

# ***MLH1, MSH2 and MSH6 Genetic Testing***



## **Customer Information Sheet**

**Genetic Technologies Corporation (GTG)**

### **Requirements for Testing**

- Peripheral blood (into EDTA tube) (9mL for comprehensive analysis, 4mL for predictive testing) or high quality DNA ( $A_{260}/_{280} > 1.7$ )\* (2µg for comprehensive analysis and 0.5µg for predictive testing);
- Completed request form (or Sample Submission Form\*\*) by referring clinician. Test request must include:
  - a. Description of test requested;
  - b. Ethnicity of the patient (when available);
  - c. Clinician full name, telephone number (and email address) for communication;
  - d. Clinician FAX number and postal address (including email address) for report;
  - e. Name and postal address (including email address) of any other individuals to whom a copy of the report should be addressed;
  - f. Invoicing institution name, contact phone number and address (for publicly funded patients);
- Copy of patient consent for the analysis\*\*;
- Completed payment form (for private funded tests). Testing can only commence once a payment has been made.

### **Description of Analysis**

#### **Comprehensive *MLH1, MSH2 and MSH6* genes analysis:**

*MLH1*: Full sequence determination in both forward and reverse directions of approximately 2,271 base pairs comprising 19 exons and approximately 570 adjacent non-coding intronic base pairs. The wild-type *MLH1* gene encodes a protein comprised of 756 amino acid residues.

*MSH2*: Full sequence determination in both forward and reverse directions of approximately 2,805 base pairs comprising 16 exons and approximately 470 adjacent non-coding intronic base pairs. The wild-type *MSH2* gene encodes a protein comprised of 934 amino acid residues.

*MSH6*: Full sequence determination in both forward and reverse directions of approximately 4,083 base pairs comprising 10 exons and approximately 510 adjacent non-coding intronic base pairs. The wild-type *MSH6* gene encodes a protein comprised of 1360 amino acid residues. The non-coding intronic regions of *MLH1, MSH2* and *MSH6* that are analysed do not extend more than 20 base pairs proximal to the 5' end and 10 base pairs distal to the 3' end of each exon. The *MLH1, MSH2* and *MSH6* comprehensive analysis also includes the detection of large genomic rearrangements (deletions and duplications). The analysis is performed with Multiplex Ligation-dependent Probe Amplification (MLPA) Assay where the amplification products are separated by capillary electrophoresis. The SALSA *MLH1* and *MSH2* MLPA (P003) probemix contains 43 different MLPA probes with amplification products between 130 and 472nt. The SALSA *MSH6* and *PMS2* MLPA (P008) (MRC-Holland) probemix contains probes for each of the 10 *MSH6* exons as well as for 13 of the 15 *PMS2* exons. Five probes are present for the *MUTYH* gene and six probes are included for *MLH3* and *MSH3* genes. In addition, one probe is included for *MSH2* exon1, two probes for the *TACSTD1* gene located immediately in front of *MSH2* gene and two probes for *MLH1* exon1. It contains 41 different probes with amplification products between 130 and 463nt. Apparent deletions/duplication of a single/multiple exon(s) are confirmed by reanalysing the DNA from the original specimen in duplicate.

**Single Site Analysis (Predictive Testing):** DNA sequence analysis (or MLPA analysis) for a specified mutation in *MLH1* and/or *MSH2* and/or *MSH6* gene.

### **Description of Method**

DNA is extracted and purified from white blood cells isolated from each sample. Aliquots of patient's DNA are each subjected to polymerase chain reaction (PCR) amplification (19 reactions for *MLH1*, 16 reactions for *MSH2* and 17 reactions for *MSH6*). The amplified products are each directly sequenced in forward and

\*For some test requests an alternative source of genetic material can be accepted for testing (please contact GTG for details)

\*\*Consent form is available on request

reverse directions using fluorescent dye direct sequencing protocol. Chromatographic tracings of each amplicon are analysed by a proprietary computer-based review followed by visual inspection and confirmation. Genetic variants are detected by comparison with a consensus wild-type sequence constructed for each gene. All potential mutations and genetic variants of unknown clinical significance are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above. For Single locus specific *MLH1*, *MSH2* and *MSH6* mutation Analysis (Predictive Testing), the duplicate PCR reactions are performed and the PCR products are analysed by direct DNA sequencing. Controls for normal and mutated sequences (where available) are co-analysed for QA purposes.

Genomic rearrangements are detected by Multiplex Ligation-dependent Probe Amplification (MLPA) Assay. MLPA reactions result in a very reproducible gel pattern with fragments ranging from 130 to 490 bp. MLPA probes are able to discriminate between sequences that differ in only one nucleotide. Comparison of this gel pattern to that obtained with a control sample indicates which sequences show an aberrant copy number. Heterozygote deletions of probe recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Alternatively, heterozygote duplication of probe recognition sequences should give a 35-50% enlarged relative peak area of the amplification product of that probe. The patient's DNA sample is tested in duplicate and compared with positive and negative controls for single/multiple exon deletions and duplications (where available). To avoid the misinterpretation of the MLPA results [due to close proximity of a mutation and/or polymorphisms to the probe ligation site] the apparent deletion's probe annealing site and nearby region (50bp upstream and 50bp downstream from the probe binding site) is always sequenced.

