Technical Note

Liquid Biopsy Solid Tumor Genetics

Oseq™ ctDNA Panel
Introduction

Human cancers typically carry several types of genomic variations, such as Single Nucleotide Variants (SNV), Copy Number Variations (CNV), Insertions and Deletions (InDels), Structural Variants (SV), and Gene Fusions. These genetic alterations infer important insights for the discovery and development of biomarkers and for use in basic cancer research, patient stratification and clinical research. Circulating tumor DNA (ctDNA), which carries tumor-specific sequence alterations, represents a variable and generally small fraction of the total circulating DNA. Studies have shown that ctDNA provides informative, inherently specific and highly sensitive biomarkers for many cancer types.

Challenges for Solid Tumor Assessment

Liquid Biopsies have become popular because of the ease of obtaining sample material with a simple blood draw instead of needle or surgical biopsy procedures, lowering risk, reducing cost and causing minimal patient discomfort. The challenges presented by Liquid biopsy samples are that only a small amount (20-100ng) of ctDNA can be isolated from 10ml of whole blood and that ctDNA is highly fragmented with an average fragment sizes in the range of 150-300bp. Traditional Digital PCR, ARMS PCR, and FISH technologies have commonly been used to detect targetable oncogenes and hotspot mutations but are limited in that most cancers have no known hotspot mutations. In addition, they are not able to detect all types of known and novel mutation in a single test.
Oseq™ ctDNA Solid Tumor Panel

BGI has developed this comprehensive NGS-based panel based on many years of cancer research experience and proprietary database development. ctDNA panels allow the use of Liquid Biopsy samples and can detect all genetic variations in a sample without bias.

The Oseq™ ctDNA panel currently consists of 636 genes that are strongly supported as clinically actionable cancer genes, exhibit high frequency alterations in the COSMIC database, or play key roles in core cancer pathways. The capture-based panel is designed to detect all types of genomic tumor alterations, including base substitutions, insertions and deletions, copy number alterations, and selected gene fusions. It is an approved clinical test in China but is for research use only elsewhere.

The Oseq™ ctDNA panel is designed for use in solid tumors, including but not limited to lung cancer, colorectal cancer, breast cancer, ovarian cancer, melanoma, and lymphoma.
Performance

Typical Sequencing depth for ctDNA analysis is > 1,000X with a blood control sequencing depth of minimal 200X after the removal of duplications. High accuracy and precision of 99% each is achieved at mutant allele frequency of 1%, to assure accurate assessment of variants in liquid biopsy samples.

<table>
<thead>
<tr>
<th>Performance</th>
<th>BGI Oseq™ ctDNA Panel</th>
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<tbody>
<tr>
<td>Gene number</td>
<td>636</td>
</tr>
<tr>
<td>Sequencing depth</td>
<td>&gt; 1,000X</td>
</tr>
<tr>
<td>Variant type</td>
<td>SNV, InDel, CNV, SV</td>
</tr>
<tr>
<td>SNP Sensitivity At Mutant Allele Frequency ≥ 3%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>At Mutant Allele Frequency 1-3%</td>
</tr>
<tr>
<td>InDel Sensitivity</td>
<td>At Mutant Allele Frequency ≥ 3%</td>
</tr>
<tr>
<td></td>
<td>At Mutant Allele Frequency 1-3%</td>
</tr>
<tr>
<td>CNV Sensitivity</td>
<td>All types</td>
</tr>
<tr>
<td>SV Sensitivity</td>
<td>All types</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
</tr>
<tr>
<td>Reproducibility Inter-batch</td>
<td>97.48%(1%-3%), 100%(&gt;3%)</td>
</tr>
<tr>
<td></td>
<td>Intra-batch</td>
</tr>
<tr>
<td>Immunotherapy Biomarker</td>
<td>TMB, MSI</td>
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Case Study

A case example, demonstrating a typical application of the Oseq™ ctDNA panel has been published as a Nature Scientific Report. The results from the report are summarized below and the full report is available here [https://www.nature.com/articles/s41598-017-14962-0]

Discovery of targetable genetic alterations in advanced non-small cell lung cancer using a next generation sequencing-based circulating tumor DNA assay
Summary

Material: 119 Patients with advanced EGFR-TKI-naive NSCLC and 15 EGFR-TKI-resistant patients
Tool: BGI Oseq™ panel-ctDNA
Results:
1) Somatic ctDNA mutations were detected in 82.8% of patients
2) 27.7% were suitable for treatment
3) 19.3% of the patients, could be targeted by agents that are in clinical trials, such as mTOR inhibitors, PARP inhibitors, and CDK4/6 inhibitors
4) Able to monitor EGFR-TKI resistance and to discover mechanisms of drug resistance

Sample Requirement

We can process your liquid biopsy blood samples with the general requirements listed below. Tumor ctDNA and PBMC gDNA will be isolated from a single tube of whole blood. PMBC gDNA will serve as the normal control for the tumor ctDNA when identifying the mutations.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>10ml, using Streck cfDNA Tube</td>
</tr>
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</table>

Oseq™ panels for gDNA, Fresh Frozen or FFPE tissue are available from BGI as well. Ask your BGI representative or visit [www.bgi.com](http://www.bgi.com) for details.

About BGI Genomics

BGI Genomics is the division of BGI Group that provides a full menu of next generation sequencing and clinical testing services to support academic research, drug development and diagnostics. The company operates service laboratories under global quality standards, including CAP and CLIA in the US, Denmark, Hong Kong and mainland China. BGI Genomics leverages its unequalled genomic research experience and massive sequencing capacity to provide customers with high-quality data, fast turnaround and affordable prices. More information is available at [www.bgi.com](http://www.bgi.com)
Request for more information

For more information, or to discuss how we can meet your specific project requirements, please visit [www.bgi.com](http://www.bgi.com), write to [info@bgi-international.com](mailto:info@bgi-international.com) or contact your local BGI representative.

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BGISEQ-500 Services are executed at our service laboratory in China.