Troubleshooting & FAQ

PCR and qPCR detection in general

Minerva Biolabs' deploys three types of PCR tests to detect microbial contaminations in biologicals and water. In this section you will find general explanations regarding the basic PCR methods.

Why do we deploy the Polymerase-Chain-Reaction as the molecular biological (diagnostic) method for the evaluation of microbial contamination?

The main drawback of classical microbiological methods is the time required to detect and identify a microbial presence. The diagnostic window (the time needed to provide a sufficient amount of microorganisms for diagnosis) can be drastically reduced with molecular biological diagnosis. The polymerase-chain-reaction reduces the diagnostic window to a few days. Further advantages of the PCR are the high sensitivity and the excellent specificity of the method. In addition, the qPCR allows the exact quantification of contaminating DNA.

A) Conventional (End-Point) PCR

Description:

Conventional PCR methods, unlike real-tim e PCR (se e below), rely on the total amount of product generated by the end of the reaction instead of the rate of product formation.

Method:

Sample

↓

Isolation of the DNA

↓

PCR with specific primers

↓

Result

Cambio Ltd, 1 The Irwin Centre, Scotland Road, Dry Drayton, Cambridge, CB23 8AR, UK
Tel :+44 (0)1954 210 200 , +44 (0)1954 210 300, email: support@ cambio.co.uk
www.cambio.co.uk

Our qualitative test is designed to detect whether a contamination (e.g. mycoplasma) is or is not present.

Why is no gene product formed?

Not every PCR is successful. There is for example a possibility that the quality of the DNA is poor, that inhibiting factors interfere with the PCR reaction, that one of the primers doesn't fit, or that there is too much or too less starting template.

Why could a gene product not be of the right size?

It is possible that there is a product, for example a band of 300 base pairs, but the expected gene should be 600 base pairs long. In that case, one of the primers probably attaches on a part of the gene closer to the other primer. It is also possible that both primers fit on a totally different gene.

Why do non-specific product bands appear?

It is possible that the primers fit on the desired locations, but that they also hybridise with other locations. In that case, you may observe different bands in one lane on a gel.

Another possibility is that the annealing temperature is too low. Increase of the annealing temperature increases specificity of the amplification process. It is also conceivable that the sample is contaminated by external DNA.

Which are the limitations of End-Point PCR?.

1. time consuming in comparison to qPCR.
2. results are based on size discrimination, which may be not very precise.
3. Reproducibility at end point of amplification is very low.
4. the end-point is variable from sample to sample.
5. Agarose Gel resolution is poor.
6. Short dynamic range (smaller than 2 logs).
7. non-automated.
8. results, obtained by staining methods, are not very quantitative.
9. Post-PCR processing required.

B) qPCR

Description:

The qPCR allows to quantitate the initial amount of the template. It monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle as opposed to conventional PCR that detects the amount of final amplified product at the end-point. The real-time progress of the reaction can be viewed in many systems.

Our quantitative test looks for contaminations and estimates the number of the contaminating particles per volume.

What do the slopes reflect?

The slopes of the detected curves reflect the amplification efficiency of the reaction, that is, how many copies of an amplicon each cycle synthesizes. When designing a qPCR experiment, it is also important to optimize this parameter making it as close as possible to a complete doubling in each cycle.

What do I observe in case of non-specific amplication?

qPCR does not detect the size of the amplicon, however, it is not influenced by non-specific amplification.

What is the advantage of quantitation?

qPCR quantitation eliminates post-PCR processing of PCR products which helps to increase throughput.

What the difference in respect to the dynamic range?

qPCR offers a much wider dynamic range of up to 10^7-fold (compared to 1000-fold in conventional PCR).

Which are the advantages of qPCR?

1. qPCR measures data at the exponential growth phase (high reproducibility in the beginning of the exponential phase), hence measurements are highly reliable and allow exact quantifications of e.g. microbial contaminations
2. The number of amplicons generated in the PCR reaction is proportional to increase in reporter dye fluorescence
3. Increased dynamic range of detection

4. No post-PCR processing
5. High sensitivity: detection is capable down to a 2-fold change
6. high specifity and objectivity
7. fully automated process