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How does Coffalyser.Net calculate and show results from mutation-specific probes?

This article was retrieved from MRC Holland Support (support.mrcholland.com) on Tuesday, 15th July 2025.

Some MLPA probemixes include one or more probes [designed to detect frequent mutations](#). These mutation-specific probes only give a signal when a specific mutation is present in the sample DNA. Because they generally do not give a signal on the majority of samples, data analysis and results display are different than for regular (copy number) probes. This background article explains how Coffalyser.Net handles mutation-specific probes.

Normalisation for mutation-specific probes

Intra ratio percentage

Most regular copy number probes are normalised against the reference probes within a sample *and* against the signals in other reference samples. This results in a final ratio that can be used to determine copy numbers. Mutation-specific probes generally do not have a signal in the majority of samples, including the reference samples. Therefore, their signals cannot be normalised against the reference samples, and no final ratio can be calculated. Coffalyser.Net solves this by only normalising to the reference probes within the sample, which produces an intra ratio percentage. If you are unsure about the difference between these two ratios, we highly recommend first reading more about [the difference between the final ratio and the intra ratio percentage](#) before continuing with this article.

Interpretation of an intra ratio percentage for mutation-specific probes

An intra ratio percentage only provides information about the relative signal height of the mutation-specific probe as compared to the reference probes in the sample. While this correlates with the number of copies of the mutation that are present, this value also depends on the relative heights of probes in the probemix. Therefore, *the intra ratio percentage cannot be used to determine the zygosity of the mutation*.

Instead, the intra ratio percentage should be used to determine whether a (significant) signal for the probe was found or not. Or, in other words, whether the sample might have the mutation the probe was designed for or not. If you need to know the zygosity of a mutation identified using MLPA, you can often investigate this using a different technique, such as sequencing.

For most mutation-specific probes, small signals (e.g. $\leq 10\%$) may represent background signals or false signals caused by electrophoresis noise, while real signals on positive samples will be considerably higher. As this may differ per application, consult the

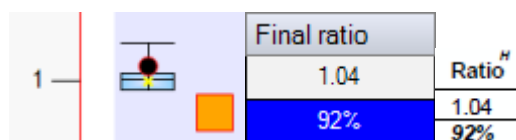
application-specific product description for more details.

Important

The intra ratio percentage *cannot* be used to determine the zygosity of a mutation. For example, an intra ratio percentage of 100% does *not* necessarily mean that there is one allele with the mutation (only that the signal happens to be similar to the signals from the reference probes).

Results display

Results for mutation-specific probes are always shown as *percentages* instead of ratios. In ratio charts, an orange box is shown rather than a probe dot. The example below shows a regular copy number probe (shown as black dot or final ratio of 1.04) and the result of a mutation-specific probe (shown as orange box or intra ratio percentage of 92%).



Intra ratio percentages are also used for other situations in which the calculation of a final ratio is not possible or not meaningful. For more details about this, read more about [how and when Coffalyser.Net shows results as final ratios or as intra ratio percentages](#).

Differences with previous versions

The way in which results of mutation-specific probes are shown has been made more consistent in recent versions of Coffalyser.Net. In v.210226.1433, v.210604.1451 and v.140721.1958, results were often shown as intra ratio percentages as well, but final ratios could be calculated for signals that were high enough if at least one reference sample also had a signal for the mutation-specific probe. This could sometimes lead to unexpected final ratios if background signals were involved. More details about the way this worked in older versions of Coffalyser.Net can be found in the relevant [Reference Manual](#). [Find which version you have installed](#).

Note

This article is about Coffalyser.Net for conventional MLPA data analysis.

Tags

Coffalyser.Net

Related Content

- [What is the difference between a final ratio and an intra ratio percentage, and when and how are they displayed in Coffalyser.Net?](#)
- [Is it possible to detect SNVs with \(digital\)MLPA?](#)

Disclaimer

The information provided in this material is correct for the majority of our products. For certain applications, the instructions for use may differ. In the event of conflicting information, the relevant instructions for use take precedence.