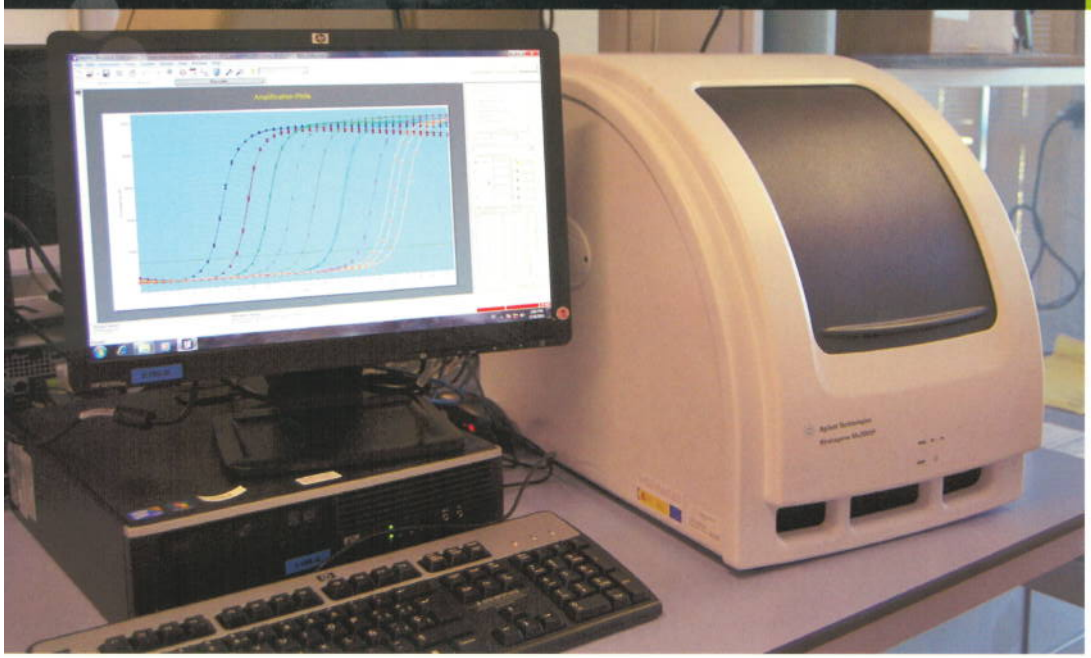


Real-time thermocycler

Real-time thermocycler

Brand
Stratagene

Model
Mx 3005P

Technical specifications

- 96-well Peltier-based block. Temperature uniformity ±0.25°C to 72°C.
- Excitation system with 5 excitation filters and quartz-tungsten lamp.
- Detection system with 5 emission filters.
- Filters: Alexa Fluor® 350, SybrGreen®/FAM™ , Cy3®, Texas Red®/ROX, Cy5®.
- Excitation range from 350 to 750nm and emission range from 350 to 700nm.
- Temperature range from 30 to 100°C.
- Sample volume from 0 to 200µL.
- Linear dynamic range to 10 orders of magnitude ($1\text{-}10^{10}$).
- Multiplex up to 5 dyes simultaneously.
- Software:
  - > View real-time amplification plots.
  - > Melting curve analysis with automatic melting temperature (Tm) calculation.
  - > Flexible Analysis Options: amplification plots of raw fluorescence data, baseline corrected, and normalized data, scatter plots, standard curve charts, dissociation curves, final call results, and text report.

SCT | Serveis Científics i Tècnics
sct@icra.cat

Technique description

Real-time polymerase chain reaction (qPCR) is a laboratory technique based on conventional PCR, which is used to amplify and simultaneously quantify a targeted DNA. A molecule labelled with a fluorescent group is added to the reaction medium. Molecules exposed to a beam of light at a particular wavelength absorb the light and then emit fluorescence. The fluorescence produced in the reaction is proportional to the amount of DNA formed.

Nowadays, qPCR can be performed using two types of flourescent molecules:

1.- Non-specific fluorescent dyes that intercalate with any double-stranded DNA. The replication of DNA during PCR leads to an increase in fluorescence intensity which is measured at each cycle. The main inconvenience is that DNA dyes will bind to all DNA, including non-specific PCR products or primer dimer. The most common fluorescent dye used is SYBR®Green.

