City of Hope

Dennis D. Weisenburger, MD
Chairman, Department of Pathology, CLIA #05D0665695

Clinical Molecular Diagnostic Laboratory
1500 East Duarte Road
Northwest Building, Second Floor, Room 2236
Duarte, CA 91010-3000
Phone 888-826-4362 Fax 626-301-8142
cmdl@coh.org http://cmdl.cityofhope.org

Assay Summary

TP53 Gene Mutation Analysis

Li-Fraumeni Syndrome

Synopsis

Germline (heritable) mutations in the TP53 gene[1] predispose individuals to various tumors (early onset sarcomas and breast cancer, brain tumors, adrenal cortical carcinomas, and leukemias) associated with Li-Fraumeni syndrome[2, 3]. Identification of TP53 gene mutations in cancer patients from Li-Fraumeni syndrome or Li-Fraumeni syndrome-like[4] families may permit identification of individuals at high risk for cancer in these families.

Indications for testing

Patients with personal and/or family history suggestive of Li-Fraumeni syndrome or Li-Fraumeni-associated cancers.

Methodology

TP53 sequencing: Coding exons and associated intron junctions are captured and enriched using custom Agilent SureSelect technology. Next-generation sequencing is performed on Illumina MiSeq. Additional Sanger sequencing is performed for any regions with insufficient depth of coverage or for verification of suspect variant calls. Targeted testing for known familial mutation is performed by Sanger sequencing.

TP53 MLPA analysis: We have incorporated the SALSA Multiplex Ligation-Dependent Probe Amplification (MLPA) kit that is a rapid, high-throughput technique for copy number quantification[7], specifically testing for large deletions/duplications for the TP53 gene in Li-Fraumeni syndrome. This assay should be considered for patients with Li-Fraumeni syndrome where full gene sequencing did not detect a mutation. The P056 kit contains probes for each of the 11 exons of the TP53 gene on 17p13.1, as well as several probes at close distances telomeric and centromeric of the TP53 gene. Two probes detect sequences in exon 1 of TP53**..**

Limitations

The sequencing analysis will not detect mutations located in regions of the TP53 gene that are not analyzed (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions). The method also will not detect gross genetic alterations including most duplications, inversions, or deletion. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance.

This MLPA kit is designed to detect deletions / duplications of one or more exons of the TP53 genes. Heterozygous deletions/duplications of probe recognition sequences should give a 35-50% reduced/increased relative peak area of the amplification product of that probe. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area.

Therefore, apparent deletions detected by a single probe always require confirmation by other methods. MLPA analysis will not detect sequence alterations or inversions.

Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes.
Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

Note:

i) for infants, a minimum of 3 ccs is sufficient.
ii) we accept DNA; at least 10 micrograms is required.

Test Request Form (TRF)

(a) A completed CMDL TRF is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
(b) Cancer Patient Information Form: Include a completed Cancer Patient Information Form for the proband and a complete pedigree.

Order Codes	***CPT Codes***	***TAT***
TP53-SEQ (TP53 gene, full gene sequencing by NGS)	81405, G0452	3 wks
TP53-CAS (TP53 gene, targeted mutation analysis, known mutation)	81403, G0452	2 wks
TP53-DEL (TP53 gene, MLPA analysis)	81479, G0452	3 wks
TP53-DEL-CAS (TP53 gene, MLPA analysis, known deletions/duplications)	81479, G0452	3 wks

References

1. Malkin, D. et al. (1990). Science 250:1233-1238.
2. Li, F. P. et al. (1988). Cancer Res. 48:5358-5362.
3. Garber, J. E. et al. (1991). Cancer Res. 51:6094-6097.
4. Birch, J. M. et al. (1994). Cancer Res. 54:1298-1304.
5. Varley, J.M. et al. (1997) Br. J. Cancer 76 :1-14
6. Bougeard, G. et al. (2008) J. Med. Genet 45(8):535-8
7. Schouten JP et al. (2002) *Nucleic Acids Res* **30**, e57

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc.