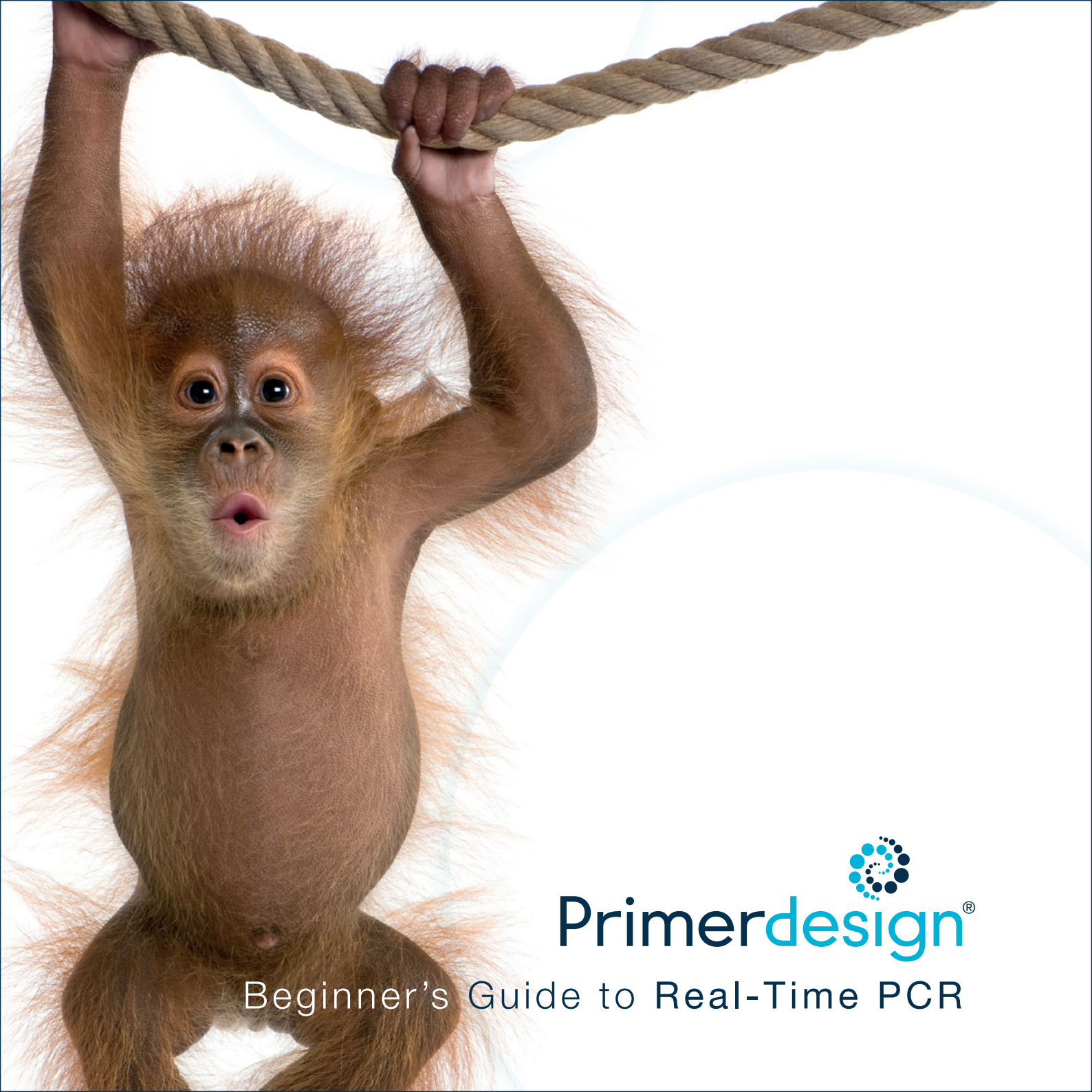


Primerdesign®

Beginner's Guide to Real-Time PCR

02 Real-time PCR basic principles

PCR or the Polymerase Chain Reaction has become the cornerstone of modern molecular biology the world over. Real-time PCR is an advanced form of the Polymerase Chain Reaction that maximizes the potential of the technique.

