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Limitations of AnderSEQ products
- AnderSEQ products are designed to detect minor variants including SNVs, indels, deletions, insertions and copy number changes of the targeted sequences. The informative output of a AnderSEQ product is restricted to the sequences targeted in that product. Variants may still exist in regions outside of the targeted regions.
- The analytical sensitivity and specificity is amplicon-dependent and also depends on the quality of the DNA samples, the choice of reference DNA samples and the quality and read depth during the next generation sequencing analysis. Hence, internal validation in your laboratory is essential and should include but is not limited to positive controls for each variant, guardbanding DNA input and quality and repetability and reproducibility studies.
- AnderSEQ cannot detect any changes that lie outside the target sequence sand will usually not detect inversions or translocations, especially if these encompass the complete amplified region. Even when AnderSEQ 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 primer annealing sequence can cause false negative results. These Mutations/SNPs can reduce the probe signal by preventing or by destabilising the binding of the target specific primer to the sample DNA leading to loss of heterozygocity.
- False positive/negative results due to contamination of DNA samples with PCR products can occur if no strict separation is kept between pre- and post-PCR areas801). HaploGNX strongly recommend including a non-templated sample into every batch/plate analyzed. 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 next generation sequencing. Always make use of calibrated instruments and pipettes.

Confirmation of novel or unexpected results

Mutations and/or polymorphisms in the DNA sequences detected by the AnderSEQ technology, impurities in the nucleic acid sample that affect the polymerase activity and/or denaturation of the sample DNA, and errors including user errors made in the AnderSEQ assay and/or data analysis, may lead to wrong estimations about variants present. For these reasons, **apparent genomic changes detected by AnderSEQ assays always require confirmation by other methods**.

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