METHYLDETECT
DNA METHYLATION ASSAYS

Technical Guide: MLH1 Gene & DNA Methylation

Introduction & Mismatch Repair Mechanism The MutL homolog 1 (MLH1) gene, located on chromosome 3p21, encodes an essential component of the eukaryotic DNA mismatch repair (MMR) pathway. Functional MMR requires the coordination of conserved MutS and MutL homolog complexes:

- **MutL-alpha Heterodimer [1]:** MLH1 primarily forms a functional heterodimer with PMS2 (termed MutL-alpha), which serves as the primary enzymatic mediator recruited following base mismatch or short insertion/deletion (indel) recognition by MutS-alpha (MSH2-MSH6) or MutS-beta (MSH2-MSH3).

- **Excision & Resynthesis:** MutL-alpha displays intrinsic endonuclease activity, creating single-strand nicks near the DNA lesion to initiate either EXO1-dependent excision (in coordination with PCNA and RPA) or EXO1-independent 5'-flap displacement via FEN-1, followed by high-fidelity DNA polymerase δ/ε resynthesis and ligation.

Carcinogenesis Pathways & Microsatellite Instability (MSI) MMR deficiency leads to the uncorrected accumulation of replication errors, particularly within repetitive mononucleotide or dinucleotide genomic tracts, driving a phenotype termed Microsatellite Instability (MSI). Colorectal cancer (CRC) progression branches across three major molecular axes:

- **Chromosomal Instability (CIN):** Classical pathway initiated by APC mutations, followed by KRAS and TP53 aberrations.

- **Microsatellite Instability (MSI):** Hypermutated phenotype driven by genomic loss of MMR function.

- **CpG Island Methylator Phenotype (CIMP):** Epigenetic hypermethylation driving global tumor suppressor silencing.

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Differential Diagnosis: Lynch Syndrome vs. Sporadic CIMP Cancer
Determining MLH1 promoter hypermethylation is a critical clinical biomarker to stratify patients with MMR-deficient / MSI-High (MSI-H) tumors:

- **Lynch Syndrome (~3% of CRCs):** Caused by germline pathogenic variants in MLH1, MSH2, MSH6, or PMS2. Tumors display MMR protein loss by IHC without MLH1 promoter hypermethylation or BRAF V600E mutations, prompting germline genetic testing.

- **Sporadic CIMP Carcinoma (~15% of CRCs):** Driven by somatic MLH1 promoter hypermethylation (frequently co-occurring with BRAF V600E). Hypermethylation confirms a sporadic etiology, ruling out hereditary Lynch syndrome for the patient and primary relatives.

- **Constitutional Mismatch Repair Deficiency (CMMR-D):** Rare pediatric cancer-predisposition syndrome resulting from biallelic germline MMR mutations. Patients present early (median age 9 years) with high-grade brain tumors, hematological malignancies, and early-onset CRCs.

EpiMelt MS-HRM Assay Applications Robust evaluation of MLH1 promoter methylation status is essential for molecular pathology workflows in CRC and endometrial cancer stratification.

MethylDetect's EpiMelt MS-HRM Assays feature proportional primer design to deliver unbiased, high-sensitivity detection (0.1% LOD) of MLH1 promoter hypermethylation directly from FFPE tumor samples or liquid biopsies using standard qPCR equipment.

For assay ordering or technical support, contact info@methyldetect.com or visit www.methyldetect.com.

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Key Literature & Reference Notes

Note on MutL-alpha Structure & Function: MutL-alpha is an obligate heterodimeric protein complex composed of MLH1 (MutL homolog 1) and PMS2 (PMS1 homolog 2, mismatch repair system component). It acts as the principal endonuclease executing DNA cleavage during eukaryotic mismatch repair.

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4. Wojdacz, T. K., Dobrovic, A., & Hansen, L. L. (2008). Methylation-sensitive high-resolution melting. *Nature Protocols*, 3(12), 1903-1908.

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