# Which factors influence variability of (digital)MLPA probes?



Variability in conventional MLPA and digitalMLPA experiments can be introduced by several factors, including sample treatment, the presence of impurities in the reaction, experimental issues, and probe(mix) characteristics.

## Background

Relative probe signals are constant as long as the reaction conditions are the same. Differences in reaction conditions do not always affect all probes equally, which can lead to changes in relative probe signals. For example, there is a relationship between relative probe signals and polymerase activity. The relative signal of some probes may decrease with reduced polymerase activity, whereas other probes may actually produce higher signals.

Typical sources of variability include:

- Impurities in the DNA sample. Impurities, such as salts, ethanol, EDTA, and phenol/trizol, can affect one or more steps in the (digital)MLPA reaction. Examples include a reduction in polymerase activity, altered denaturation efficiency or a reduced ligase activity. Reducing the amount of DNA or performing an extra DNA clean-up step may have a beneficial effect on the variability because it also decreases the concentration of the impurity. Read more about reducing the effect of impurities.
- Differences in sample treatment. DNA extracted from a different tissue source or with a different extraction method may have different properties or impurities, which can introduce variability. It is important to treat all samples in an experiment as similarly as possible. This is especially critical for DNA extracted from formalin-fixed, paraffin embedded (FFPE) tissues, as this DNA is often relatively impure and as it may have been chemically modified. Read more about the use of DNA from FFPE material.
- Experimental issues, such as pipetting errors, improper mixing, or equipment malfunction can also introduce variability. It is important to prepare master mixes whenever possible, to mix everything properly, and to ensure that calibrated pipettes and thermocyclers are used. Evaporation can also lead to variability. To minimize evaporation, use PCR tubes/plates suitable for your thermocycler, make sure that the lids are not deformed, and minimize the time that the tubes are open while pipetting the ligation reaction by using a multichannel pipette.

Relative probe signals are not strongly influenced by the amount of DNA used, as long as this falls within the recommended range and as long as the DNA is free from impurities.

Note

Many sources of variability can be identified by paying close attention to the control probes included in the (digital)MLPA probemix and the quality warnings given by Coffalyser.Net or Coffalyser digitalMLPA.

Tags

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