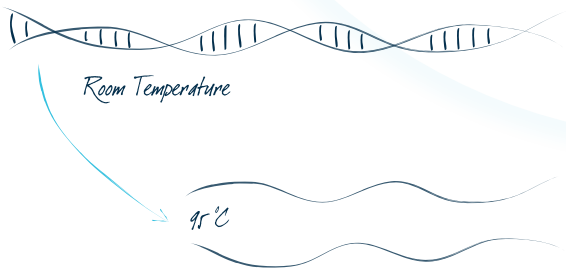
To understand real time PCR it is easier to begin with the principles of a basic PCR:
PCR is a technique for amplifying DNA. There are 2 reasons why you may want to amplify DNA. Firstly you may want to simply create multiple copies of a rare piece of DNA. For example a forensic scientist may want to amplify a tiny piece of DNA from a crime scene. More commonly however you may wish to compare 2 different samples of DNA to see which is the more abundant. DNA analysis requires amplification in order for there to be enough DNA to give a detectable signal for quantification. If you amplify both samples at the same rate, you can calculate which sample had the highest copy number of the target of interest to begin with.

It is a thermostable polymerase enzyme that drives a PCR. A polymerase will synthesize a complementary sequence of bases to any single strand of DNA providing it has a double stranded starting point.

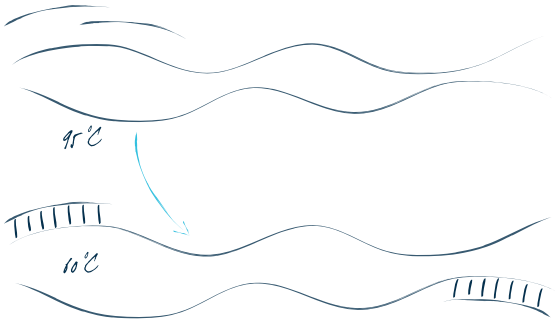
This is very useful because you can choose which gene you wish the polymerase to amplify in a mixed DNA sample by adding small pieces of DNA complimentary to your gene of interest. These small pieces of DNA are known as primers because they prime the DNA sample ready for the polymerase to bind and begin copying the gene of interest.

During a PCR, changes in temperature are used to control the activity of the polymerase and the binding of primers.

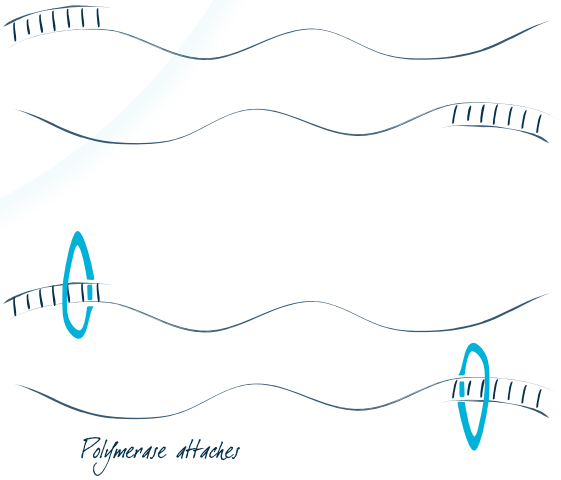
To begin the reaction the temperature is raised to 95°C. At this temperature all double stranded DNA is "melted" in to single strands:

