

Sarcoma Fusion Next-Generation Sequencing Panel

Background

Benign and malignant mesenchymal tumors (sarcomas and their mimics) are difficult to diagnose with many benign and malignant entities that differ in their clinical behavior and response to therapy. Many of these tumors harbor gene fusions that are crucial to establishing a definitive diagnosis. The Sarcoma Fusion Next-Generation Sequencing (NGS) Panel is a custom designed, 34-gene panel, high complexity laboratory developed test (LDT) designed for targeted sequencing of benign and malignant soft tissue neoplasms. This assay identifies fusion transcripts in targeted regions of RNA from total nucleic acid (TNA) isolated from formalin-fixed, paraffin-embedded (FFPE) tissue specimens.

The test will identify the vast majority of known fusions in benign and malignant mesenchymal tumors, but also has the ability to identify a limitless number of as-of-yet undiscovered gene fusions. This technology only “primes” from one partner of the gene fusion, allowing for discovery of new gene fusion partners.

Highlights of Sarcoma Fusion NGS Panel

- Comprehensive detection of gene fusions across 34 targeted genes aids in determining diagnosis, prognosis, and therapeutic options.
- FFPE tissue removes need to send fresh or frozen specimens.

The targeted genes included in the panel are:

<i>ALK</i>	<i>GLI1</i>	<i>PLAG1</i>
<i>BCOR</i>	<i>HMGA2</i>	<i>ROS1</i>
<i>CAMTA1</i>	<i>JAZF1</i>	<i>SS18</i>
<i>CCNB3</i>	<i>MEAF6</i>	<i>STAT6</i>
<i>CIC</i>	<i>MKL2</i>	<i>TAF15</i>
<i>CSF1</i>	<i>NCOA2</i>	<i>TCF12</i>
<i>EPC1</i>	<i>NTRK1</i>	<i>TFE3</i>
<i>EWSR1</i>	<i>NTRK2</i>	<i>TFG</i>
<i>FOS</i>	<i>NTRK3</i>	<i>USP6</i>
<i>FOSB</i>	<i>NUTM1</i>	<i>YWHAE</i>
<i>FOXO1</i>	<i>PAX3</i>	
<i>FUS</i>	<i>PDGFB</i>	

Clinical Indications

This test is intended for the diagnosis of benign or malignant mesenchymal tumors (sarcomas and their benign mimics).

Interpretation

The results of this test are to be interpreted in the context of the histological, immunohistochemical, and clinical features of the neoplasm.

Methodology

This test relies on Anchored Multiplex PCR (AMP™) technology to generate scalable, target-enriched libraries for NGS from formalin-fixed, paraffin embedded tissue sections. In AMP, unidirectional gene-specific primers (GSPs) are used to enrich libraries for known and unknown mutations. Adapters that contain both molecular barcodes and sample indices enable quantitative multiplex data analysis, read de-duplication, and accurate variant calling. Libraries are sequenced on the Illumina MiSeq instrument, which employs “sequencing by synthesis;” a fluorescence, image-based, reversible-terminator technology to sequence targeted regions of the 34 genes included in the panel.

Sequencing data are analyzed for fusion variant detection using Archer® Analysis bioinformatics tools. Specimen quality control is monitored and recorded by in-house developed software (scripts). Raw sequencing data are de-multiplexed based on unique index sequence using the Illumina bcl2fastq program. The fastq.gz files are de-duplicated according to the unique molecular barcode present and aligned to the human reference genome hg19. Part of the fusion calling and annotation is performed utilizing the Archer® Quiver™ Fusion Database.

Limitations of the Assay

This test does not detect missense mutations, insertions, deletions, or copy number changes, and does not distinguish between variants that are inherited versus acquired.

References

1. Archer Dx, FusionPlex Anchored MultiPlex PCR (AMP) technology <http://archerdx.com/fusionplex/> [Accessed: July 2018]
2. MiSeq System user Guide, Publication Number 15027617 Rev.0. *Illumina*, San Diego, CA. 9/2014.
3. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®), *Soft Tissue Sarcoma*, version 1.2019
4. Taylor BS, Barretina J, Maki RG, Antonescu CR, Singer S, Ladanyi M. Advances in sarcoma genomics and new therapeutic targets. *Nat. Rev. Cancer*. Jul 14 11(8), 541-57 (2011).

Test Overview

Test Name	Sarcoma Fusion NGS Panel
Ordering Mnemonic	SRCNGS
Methodology	Next-Generation Sequencing
Specimen Requirements	Formalin-fixed paraffin-embedded slides. Transport and store slides at ambient temperature. 10 unstained sections FFPE on charged, unbaked slides plus one H&E stained slide with best tumor area circled by a pathologist.
Stability	Ambient: Formalin-fixed paraffin-embedded slides. Transport and store slides at ambient temperature. Frozen: Unacceptable Refrigerated: Unacceptable
Days Performed	1 day per week
Days Reported	14 days
CPT Codes	81445

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