

The real-time polymerase chain reaction (PCR) is one of the most widely used techniques in modern molecular biology. This method is based on fluorescent monitoring of DNA amplification by using some detection system specific for reaction product. Since its development in the 1990s many different detection formats have been developed. These include dsDNA specific dyes, which are very simple and cheap, but not sufficiently specific, and various types of sequence-specific fluorescent oligonucleotide probes or modified primers, which provide a high-level of specificity, but are relatively expensive and require careful optimization of reaction conditions. An alternative approach to monitoring of real-time PCR reactions is presented in this study. Its function is based on the observation that guanine nucleotide can quench fluorescence of some fluorescent labels. The approach makes use of an oligonucleotide primer containing a labelled cytosine nucleotide at its 5' end. When such a primer is incorporated into the product of amplification, its fluorescence is quenched by the guanine nucleotide complementary to the modified cytosine. This system of "5' labelled primers" is easy and low-cost like the dsDNA specific dyes, but it is more specific, because non-specific products of amplification are not detected. Different variants of this system were tested with primer sequences targeted to DNA of model microorganism *Bacillus subtilis*. Detection limit 10 copies of template DNA per sample and standard curve with correlation coefficient higher than 0.98 was acquired with FAM-labelled primer. The principle of the method was finally applied to development of detection assay for dangerous pathogen *Bacillus anthracis*. Melting curve analysis showed that specific product was synthesized in samples containing DNA of *Bacillus anthracis* isolates, but not in samples containing DNA of other species. This technology simplifies the amplification assay and opens new possibilities for real-time PCR quantification.