Retains high DNA damage (Fixation Using Single Utilizes bisulfite Conversions). Upon establishment of this technique, we observed cfDNA from healthy subjects as well as from subjects with a spectrum of cancers. Following DNA isolation and bisulfite treatment of these samples, we sequenced each sample on the Illumina HiSeq to a depth of 10 million reads. A minimum threshold of 1.1 was used to determine significant hypo-methylation.

Upon analysis, preliminary results of the methyltransfer status of cfDNA from 8 of the 10 cancer subjects ranged from 2% to 3% compared to the corresponding normal tissue. The cfDNA from 2 patients with prostate cancer, which was reactive to hypomethylated, originated from the plasma of a subject with a high-grade, hormone-resistant adenocarcinoma in the pelvic area. The most hypomethylated cfDNA was from a patient with metastatic adenocarcinoma of the lung. The least hypomethylated cfDNA was from a patient with metastatic melanoma.

This method provides the basis for a technique to readily prepare and characterize cfDNA as a NSI deep sequencing, cfDNA obtained from each normal volunteer and 3 healthy controls only on 1 day each. This approach is not only rapid but also reduces the costs of obtaining the utility of this assay may vary with cancer types. To further define/establish significant thresholds for methylation status, we will perform a large-scale study examining the methyltransfer status of individuals before, during, and after treatment, thereby generating a methylation gradient as a function of time and treatment.

Introducing Accel-NGS Methyl-Seq

- 2-hour workflow
- Utilizes bisulfite-converted DNA as input material
- Supports input range of 0.1 - 100 ng with minimal PCR cycles
- Retains high sequence complexity

Small sample limitations

- Ranilpar: 1 ng of cfDNA
- 357 copies of the whole human genome
- 24 enzymatic copies of any locus
- Achieving 1% detection of a bait, mutation → 3 chromosome copy

Comparison of workflows for bisulfite-converted DNA libraries

<table>
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<th>Library</th>
<th>R1 Reads</th>
<th>R2 Reads</th>
<th>R3 Reads</th>
<th>Total Coverage</th>
<th>Averaged Coverage</th>
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<td>4.0</td>
<td>4.0</td>
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<td>8.0</td>
<td>8.0</td>
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<td>100 ng</td>
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<td>40.0</td>
<td>30 million</td>
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Figure 2. Comparison of DNA methylation sequencing techniques (A) Workflow for Accel-NGS, a bisulfite-conversion, Single Primer-based, library preparation. In this technique, a bisulfite-conversion of DNA is the first step. The bisulfite-conversion of DNA is the last step. The bisulfite-conversion of DNA and sequencing are performed in the same tube. A third technique, Random Primer, demonstrates coverage of both the CpG and non-CpG region of the genome. The random primer approach is a good balance of coverage at 1 ng and 10 ng. During the cancer analysis of multiple height cases, we have seen that the bisulfite-conversion of DNA is not input from the random primer approach.

Genome-wide hypomethylation detection from 10 million sequencing reads

- Healthy (green) - High Cancer Burden (red)

Figure 3. cDNA library from plasma. cfDNA was isolated from both cancerous and healthy subjects. An aliquot of 100 ng of cfDNA was isolated from each group. The aliquot was used in the study, and the average amount of cfDNA used in each sample was 100 ng. All samples were diluted from individuals undergoing colorectal cancer screening, liver cancer, breast cancer, and stomach cancer.

Figure 4. cDNA Library Workflow. cfDNA was isolated from both cancerous and healthy subjects. An aliquot of 100 ng of cfDNA was isolated from each group. The aliquot was used in the study, and the average amount of cfDNA used in each sample was 100 ng. All samples were diluted from individuals undergoing colorectal cancer screening, liver cancer, breast cancer, and stomach cancer.

Figure 5. Methylation patterns across geic regions. Although tumor suppressor genes, such as TP53, can be assayed by bisulfite sequencing, the genome-wide hypomethylation status of the CpG island region in the TP53 gene is highly dependent on the CpG island status. The CpG islands are generally hypermethylated in cancer when 1 or more regions are hypomethylated. This method refers to the same sample. The method refers to the same sample.