Solis Biodyne Multiplexing PCR

With multiplexing the PCR you are able to save time, money and precious samples. A the same time you will get more reliable results by having target and reference in the same reaction.

What is multiplex PCR?

First used as a method in detecting deletions in the dystrophin gene back in 1988[1], multiplex PCR has since made its way into many protocols across a variety of research areas. While still used in gene deletion analyses, multiplex PCR as well as qPCR is an incredibly useful tool in many applications, such as SNP genotyping, pathogen detection and food analysis to name just a few.

Multiplex allows for the researcher to detect multiple targets in a single reaction well. The prerequisite for this is using more than one primer pair for simultaneous target amplification and analysis

Why to multiplex?

Multiplex PCR is a useful tool in any laboratory working with repeating samples and targets. Incorporating it into your workflow allows you to work through your samples faster while using less sample as well as consumables.

Multiplexing can be applied both in endpoint PCR as well as in real-time qPCR. In endpoint PCR, gene targets are discriminated by amplicon size and detected typically via gel electrophoresis.

The amplicon size may be limited by the properties of a DNA polymerase. Typical Taq DNA polymerases allow amplification of up to 5 kb fragments. With endpoint PCR generally more targets can be detected in one reaction compared to real-time qPCR. In qPCR, the amplicon length is rather limited, typically between 100-200 bp, and the amplicons are detected in real-time using mostly target-specific hydrolysis probes (i.e TaqMan) or dsDNA intercalating dyes (i.e. SYBR® Green, EvaGreen®, or other similar dyes).

Multiplex qPCR with hydrolysis probes is highly adopted in the diagnostic sector, where it is used for detecting several pathogens as well as an internal control in the same sample in an extremely specific manner. Its time saving, cost-efficient and increased reliability features make it highly beneficial for routine and high throughput experiments.

Each target gene must bind a differently labeled probe to set its amplification signal apart from the other targets in the same reaction, as each label emits fluorescent light at a different wavelength that is detected by specific channels in the qPCR cycler.

Choosing the right tools

Using regular qPCR mixes in highly multiplexed assays carries a number of risks, such as late Ct values, low efficiency, low sensitivity and low fluoresence. As regular qPCR mixes do not have sufficient amount of components for amplifying more than 2 targets simultaneously, you are running the risk of getting false negative results. Using specifically designed multiplex mixes will greatly increase your success.

Read more: Multiplex PCR – maximize your through put

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