

# MYH Technical Specifications

Myriad Genetic Laboratories, Inc. Updated: 13 December 2004

## TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS:

### Description of Analysis

**MYH Mutation Panel:** DNA sequence analysis of specific portions of *MYH* exons 7 and 13 designed to detect the mutations Y165C and G382D. Specimens testing positive for only one mutation are sequenced as described below.

**MYH Sequencing:** Full sequence determination in both forward and reverse directions of approximately 1608 base pairs comprising 16 exons and approximately 450 adjacent non-coding intronic base pairs.

The non-coding intronic regions of *MYH* that are analyzed 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.

**Single Site Analysis:** DNA analysis, by direct sequence methods, for a specified mutation in *MYH*.

### Description of Method:

Blood samples are assigned a unique bar-code for robotic specimen tracking. DNA is extracted and purified from white cells isolated from each sample. Aliquots of patient DNA are each subjected to polymerase chain reaction (PCR) amplification reactions. The amplified products are each directly sequenced in forward and reverse directions using fluorescent dye-labeled sequencing primers. Chromatographic tracings of each amplicon are analyzed by visual inspection. Genetic variants are detected by comparison with a consensus wild-type sequence constructed for each gene. All potential genetic variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above.

### Performance Characteristics:

**Analytical sensitivity:** 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). No false-positive results were seen in a sample set consisting of ten DNA samples obtained from individuals that were analyzed for the two mutations in the *MYH* mutation panel using the sequencing method described above.

**Analytical sensitivity:** Failure to detect a genetic variant or mutation in the analyzed DNA regions may result from errors in specimen handling and tracking, amplification and sequencing reactions, or computer-assisted analysis and data review. The sequencing method described above accurately identified each of twenty-six samples that had been analyzed for the two mutations in the *MYH* mutation panel.

**Limitations of method:** There may be limited portions of *MYH* 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 large genomic rearrangements and some types of errors in RNA transcript processing.

### Description of Nomenclature:

All mutations and genetic variants are named according to the convention of Beaudet and Tsui. (Beaudet AL, Tsui LC. A suggested nomenclature for designating mutations. *Hum Mut* 1993; 2:245-248). Nucleotide numbering starts at the first translated base of *APC* and *MYH*.

### Interpretive Criteria:

**“Positive for two *MYH* mutations”:** Includes observations of two *MYH* mutations, or observations of two alleles of one mutation. The presence of two *MYH* mutations has been documented in recent literature to be associated with colorectal polyposis and cancer.

Causal mutations include nonsense and frameshift mutations, as well as specific missense mutations and non-coding intervening sequence (IVS) mutations recognized as deleterious on the basis of data derived from functional assays, biochemical evidence, demonstration of abnormal mRNA transcript processing and/or segregation analysis in families.

**“Positive for two *MYH* mutations, clinical significance uncertain”:** Includes observations of two *MYH* mutations but it cannot be determined from this analysis alone whether these two mutations are on opposite alleles. Testing one of this patient's parents or children will determine if these mutations are on opposite alleles, thereby indicating if this patient is at increased risk of colorectal polyposis and cancer. If these mutations are on the same allele, it is currently unknown if this patient is at some measure of increased risk for colorectal polyposis and cancer.

**“One *MYH* mutation detected, colorectal polyposis and cancer risk unknown”:** Includes observations of one allele of a causal mutation. It is currently unknown whether individuals who carry a single *MYH* mutation are at some measure of increased risk for colorectal polyposis and cancer. Patients with one *MYH* mutation detected through the *MYH* mutation panel will automatically receive full sequence analysis of the *MYH* gene.

**“Genetic variant of uncertain significance”:** Includes missense mutations and mutations that occur in analyzed intronic regions whose clinical significance has not yet been determined.

**“No mutation detected” (full sequence analysis):** Includes non-truncating genetic variants observed at an allele frequency of at least 2% 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. Data on polymorphic variants are available upon request.

There may be uncommon genetic abnormalities in *MYH* that will not be detected by full sequence analysis (see **Limitations of method**, above). This analysis, however, is believed to rule out the majority of abnormalities in this gene.

**“Specific variant/mutation not identified”:** Indicates that specific and designated mutations or variants are not present in the individual being tested. If two mutations have been identified in a family member, a negative analysis for the specific mutations indicates that the tested individual is at the general population risk of developing colorectal polyposis and/or cancer.

### Change of interpretation and issuance of amended reports:

Whenever there is a change in the overall clinical interpretation of a patient's test result, an amended report will automatically be provided by Myriad Genetic Laboratories.