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Is the purity and quality of the sample DNA important for MLPA and digitalMLPA?

Sample purity is very important for MLPA and digitalMLPA while sample quality is usually relatively unimportant for successful reactions.

Purity

Sample purity is of utmost importance for both MLPA and digitalMLPA. Both techniques are based on a multiplex reaction, and have a higher sensitivity to impurities than some conventional PCR tests. Impurities such as ethanol, metal ions, salt, EDTA or phenol/Trizol remnants can decrease the activity of one of the enzymes or alter the properties of some probes, which can lead to poor or variable results.

For example, some probes are more sensitive to polymerase activity than others, which also makes them more sensitive to impurities that inhibit the polymerase. In MLPA, longer amplicons are generally more sensitive to reduced polymerase activity than shorter fragments, which can lead to a characteristic sloping pattern.

The presence of NaCl or other ionic impurities, such as Mg^{2+}, can also result in incomplete denaturation of the sample DNA. If the sample DNA is not completely denatured during the initial denaturation step in the protocol, probes may not be able to bind to their target sequences as efficiently. Probes that target GC-rich regions, which are more difficult to denature, will be more severely affected.

Read more about ways to improve the purity of DNA samples.

Quality

The quality of the DNA tends to have less of an effect on relative probe signals. For example, fragmentation does not strongly affect most probes because the target sequences are relatively short. Sample DNA that has degraded to the extent that a 1000 nt PCR is no longer possible may still produce good MLPA and digitalMLPA results.

Some chemical modifications of the DNA can have a more profound effect on MLPA and digitalMLPA results. Depurination, for example, affects probes differently depending on the amount and location of purines in the probes' target sequences. You can read more about depurination and how to prevent it in this article.

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