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Limitations of SALSA® MLPA® products

- SALSA® MLPA® products are designed to detect copy number changes of the sequences detected by the SALSA® MLPA® probes. The informative output of a SALSA® MLPA® product is restricted to the sequences detected by the probes included in that product. Copy number changes may still exist in regions outside of the probe's recognition sequence. Unless explicitly stated otherwise, SALSA® MLPA® probes are not designed to detect point mutations.
- The analytical sensitivity and specificity is probe-dependent and also depends on the quality of the DNA samples, the choice of reference DNA samples and the quality of the capillary electrophoresis. Internal MLPA technique validation in your laboratory is essential and should indicate a standard variation of each probe of 0.10 or lower when DNA samples of healthy individuals are tested.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will usually not detect inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that changes in that gene or chromosomal region do exist but have gone undetected.
- Sequence changes (SNPs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (at least up to 20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of the probe oligonucleotide to the sample DNA. The complete sequence detected by the probes is available on www.mlpa.com. The product descriptions contain only partial sequences detected by the probes.
- False positive duplication results due to contamination of DNA samples with PCR products have been described (Varga et al, 2012 Anal Biochem 421:799-801). In case of doubt, a second, independently purified DNA sample should be tested.
- Results can be compromised by, among others: impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis and a poor data normalisation procedure.

- Not all deletions and duplications detected by MLPA will be pathogenic. For some genes, in-frame deletions that resulted in very mild, or no disease, have been described. In certain cases, testing of DNA samples from the parents might be necessary for correct interpretation of results.

Confirmation of results

Mutations and/or polymorphisms in the DNA sequences detected by the SALSA® MLPA® probes, impurities in the nucleic acid sample that affect the polymerase activity and/or denaturation of the sample DNA, and errors made in the MLPA reaction, fragment separation and/or data analysis, may lead to wrong estimations about copy numbers present. For these reasons, **apparent copy number changes detected by MLPA always require confirmation by other methods**.

Technical Service

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