

# Panexia™ Technical Specifications

Myriad Genetic Laboratories, Inc. Updated: August 2012

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

## Description of Analysis

### **Comprehensive Panexia™:**

**PALB2:** Full sequence determination in both forward and reverse directions of approximately 3950 base pairs comprising 13 coding exons and approximately 390 adjacent base pairs in the non-coding intervening sequences (introns). The wild-type *PALB2* gene encodes a protein comprised of 1186 amino acids.

**BRCA2:** Full sequence determination in both forward and reverse directions of approximately 10,200 base pairs comprising 26 coding exons and approximately 900 adjacent base pairs in the non-coding intervening sequence (intron). Exon 1, which is non-coding, is not analyzed. The wild-type *BRCA2* gene encodes a protein comprised of 3418 amino acids.

The non-coding intronic regions of *PALB2* and *BRCA2* 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.

**Large rearrangement analysis:** All coding exons of *PALB2* are examined for evidence of deletions and duplications using microarray comparative genomic hybridization analysis (microarray-CGH). Large rearrangements for *BRCA2* can be identified by BRACAnalysis® Rearrangement Testing, which is a separate test from Panexia™.

**Single Site Panexia™:** DNA sequencing analysis is performed for a targeted gene region containing the specified variant in *PALB2* or *BRCA2*. Microarray-CGH analysis is performed for all requests for single site mutation analysis of a large rearrangement in *PALB2*.

## Description of Method:

Patient samples are assigned a unique bar-code for robotic specimen tracking. DNA is extracted and purified from peripheral blood samples or buccal mouthwash samples (for *BRCA2*), submitted for molecular testing.

**Full sequence analysis:** Aliquots of patient DNA are each subjected to polymerase chain reaction (PCR) amplification (22 reactions for *PALB2*, 47 reactions for *BRCA2*). 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 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 genetic variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above.

**PALB2 Large Rearrangement Analysis:** Genomic DNA from patients is analyzed by microarray-CGH analysis to determine copy number abnormalities indicative of

deletion or duplication mutations across the *PALB2* gene. Approximately 400 probes have been designed to interrogate all coding exons, and limited flanking intron regions of the *PALB2* gene. Each probe is analyzed using proprietary software that compares the ratio of bound patient DNA to that of a reference DNA to indicate regions of altered copy number. The microarray design includes probes to detect deletions and duplications in multiple genes tested by MGL; however, a data masking feature is used to limit the analysis only to specific genes for which testing has been requested.

Patient samples positive for deletions or duplications are confirmed by repeat microarray analysis of the genes.

## Performance Characteristics:

**Analytical specificity:** 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%). For *PALB2* Large Rearrangement analysis, no false positive results were obtained through the large rearrangement testing process that uses microarray-CGH on a set of 310 individual DNA samples.

**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 rate of such errors is estimated from validation studies to be less than one percent (<1%). The analytical sensitivity of DNA sequencing performed in both directions is estimated to be >99%. The large rearrangement testing process, using microarray-CGH correctly identified a synthetic positive run in four replicates among 310 samples. The synthetic positive sample was created from genomic DNA that was digested with specific restriction enzymes.

**Overall test accuracy:** For a patient with at least a 10% probability of a positive test based on a personal or family history of cancer, the chance of an incorrect test result is less than 1%.

**Limitations of method:** There may be limited portions of *PALB2* or *BRCA2* 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. The large rearrangement analyses described above will detect deletion and duplication rearrangements involving the promoter and coding exons of *BRCA2* and solely the coding exons of *PALB2*. These assays will not detect some types of errors in RNA transcript processing, regulatory mutations, or balanced rearrangements (i.e. inversions). Insertions that do not result in duplications will generally not be detected by microarray-CGH.

## 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 *PALB2* and the first transcribed base of *BRCA2* according to GenBank entries AC008870 and U43746, respectively.

### **Interpretive Criteria:**

The classification and interpretation of all variants identified in the assay reflects the current state of scientific understanding at the time the report is issued. In some instances, the classification and interpretation of variants may change as scientific information becomes available.

**“Positive for a deleterious mutation”:** Includes all nonsense and frameshift mutations that occur at or before the last known deleterious amino acid positions of *PALB2* and *BRCA2*, respectively.

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, statistical analysis, biochemical evidence and/or demonstration of abnormal mRNA transcript processing.

Deletions and duplications of an entire exon(s) in *PALB2* identified by microarray-CGH may also be interpreted to be deleterious. Deleterious large genomic rearrangements include single exon and multi exonic deletions and duplications that are out of frame. In frame deletions/duplications are interpreted on an individual basis and the specific evidence supporting the classification of these mutations is included in the individual patient report.

**“Genetic variant, suspected deleterious”:** Includes genetic variants for which the available evidence indicates a likelihood, but not proof, that the mutation is deleterious. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

**“Genetic variant, favor polymorphism”:** Includes genetic variants for which available evidence indicates that the variant is highly unlikely to contribute substantially to cancer risk. Includes *PALB2* variants identified in trans with a deleterious mutation if the individual has not been diagnosed with Fanconi anemia complementation group N (FANC N), and *BRCA2* variants identified in trans with a deleterious mutation if the individual has not been diagnosed with Fanconi anemia complementation group D1 (FANC D1). Also includes missense mutations in *BRCA2* that occur at or distal to amino acid 3326. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

**“Genetic variant of uncertain significance”:** Includes missense variants and variants that occur in analyzed intronic regions whose clinical significance has not yet been determined, as well as nonsense and frameshift mutations that occur distal to the last known deleterious amino acid positions of *PALB2* and *BRCA2* respectively.

**“No deleterious mutation detected”:** Includes 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. Includes truncating mutations in *BRCA2* that occur at and distal to amino acid 3326. 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 *PALB2* and *BRCA2* that will not be detected by Panexia™ (see **Limitations of method**, above). This analysis, however, is believed to rule out the majority of abnormalities in these two genes.

**“Specific variant/mutation not identified”:** Indicates that specific and designated mutations or variants 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 likely at the general population risk of developing breast, ovarian or pancreatic cancer.

**Change of interpretation and issuance of amended reports:** Whenever there is a change in the interpretation of a patient’s test result, an amended report will be provided by Myriad Genetic Laboratories.