Comparison of enzymatic and bisulfite conversion to map the plasma cell-free methylome in cancer

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Highlights

- Aberrant methylation is pervasive in cancer and a promising source of diagnostic and predictive biomarkers in cell-free DNA (cfDNA).
- We tested a new Enzymatic Methyl-seq (EM-seq) method and compared to the standard bisulfite treatment of cfDNA from 22 patients (7 healthy controls, 15 patients with predominantly late stage cancer).
- Libraries made with EM-seq showed higher levels of cytosine conversion, increased alignment quality, less DNA fragmentation and higher overall genome coverage.
- Methylation levels of CpG islands and CpG shores are highly correlated regardless of conversion method or cancer status.

Experimental Design

A. 22 patients with late stage cancer.

B. Age (years) 

<table>
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<tr>
<th>Mean</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>65</td>
<td>43</td>
<td>87</td>
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C. Cancer Site

<table>
<thead>
<tr>
<th>1. Lung</th>
<th>2. Breast</th>
<th>3. GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
<td>3</td>
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D. Sex

<table>
<thead>
<tr>
<th>1. Male</th>
<th>2. Female</th>
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<tr>
<td>3</td>
<td>12</td>
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Copy number assessment

Figure 2. Paired bisulfite (yellow) and EM-seq (black) whole genome sequencing data was processed through alignment and custom analysis pipeline. A) The conversion rate was calculated as the fraction of cytosines in non-CpG contexts that were detected as not methylated. We looked at the fraction of high quality sequence bases. B) The fraction of aligned bases with a quality greater than 30 is significantly higher in EM-seq libraries. C) Average genome coverage is consistently higher in EM-seq libraries, even when read depth is considered. D) Bisulfite uniformly yields shorter average fragments than paired EM-seq treated libraries regardless of cancer status.

Data quality

EM-seq performed better in key technical metrics, yielding higher quality whole genome methylation data.

Copy number assessment

Figure 4. Copy number aberrations (CNAs) are detectable in methyl sequencing libraries. A) An example copy number profile from a cancer patient are shown for bisulfite (left) and EM-seq (right). B) A strong agreement in normalized read depth is observed for bisulfite (x-axis) and EM-seq (y-axis) (p=6.3e-10) along the identity line. Considering all affected patients, tumor fraction ratios between EM-seq and paired bisulfite libraries were largely comparable. C) The histogram showing the CNA tumor fraction ratios shows four patients with a reduced tumor fraction (right) conditions. B) A strong agreement in normalized read depth is observed for bisulfite (x-axis) and EM-seq (y-axis) (p=6.3e-10) along the identity line. Considering all affected patients, tumor fraction ratios between EM-seq and paired bisulfite libraries were largely comparable. D) The MAD is higher in WGBS (orange) libraries, across the read depth range.

Methylation comparison

Methylation levels in healthy and late stage cancer patients are highly concordant. Both methodologies discriminate affected from healthy participants by cancer associated methylation changes. Methylation levels in healthy and late stage cancer patients are highly concordant. Both methodologies discriminate affected from healthy participants by cancer associated methylation changes. Methylation levels in healthy and late stage cancer patients are highly concordant. Both methodologies discriminate affected from healthy participants by cancer associated methylation changes.

Conclusions

- Enzymatic methylation treatment resolves methylation levels in cfDNA without signs of bisulfite-associated DNA damage.
- Better quality reads, and higher overall coverage are technical advantages of the enzymatic methylation approach.
- Unlike whole genome sequencing, control EM-seq libraries had less variability in genome-wide coverage distributions and preserved the canonical cfDNA fragment length.
- WGBS and EM-seq detect cancer associated methylation changes with indistinguishable performance.

Acknowledgments

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