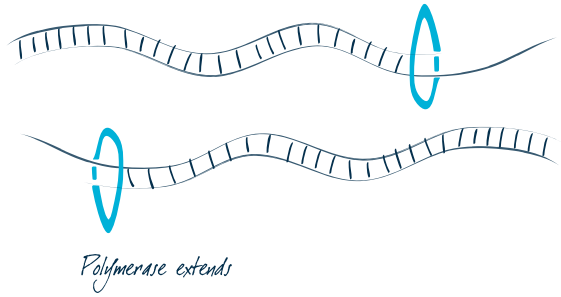


The temperature is then lowered to ~60°C. This allows the primers to bind to your gene of interest:



Thus the polymerase has somewhere to bind and can begin to copy the DNA strand:





The optimal temperature for the polymerase to operate is 72°C so at this point the temperature is sometimes raised to 72°C to allow the enzyme to work faster.

There are now twice as many copies of your gene of interest as when you started:

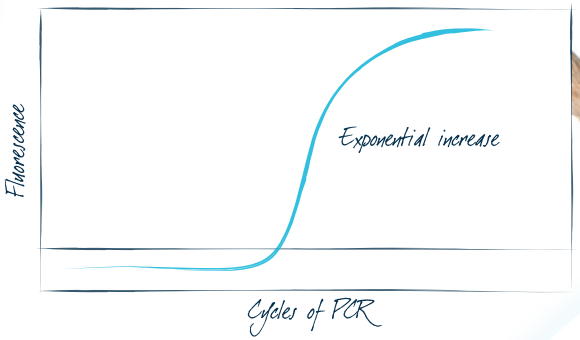
This temperature change is repeated through around 40 'cycles'. Thus one copy becomes 2, 2 become 4, 4 become 8, and so on until billions of copies are created.

After amplifying your gene it is possible to run the amplified DNA out on an agarose gel and stain it with a dye which makes it visible. The brighter the visible band, the more copies of your target you have created.

Real-time PCR

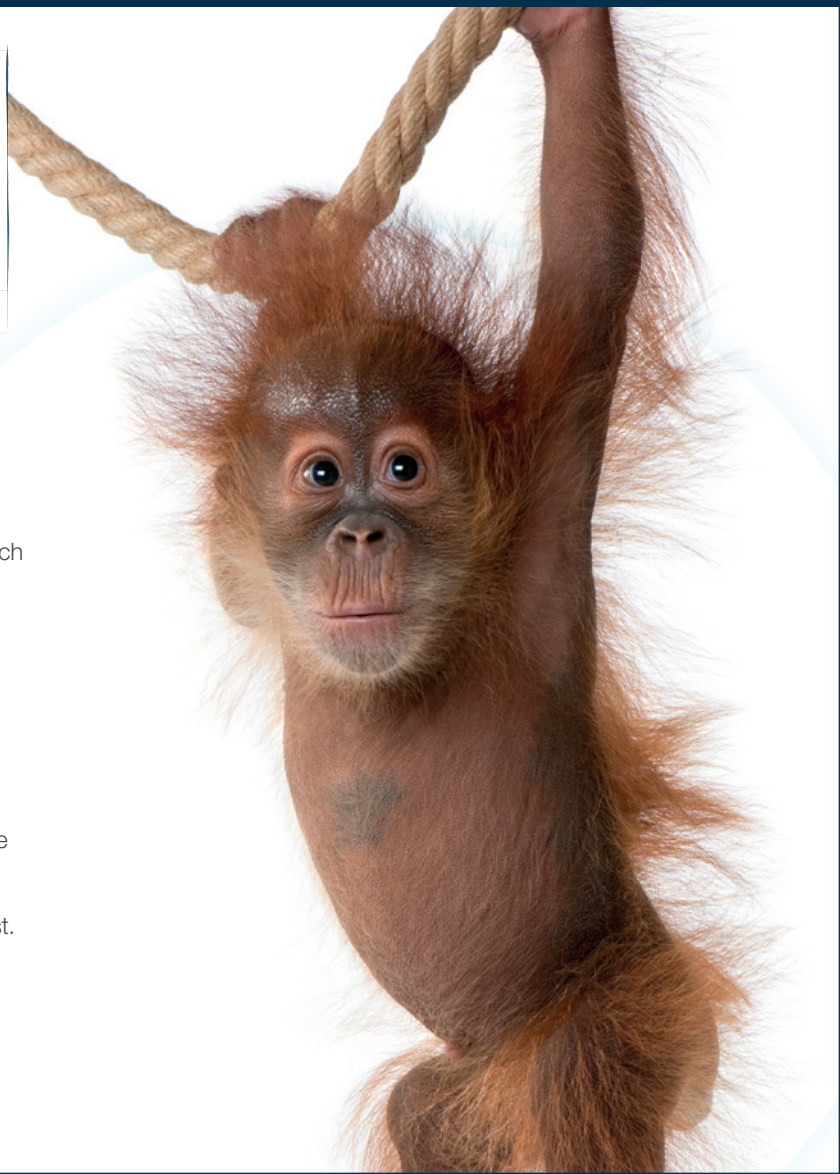
This same principle of amplification is employed in real-time PCR. But instead of looking at bands on a gel at the end of the reaction, the process is monitored in "real-time". Literally, the reaction is placed in to a real-time PCR machine that watches the reaction occur with a camera or detector.

