Techniques for Measuring Cancer Burden in Liquid Biopsy Samples

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Abstract

Liquid biopsy is a non-invasive sample source that can be utilized to assess cancer burden by measuring the tumor-derived fraction of circulating, cell-free DNA (cfDNA) from plasma. We evaluated two assays to monitor cancer burden using cfDNA: whole genome bisulfite sequencing (WGBS) and targeted amplicon sequencing for 56 oncology-related genes. We tested samples with both assays to characterize their efficacy across a broad spectrum of cancer types, stages, and treatment regimens. cfDNA was extracted from tumor-bearing patients and normal controls. To monitor methylation density, WGBS was performed using 5 ng of bisulfite-converted cfDNA with the Accel-NGS® Methyl-Seq DNA Library Kit. To detect tumor-specific mutations, 10 ng of cfDNA was used for the Accel-AmpliSeq® 56G Oncology Panel. Six out of eight cancer samples demonstrated significant hypomethylation in cfDNA, ranging from 2-40% when compared to healthy controls. The 56 gene amplicon panel identified point mutations in the cfDNA of only three samples, which also had the highest observed hypomethylation (18-40%). For all but two cancer samples, corresponding mutations were also found in the primary tumor at allele frequencies significantly higher than in the cfDNA fraction (e.g., 22% in tumor vs. 5% in cfDNA). The three cancer samples that had primary tumor mutations that were not detected in cfDNA also had the lowest observed hypomethylation. Therefore, a correlation between hypomethylation and detection of tumor mutations in the cfDNA fraction may exist. Further studies will elucidate which assay is more sensitive at detecting tumor burden in cfDNA.

Experimental Design

Tumor bearing blood, n = 8 (Streck Cell-Free DNA BCT®)
Healthy control blood, n = 5 (Streck Cell-Free DNA BCT)
Isolate cfDNA with QiAmp® Circulating Nucleic Acid kit

Assay Validation

- Validation panels:
  - 1 ng Arabidopsis DNA Input
  - 200 bp synthetic DNA
  - 2 ng of human DNA
  - 4 ng of human DNA in 40 ng of human cfDNA

- Detects point mutations accurately across a range of 100% to 0% sequencing coverage.

- HoLoFreq)).

Two Liquid Biopsy Assays: Genome-Wide Hypomethylation and Mutation Detection

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<th>Assay</th>
<th>cfDNA Methylome (G)</th>
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<td>Accel-NGS Methyl-Seq</td>
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<td>Accel-AmpliSeq 56G Oncology Panel</td>
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<td>Bisulfite conversion and Accel-NGS Methyl-Seq library construction</td>
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<td>Calculate hypomethylation status of cancer samples compared to healthy controls from 10 ng Illumina® MiSeq® reads using Methyplex.</td>
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<td>Accel-NGS Methyl-Seq Kit</td>
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| - 2-hour library workflow
- Post-bisulfite prep uses efficient, adaptive cfDNA library technology
- Supports input range of 100 pg to 100 ng with minimal PCR step
- Retains high sequence complexity for comprehensive and uniform methylation coverage
| Accel-NGS 2X Hyb Kit | | | | |
| - Simple with-bead protocol
- Broad input range 10 pg - 1 µg
- Repairs both 5' and 3' termini to enhance ligation efficiency
- Compatible with cfDNA and FFPE samples
- Increased library complexity
- Balanced coverage at AT/GC-rich regions

Conclusions

- Accel-NGS Methyl-Seq DNA Library Kit:
  - Provides uniform, comprehensive methylation coverage.
  - Enables liquid biopsy for genome-wide hypomethylation from 5 ng cfDNA.

- Accel-AmpliSeq 56G targeted sequencing panel:
  - Provides uniform, comprehensive genome coverage.
  - Enables a limit of mutation detection of 1% for liquid biopsy from 10 ng cfDNA.

Using both kits for liquid biopsy, a correlation was observed between percent hypomethylation and mutation detection for the tumor bearing cfDNA sample set presented.

The Accel NGS 25 DNA Library Kit:
- Provides unique, comprehensive genome coverage.
- Enables PCR-free cfDNA sequencing from 10-15 ng input.

Disclosure: All authors are employees of Swift Biosciences, Inc.