. The false positives limit the effective application of conventional assay methods. Another value of the future genetic detection is that the mutation sensitive molecular switches mediated by high-fidelity DNA polymerase can also be used for mutation load analysis. Different from sub-health with only temporary changes in phenotype, sub-health with higher mutation load have increased risk suffering from a variety of gene-associated diseases and important gene mutation

can lead to leukemia and many other tumors.

The mutation sensitive molecular switches used for mutation load analysis can monitor radiation-induced DNA damage and provide real-time monitoring at the molecular level, and can also offer early effective warning for high-risk group which tend to be exposed to carcinogenic and mutagenic agents. Moreover, the detection of gene mutation load at the molecular level has important guiding significance for anti-radiation medicine to protect from gene mutation. Some mitochondrial DNA (mtDNA) mutations show a dose-dependent pattern in causing diseases. The existence of homoplasmy and heteroplasmy in mitochondria mutation and their close relationship with the development of disease strongly suggest the value of testing the mutation load of mtDNA in future studies of personalized medicine.

Concluding remarks

In general, the single nucleotide operated on/off mutation sensitive molecular switch is easy to operate, economic, and can combine with other platforms such as agarose gel electrophoresis, real-time polymerase chain reaction (PCR) and DNA microarray. These advantageous features make the on/off switch technology suitable for automation and high throughput screening, having promising benefits in clinical genetic detection and mutation screening for gene diagnosis and therapeutic prediction, which is suitable for the requirement of personalized medicine. In addition, the on/off switch can also be used to study the relationship between the drug effect and genetic influences in finding the target gene of a drug effects, and greatly benefit pharmacogenetics and the individual treatment. The versatility of proofreading genotyping assays might greatly enhance mutation analysis in the post-genomic era as well as accelerate other fundamental and clinical applications.

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