MRC Holland Support

How many samples do I need to include in a digitalMLPA experiment with no dedicated reference samples?

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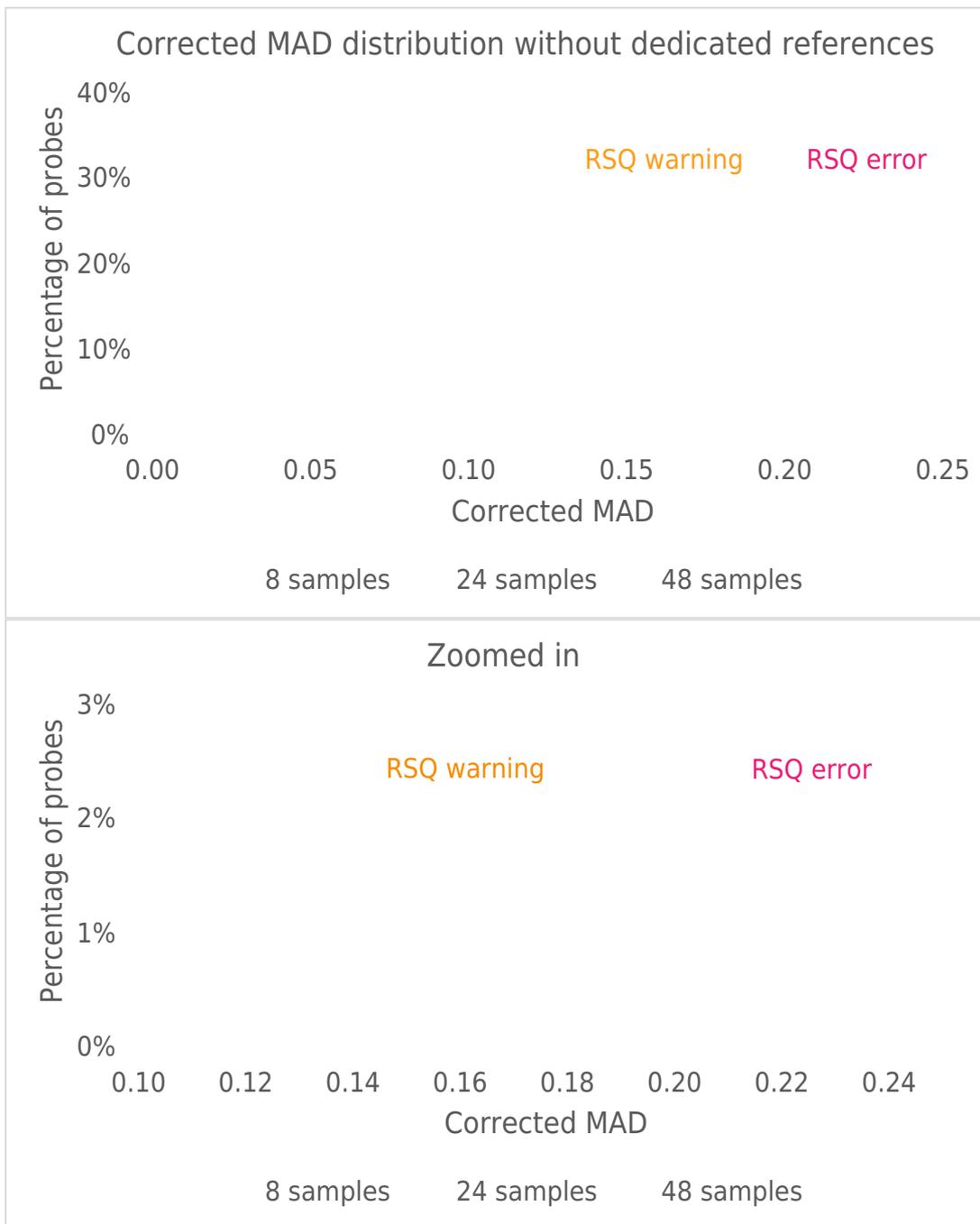
The minimum number of samples required in a digitalMLPA™ experiment without dedicated reference samples depends on several factors, including the experimental conditions, the quality of the samples, probemix characteristics and the overall reproducibility in the experiment. Therefore, the minimum number of required samples needs to be determined experimentally under your conditions.

Background

The use of dedicated reference samples is recommended for most digitalMLPA experiments. In certain situations, e.g. when a large number of samples are used and copy number changes are expected to be (very) rare, it is possible to omit dedicated reference samples and to rely on all test samples for inter-normalisation.

The reference sample quality (RSQ) score is one of the most important readouts to determine if the number of samples was high enough for reliable normalisation. The RSQ measures the variation of probes in the sample population used for normalisation. Depending on the amount of variation between samples in your experiment, it may be necessary to include more samples to avoid an RSQ warning or error. Read more about how the RSQ is calculated.

The figure below shows an example of the effect of the number of samples in an experiment without dedicated reference samples. In this experiment, an RSQ error was triggered when eight samples were included in the analysis, an RSQ warning was (just) triggered when 24 samples were included, and no warning was triggered when 48 samples were included. The inclusion of more samples leads to a narrower distribution of corrected MAD scores, and reduces the chance that an RSQ warning or error is triggered due to inherent variation.

Corrected MAD distribution without dedicated references

Percentage of probes

40%

30% RSQ warning   RSQ error

20%

10%

0%

0.00   0.05   0.10   0.15   0.20   0.25

Corrected MAD

8 samples   24 samples   48 samples

Zoomed in

Percentage of probes

3%

RSQ warning   RSQ error

2%

1%

0%

0.10 0.12 0.14 0.16 0.18 0.20 0.22 0.24

Corrected MAD

8 samples   24 samples   48 samples

Tags

Coffalyser digitalMLPA

digitalMLPA

Related Pages

- [How do I troubleshoot RSQ warnings or errors in my digitalMLPA experiment?](#)
- [What does the RSQ warning on my Coffalyser digitalMLPA report indicate and what is it based on?](#)

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