There are a many different techniques that are used to allow the progress of a PCR reaction to be monitored but they all have one thing in common. They all link the amplification of DNA to the generation of fluorescence which can simply be detected with a camera during each PCR cycle. These different techniques are discussed on page 38. Hence, as the number of gene copies increases during the reaction, so the fluorescence increases.



Real time PCR has many benefits over the old fashioned approach:

- Firstly it gives you a look in to the reaction. You can literally see which reactions have worked well and which have failed.
- The efficiency of the reaction can be precisely calculated.
- There is also no need to run the PCR product out on a gel after the reaction as the melt curve analysis effectively does this for you.
- The greatest advantage of all however, is that real-time PCR data can be used to perform truly quantitative analysis of gene expression. In comparison, old fashioned PCR was only ever semi-quantitative at best.



Kits, reagents and expertise

Real-Time PCR is an exceptionally powerful research tool. With the correct kits, reagents and experimental design it is quick and easy to generate high quality meaningful data in no time with real-time PCR. Whether you are an expert or a complete beginner PrimerDesign Ltd can make your life very easy. We are experts and can help you by providing everything you need to generate the very best possible real-time PCR data.

What will you need?

Precision™ Reverse Transcription kits

High efficiency Reverse Transcription (RT) ensures the ability to detect rare transcripts and obtain the strongest possible Real-Time PCR signals. As a complete solution, the PrimerDesign Reverse Transcription kit contains all of the reagents necessary for the RT step.

geNorm™ Reference gene selection kits

geNorm is a system for selecting the best candidate reference gene for a given experimental scenario. The kit contains range of assays for reference genes and access to the geNorm software in qbase+.

Custom designed Real-Time PCR assays for any gene in any species

PrimerDesign specialise in the custom design and validation of Real-Time PCR primer assays. Simply supply the name or accession number of your target gene of interest and our team will design the best possible Real-Time PCR primers for that unique sequence.

Reference gene assays

To compliment your custom gene of interest assay, PrimerDesign has a wide range of reference gene assays which can be used as a normalising signal.

Precision™ 2 x Real-Time PCR Mastermix

Precision FAST 2 x Real-time PCR Mastermix is an ultra-fast, cost-saving mix for qPCR. The mix is designed for rapid cycling protocols that can dramatically shorten run times.

BrightWhite™ Real-Time PCR plasticware

The best possible Real-Time PCR is performed using white, opaque plasticware. BrightWhite plasticware channels all of the fluorescent output from your reaction straight back to the detector. Brighter, better data.

Please feel free to contact us for free advice or technical support.

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PrimerDesign works to an ISO 9001:2008 certified quality management system

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Products for Real-Time PCR