PathogenDx

Setting the standard in DNA testing

PCR: The Important Difference Between "qPCR" and "Endpoint PCR" Methods

Landmarks in PCR

In the well-known Polymerase Chain Reaction (PCR) an enzyme (Taq) and a number of DNA building blocks are mixed with a small amount of DNA starting material, as a "template" for the PCR reaction. The DNA "template" is then subjected to a cycle of heating and cooling (i.e. a thermal cycle) typically the series such as [95C, 60C, 72C] which is then repeated up to 40 times, i.e. up to 40 thermal Cycles (see Figure). In each of those thermal cycles, the original DNA template is copied by factors of two. In the beginning of that PCR Reaction, the amount of DNA product doubles during each cycle. Near the end of the reaction (typically at around cycle number 35-40) one or more of the reagents needed to support the PCR reaction is consumed, thus creating a situation where the PCR reaction reaches an "Endpoint" (Green line in the Figure) which serves as a first useful landmark for the reaction.

Early in the PCR reaction, there is a point where the amount of new DNA being produced from the small amount of the original template becomes just large enough to be detected. The cycle number where the DNA is first detected above background is called "Cq" and is a very useful parameter because it can be used to (back calculate) the amount of template DNA that was started with in the reaction.

Real Time PCR (qPCR) Methods

In one very well known version of PCR, the device used to perform the PCR thermal cycling reaction can also measure the amount of amplified DNA being produced at the end of each thermal cycle, in real time, thereby producing a curve that is very similar to the cartoon depicted in the Figure. Such devices analyze the amplified DNA curve as in the Figure and generate the (Cq) value of interest, which can then be used to detect and to quantify the original template DNA. The strength of such qPCR analysis, and the reason that it is widely used in Cannabis and Ag testing more generally is that it is a "one-pot" reaction in that the important information (Cq) is obtained in real time during the PCR reaction itself. Hence the name "Real Time" PCR, as it is often described.

Endpoint PCR Methods

There are a number of alternative PCR methods which are all based on allowing the PCR reaction to proceed to completion, i.e. to reach the "PCR Endpoint" depicted in the Figure (right). The DNA thus obtained must subsequently be measured by other types of physical analysis, after the PCR reaction is over. Thus, as a class, they are referred to as "Endpoint PCR Methods".

Functional Comparison Between the Two Major Classes of PCR Method.

qPCR

In general, the major strength of methods based on Real Time PCR (qPCR) is that the analysis is a type of elegant, one-pot reaction that is very well suited for routine laboratory analysis. qPCR is also attractive in that, with proposer controls and standards, the seminal Cq value obtained from it is relatable to the amount of the template DNA present in the beginning of the reaction. Thus, in the present case of microbial DNA analysis in Cannabis or Hemp, qPCR is well suited to detect the microbial DNA of interest and also to give a good estimate of how much of the microbial DNA of interest was in the original sample being tested.

Endpoint PCR

In general, any method based on Endpoint PCR will require an extra step for physical analysis. Well known second steps of that kind are gel electrophoresis, or DNA sequencing or DNA microarrays. However, the "Pay-back" for that extra step is an enormous increase in the sensitivity of the PCR assay.

That very large sensitivity differential is easily seen in the Figure, if attention is paid to the two important landmarks previously discussed. (Cq) the key parameter of qPCR (left side of the Figure) is the point at which the amount of PCR product just emerges as being greater than background.

Thus (Cq) occurs early in the PCR reaction, while the PCR Endpoint, the key parameter of all "Endpoint PCR" based methods (right side of the Figure) occurs much later in the reaction (typically 10-15 cycles later). During the execution of those 10-15 cycles of extra PCR amplification, the amount of DNA product increases @100 fold (see left Axis of the Figure). As a result, the amount of DNA available for analysis by the "Endpoint PCR" methods is usually 100x or more greater than the amount of DNA present when the data of interest in qPCR (Cq) is being calculated.

Thus "Endpoint PCR" methods are generally about 100x more sensitive than is the case for qPCR.

That important sensitivity difference is the reason that Endpoint PCR is often used as the "Front End" for mission-critical DNA analysis, when sensitivity is of the greatest importance. The best known example of the need for such mission-critical sensitivity is **"Endpoint PCR + Capillary Electrophoresis"**, generally known as the "Identifier Assay" which, based on its sensitivity and specificity, has become the world's Gold-Standard for DNA-based human ID in Forensics. Similarly, **"Endpoint PCR + DNA Sequencing"** has grown to be the Gold Standard for analysis of complex microbial populations (i.e. "microbiome" analysis). Most recently **"PCR+Microarrays"** has been deployed by Pathogendx as the emerging standard for high sensitivity analysis of the sort of complex microbial populations which can contaminate cannabis and hemp in regulated markets.