METHYLDETECT
DNA METHYLATION ASSAYS

Technical Guide: PCR Bias in Methylation Experiments

Introduction & Mechanism

The methylated and non-methylated locus after bisulfite modification differs in the number of cytosines (for detail see: What is bisulfite modification?). Different base compositions of the template may lead to differences in the efficiency of PCR amplification of those two versions of a locus, and this phenomenon is referred to as **PCR bias**. PCR bias in methylation studies was first identified by Warnecke PM et al. (PMID: 9336479).

This phenomenon was shown to present a serious pitfall in PCR-based methylation detection, leading to an underestimation of the methylation content of the screened sample.

Challenges in Low-Frequency Detection

PCR bias presents a serious challenge especially in experiments that aim to detect methylated alleles in a sample where they are present at low frequency in a background of non-methylated alleles. In those samples, higher efficiency of PCR amplification of the non-methylated allele may even lead to a complete lack of ability to detect the methylated allele and consequently a false-negative result, described in PMID: 19483476.

Sample material with a low content of methylated alleles of interest and a high background of non-methylated alleles includes:

- **Whole blood**, where only a specific type of cells may contain the methylated allele of interest and the vast majority of cells contain non-methylated alleles.
- **FFPE samples**, where the methylated allele in the DNA sample is underrepresented due to significant DNA degradation.
- **Liquid biopsies**, where only a few copies of the methylated allele may be present due to the nature of the sample

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Overcoming PCR Bias with MS-HRM

The innovative primer design for Methylation-Sensitive High-Resolution Melting technology (MS-HRM) allows researchers to overcome PCR bias and enables highly sensitive methylation detection even in samples where the methylated allele is significantly underrepresented. For details of primer design, see PMID: 18710507.

Assay Support & Applications

MethylDetect is dedicated to making MS-HRM technology accessible to clinical and academic researchers worldwide. We offer pre-validated, ready-to-use DNA methylation assays and reagents compatible with standard qPCR equipment.

For technical assistance or to request a quote, contact our team at info@methyldetect.com or visit www.methyldetect.com.

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