MutS as a tool for mutation detection

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Detection of point mutations may be invaluable in diagnosis, therapy and prophylaxis of genetic diseases and neoplasms that together are reported to afflict almost 40% of human population. SNP (single nucleotide polymorphism) analysis could be helpful in prediction and elimination of drug side effects. As the frequencies of individual mutations are very low, a huge number of samples have to be screened for a defined mutation. Numerous efforts are made to introduce a cheap, rapid and reliable screening method for mutation detection and there is still a demand for new solutions and improvements.

MutS protein is the cellular guard of replication fidelity, so it seems to be a very promising tool for mutation detection. MutS recognizes mispaired and unpaired bases in DNA. The DNA fragment examined for mutation (e.g. a PCR product) should be mixed with the reference DNA (without mutation), heated and cooled to form heteroduplexes. In case of mutation, the resultant heteroduplexes contain mismatches recognized by MutS. We developed three approaches to the detection of point mutations exploiting MutS ability to recognize DNA mismatches: DNA retardation, protection of mismatched DNA against exonuclease digestion, chimeric MutS proteins.

DNA retardation in polyacrylamide gels stained with SYBR-Gold allows mutation detection using 1-3 μg of *Thermus thermophilus* his$_6$-MutS protein (obtained in mg amounts per 1 litre of *E. coli* culture) and 50-200 ng of PCR product. The method enables the search for a broad range of mutations: from single up to several nucleotides, as mutations above three nucleotides are detected even without MutS, due to mobility shift caused by large insertion/deletion loops in heteroduplex DNA.

MutS binding DNA mismatches protects the complexed DNA against exonuclease digestion. Direct addition of fluorescent dye SYBR-Gold allows mutation detection in one tube assay. The limited efficiency of T4 DNA polymerase as exonuclease hampers the application of the method in practice.

MutS binding to mismatched DNA immobilized on solid phase could be observed thanks to the activity of a reporter domain linked to MutS. We obtained chimeric bifunctional proteins consisting of *Thermus thermophilus* MutS and reporter domains, like β-galactosidase and GFP. Very low detection limits for β-galactosidase could theoretically enable mutation detection not only by the examination of PCR products, but even of genomic DNA.