# Polymerase Chain Reaction (PCR)

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## Background

The polymerase chain reaction (PCR) is an indispensible molecular biology technique used in many areas such as cloning, analysing gene expression (**RT-PCR**), ***forensics*** and diagnosis of disease. PCR is a simple yet powerful technique that allows an extremely large number of copies of DNA to be produced from a very small number of starting copies.

## PCR reaction mixture

The first step of PCR is to chemically synthesize very small, single-stranded pieces of DNA (termed **primers**) that complement sequences that flank the DNA sequence of interest; there are many web-based tools that help with the design of these. A PCR reaction is made up of **target DNA** (i.e. genomic DNA, cDNA made from RNA), and a pair of primers that bind on opposing strands, flanking the target. The reaction mixture also contains a **DNA polymerase** to synthesize new DNA and deoxyribonucloside triphosphates (*dNTPs* – dATP, dTTP, dGTP and dCTP) required for new DNA generation, in an appropriate salt-containing buffer.

## The PCR reaction

The PCR reaction is made up of three main steps (**Figure 1**):

*Denaturation*: The mixture is heated to 95ºC for 30 s to denature the DNA, making single strands that act as templates for DNA synthesis.

*Annealing:* The mixture is cooled to between 55-72ºC for a short amount of time (approximately 30 s). This temperature depends on the primer sequence. Once this temperature is optimized, the primers anneal to the single-stranded DNA specifically at the regions flanking the DNA sequence of interest.

*Elongation:* This step is carried out at 72ºC (usually), the optimal temperature for the DNA polymerase. During this step, the DNA polymerase synthesizes an exact copy of both of the single-stranded target sequences starting at each primer using the dNTPs. The length of time spent at this step depends on the length of the target DNA sequence; the longer it is the longer this time needs to be.

The amount of DNA in the reaction mix therefore increases exponentially at the end of step 3. These three steps (collectively known as a **cycle**) are then repeated using an automated heating block 25-35 times (depending on the amount of initial target DNA) so producing a vast number of synthesized identical copies of the original target. These products can then be visualised by **electrophoresis** through an agarose gel containing ethidium bromide (**Figure 2**).

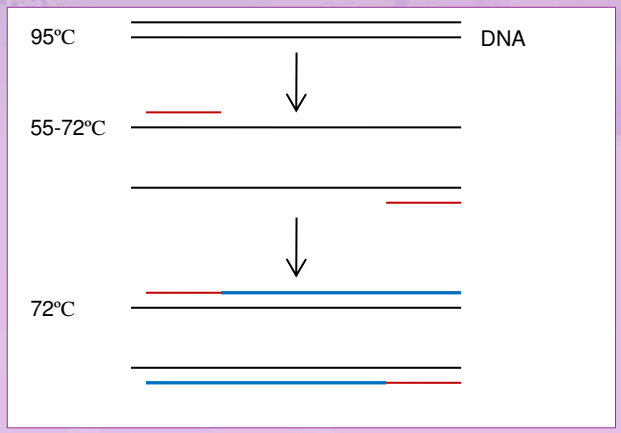


**Figure 1.** A diagram showing the three steps of PCR. One PCR cycle produces twice as many products.

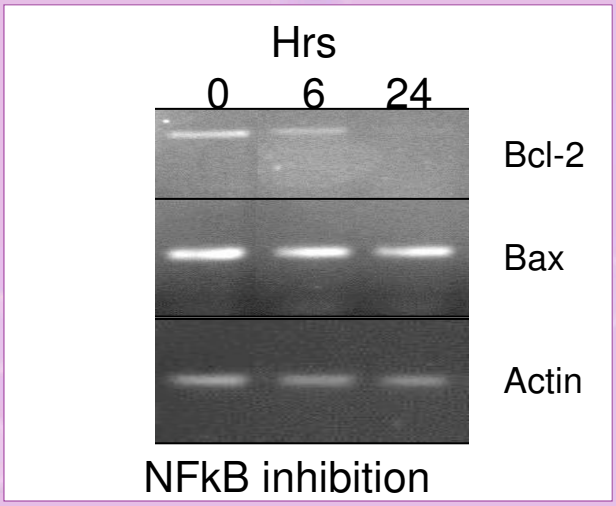


**Figure 2.** An agarose gel showing three RT-PCR products, actin being the control . This shows that the control stays the same, therefore the same amount of cDNA was added in all samples. It also shows that NFkB inhibition over time has no affect on Bax but leads to a decrease in Bcl-2.