2.- Specific hybridization probes labelled with two types of fluorochromes, a donor (fluoropher) and acceptor (quencher). The fluorophore transfers its energy to the quencher; the energy is released from the quencher as fluorescence of a higher wavelength. The most widely used types of probe are: TaqMan, molecular beacons and FRET probes.

The amplification curve is usually the first graph we look at. It shows the increase of fluorescence level on the Y axis, compared to the run cycle number on the X axis. The curve consists of an initial phase where the production of DNA is below the detection level of the equipment. The linear portion of each curve is in the exponential phase of PCR, where the amount of product, and therefore the signal, doubles after each cycle. The top section of the curve shows minimal signal increase, as PCR slows due to the depletion of reaction components.

Applications

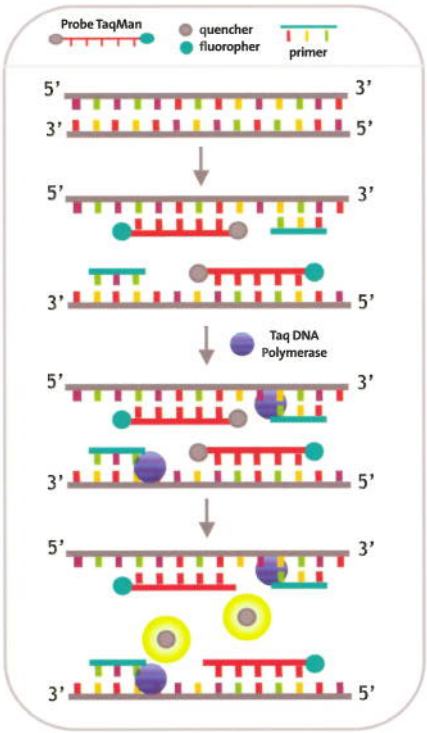
> In research situations, qPCR is mainly used to provide quantitative measurements of gene transcription. The technology may be used in determining how the genetic expression of a particular gene changes over time, such as in the response of tissue and cell cultures to the administration of a pharmacological agent, progression of cell differentiation, or in response to changes in environmental conditions.

> Absolute or relative quantification of DNA or RNA.

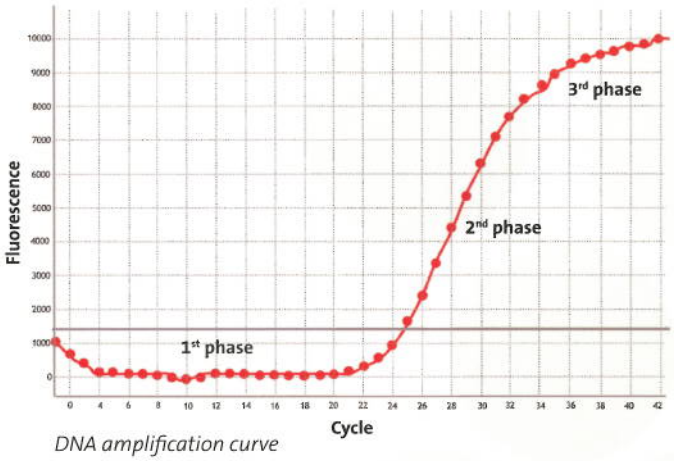
> Detection of single nucleotide polymorphisms.

> Analysis of gene expression.

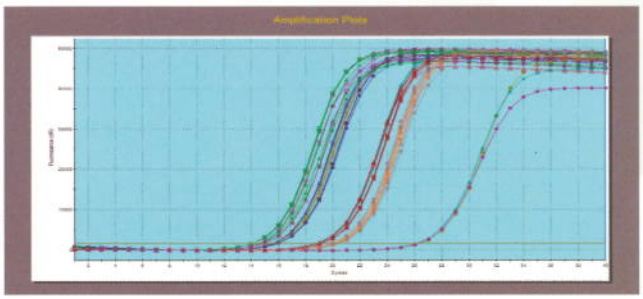
> Detection of genetically modified organisms.



Example of TaqMan probe



DNA amplification curve



Amplification curves for the quantification of gene 16SrDNA