

## Assay Summary

### TP53 Gene Mutation Analysis

#### Li-Fraumeni Syndrome

##### *Synopsis*

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

##### *Indications for testing*

Cancer patients with family history indicative of Li-Fraumeni syndrome, with genetic counseling, may consider TP53 gene sequence analysis.

##### *Methodology*

**TP53 sequencing:** All coding exons (2-11) and associated intron junctions of the TP53 gene are analyzed by direct DNA sequence analysis using an automated fluorescent sequencing machine. When a mutation is detected, confirmation is carried out on an independent amplification of PCR using a second prep (B-prep) by sequencing in the opposite direction. If no mutation is found, sequence analysis is performed in both directions. At-risk family members can be offered DNA sequence analysis of only the region of the gene with the previously identified mutation.

**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<sup>7</sup>, 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. The precision and accuracy of the method as a whole has been previously established at >95%, and >99%, respectively.

##### *Performance*

Alterations in the TP53 gene are detected in approximately 70% of Li-Fraumeni patients<sup>5</sup>. The sensitivity of DNA sequence analysis for detection of heterozygous point mutations is estimated to be greater than 99%. The specificity of the analysis is estimated to be greater than 98%. Large deletions are estimated to account for approximately 5% of the alterations observed in patients with LFS<sup>6</sup>. The assay sensitivity of deletion detection is >99%.

### **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**

(a) 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.

(b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W. A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

### **Test Request Form (TRF)**

- (a) A completed MDL [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.

<b>Order Codes</b>	<b>CPT Codes</b>	<b>TAT</b>
TP53-SEQ (TP53 gene, full gene sequencing)	83890, 83898(x5), 83904(x5), 83894, 83912	4 wks
TP53-CAS (TP53 gene, targeted mutation analysis, known mutation)	83890, 83898, 83904, 83894, 83912	3 wks
TP53-PD (TP53 gene, known mutation detection, prenatal)	83890, 83898, 83894, 83904, 83912	2 wks
TP53-DEL (TP53 gene, MLPA analysis)	83890, 83896(x12), 83909, 83912	3 wks
TP53-DEL-CAS (TP53 gene, MLPA analysis, known deletions/duplications)	83890, 83896(x12), 83894, 83912	3 wks
TP53-DEL-PD (TP53 gene, MLPA analysis, prenatal)	83890, 83896(x12), 83894, 83912	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.