

# An Introduction to Real-Time PCR

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## Abstract

The development of instruments that allowed real-time monitoring of fluorescence within PCR reaction vessels was a very significant advance. The technology is very flexible and many alternative instruments and fluorescent probe systems have been developed and are currently available. Real-time PCR assays can be completed very rapidly since no manipulations are required post-amplification. Identification of the amplification products by probe detection in real-time is highly accurate compared with size analysis on gels. Analysis of the progress of the reaction allows accurate quantification of the target sequence over a very wide dynamic range, provided suitable standards are available. Further investigation of the real-time PCR products within the original reaction mixture using probes and melting analysis can detect sequence variants including single base mutations.

Since the first practical demonstration of the concept real-time PCR has found applications in many branches of biological science. Applications include gene expression analysis, the diagnosis of infectious disease and human genetic testing.

Due to their capability in fluorimetry the real-time machines are also compatible with alternative amplification methods such as NASBA provided a fluorescence end-point is available.

## PCR: The Early Years

The theoretical concept of producing many copies of a specific DNA molecule by a cycling process using DNA polymerase and oligonucleotide primers was first expounded in a paper by Kleppe and colleagues in 1971 (Kleppe *et al.*, 1971). At that time, the practical exploitation of such a process must have seemed remote to the biologists who read the paper. This was due to the difficulty and cost of producing oligonucleotides, the non-availability of thermostable DNA polymerases and the lack of automated thermocycling instruments. By the time of the first demonstration of the PCR process by Saiki and colleagues in 1985 (Saiki *et al.*, 1985) automated oligonucleotide synthesisers were commonly available. This meant that the potential of PCR in a wide range of applications was recognised. However, it was still necessary to inject fresh thermo-labile polymerase prior to each elongation step and thermal cyclers were still in development. Consequently, the key step in realising the potential of the PCR was probably the use of a thermostable polymerase which was first described in 1988 (Saiki *et al.*, 1988). Since the first description of a practical DNA amplification process many refinements have been described and automatic thermal cyclers have become standard laboratory equipment. PCR is now an essential tool for many biologists and the standard protocols are very simple and user friendly. The exponential amplification process provides nanogram quantities of essentially identical DNA molecules starting from a few copies of a target sequence. The amplified material (the PCR amplicon) is available in sufficient quantity to be identified by size analysis, sequencing or by probe hybridisation. It can also be cloned readily or used as a reagent.