### **Limitations of Method**

There may be limited portions of either *MLH1*, *MSH2* or *MSH6* gene for which sequence determination can be performed only in the forward or reverse direction. Unequal allele amplification may result from rare polymorphisms under primer sites. This assay will not detect any types of errors in RNA transcript processing. There may be uncommon genetic abnormalities in *MLH1*, *MSH2* and *MSH6* genes that will not be detected by the methods used. This analysis, however, is believed to rule out the majority

of abnormalities in these genes, which are believed to be responsible for most hereditary susceptibility to hereditary colon cancers (HNPCC). The incidence of a false report of a genetic variant or mutation resulting from technical error is considered negligible because of independent confirmation of all genetic variants (see above). The incidence of a false report of a genetic variant or mutation resulting from errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%). Confirmation testing using a second blood sample for all mutations identified is recommended.

### **Interpretive Criteria**

#### **“Positive for a deleterious (harmful) mutation”**

A positive test result means that the laboratory found a change in the *MLH1*, *MSH2* or *MSH6* gene. Depending on the purpose of the test, this result may confirm a diagnosis, indicate that a person is a carrier of a particular genetic mutation, identify an increased risk of developing a disease in the future, or suggest a need for further testing. Because family members have some genetic material in common, a positive test result may also have implications for certain blood relatives of the person undergoing testing. It is important to note that a positive result of a predictive or presymptomatic genetic test usually cannot establish the exact risk of developing a disorder. A positive test result does not mean the tested individual will acquire the disease, it only shows the greater risk of developing the disorder. Positive test result indicates the presence of the mutations (nonsense, insertions, deletions) that prematurely terminate (“truncate”) the protein product of *MLH1*, *MSH2* or *MSH6* gene. In addition, specific missense mutations and non-coding intervening sequence (IVS) mutations are recognized as deleterious on the basis of data derived from linkage analysis of high risk families, functional assays, biochemical evidence and/or demonstration of abnormal mRNA transcript processing.

#### **“Genetic variant of uncertain significance”:**

A genetic variation (or variant) of unknown significance may or may not be associated with disease. A possible disease variant is suspected to be associated with disease, but this association remains quite uncertain. More information is needed to clarify the significance of such genetic variations. Over time, this information may become available in the literature. Genetic testing of affected individuals within the

patient's extended family (known as concordance testing) may also yield this information. If all affected family members harbor the genetic variation in question, it is likely to be associated with the inherited disorder. If some affected family members do not harbor the genetic variation in question, it is less likely to be associated with the inherited disorder. Clarifying the significance of a genetic variation through concordance testing in a family not only benefits this particular family, but also other families who harbor the same genetic variation. Genetic variants of uncertain significance include missense mutations and mutations that occur in analysed intronic regions whose clinical significance has not yet been determined, as well as chain-terminating mutations that truncate *MLH1*, *MSH2* and *MSH6* distal to amino acid positions 756, 934 and 1360, respectively. A genetic variant of uncertain significance is considered to be less likely to be deleterious if it has been observed in one or more individuals with a known deleterious mutation in the same gene. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

**“No deleterious mutation detected”**

A negative test result means that the laboratory did not find a dangerous copy of the *MLH1*, *MSH2* or *MSH6* gene. This result can indicate that a person is not affected by a particular disorder, is not a carrier of a specific genetic mutation, or does not have an increased risk of developing a disease. It is possible, however, that the test missed a disease-causing genetic alteration because many tests cannot detect all genetic changes that can cause a particular disorder. Further testing may be required to confirm a negative result. Negative test result includes that the non-truncating genetic variants observed at an allele frequency of approximately 1% of a suitable control population (providing that no data suggest clinical significance), as well as all genetic variants for which published data demonstrate absence of substantial clinical

significance. Also includes mutations in the protein-coding region that neither alter the amino acid sequence nor are predicted to significantly affect exon splicing, and base pair alterations in non-coding portions of the gene that have been demonstrated to have no deleterious effect on the length or stability of the mRNA transcript. In some cases, a negative result might not give any useful information. This type of result is called uninformative, indeterminate, inconclusive, or ambiguous. Uninformative test results sometimes occur because everyone has common, natural variations in their DNA, called polymorphisms that do not affect health. If a genetic test finds a change in DNA that has not been associated with a disorder in other people, it can be difficult to tell whether it is a natural polymorphism or a disease-causing mutation. Data on polymorphic variants are available upon request. An uninformative result cannot confirm or rule out a specific diagnosis, and it cannot indicate whether a person has an increased risk of developing a disorder. In some cases, testing other affected and unaffected family members can help clarify this type of result.

**“Specific variant/mutation not identified”**

Specific and designated deleterious mutations or variants of uncertain clinical significance are not present in the individual being tested. If one (or rarely two) specific deleterious mutations have been identified in a family member, a negative analysis for the specific mutation(s) indicates that the tested individual is at the general population risk of developing breast or ovarian cancer.

**Change of Interpretation and Issuance of Amended Reports**

If and whenever there is a change in the clinical interpretation of a specific reported variant, an amended test report will automatically be provided by Genetic Technologies.

**Description of Nomenclature:**

In the Human Genome Variation Society (HGVS) standard nomenclature (<http://www.hgvs.org>) the nucleotide numbering is in relation to the translation initiation codon, starting with number 1 at the A of the ATG translation initiation codon. The GenBank Accession numbers for *MLH1*, *MSH2* and *MSH6* genes are listed below:

	Genomic	Transcript	Protein
<i>MLH1</i>	AC011816.17	NM_000249.2	NP_000240.1
<i>MSH2</i>	AC079775.6	NM_000251.1	NP_000242.1
<i>MSH6</i>	AC006509.15	NM_000179.1	NP_000170.1

**Quality Assurance:**

All tests are performed according to ISO15189/ISO17025 standards in NATA and RCPA accredited laboratory. Accreditation No: 15370.

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