Detection of Ultra-rare Mutations in vivo Establish Biomarkers of Endogenous and Environmental Exposure

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DNA Sequencing of Ultra-Rare Mutations

Endogenous and environmental processes alter the genetic record through damage and mutation. Next-generation sequencing (NGS) technologies have been revolutionary in describing the genetic differences between clonal populations but are too error-prone to detect ultra-rare mutations.

We introduce the TwinStrand Duplex Sequencing™ assay that is sensitive enough to directly measure the faint signal of a mutagen within days of animal or cellular exposure using only bulk-extracted genomic DNA.

Sequencing Errors Obscure Truth

Next-Generation Sequencing (NGS)

Single Strand

Error-Corrected NGS

Duplex Sequencing

TwinStrand Duplex Sequencing™ Technology

A DuplexSeq™ Adapter has:

1. Identical (or relatable) degenerate tags in each strand.

Measuring Genotoxicity

Common Sources of Error

- Sequencer Artifact
- PCR Misincorporation
- DNA Damage
- 8-oxoguanine
- Deaminated cytosine
- Ambient sites
- Many others...

The error rate of NGS is ~0.1% which creates a background that obscures rare mutations. Duplex Sequencing overcomes these errors by forming consensus among PCR duplicates from the same source molecule.

Accuracy is Required

Experimental Design

<table>
<thead>
<tr>
<th>Tg-RasH2 Mouse</th>
<th>BigBlue® Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissues</td>
<td></td>
</tr>
<tr>
<td>Lung (10)</td>
<td>Liver (15)</td>
</tr>
<tr>
<td>Splenic (10)</td>
<td>Marrow (17)</td>
</tr>
<tr>
<td>Blood (10)</td>
<td></td>
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<tr>
<td>Urethane (10)</td>
<td></td>
</tr>
<tr>
<td>VC (15)</td>
<td>BJ(BP) (10)</td>
</tr>
<tr>
<td>ENU (11)</td>
<td>VC (11)</td>
</tr>
</tbody>
</table>

Genomic Targets

- Pol1c, Rho, Clbn1
- Hsp, Nia, Kias

Duplex BP

4,923,565,684
4,716,990,836

Mutation Frequencies

- Mutation induction varies considerably between mutagen and tissue types.
- The fold-change mutation frequency in the assay reflects those found by using the BigBlue Transgenic Rodent (TGR) assay.

Base Substitution Spectra

The Trinucleotide Spectra of base substitutions reveals a unique fingerprint for each treatment group. These fingerprints are consistent with known signatures of clonal mutation obtained in tumors caused by cigarette exposure. In uninfected animal liver tissues it is standard for CG→AT and CG→GC which is likely caused by oxidation and CG→TA caused by deamination of cytosine and 5-methylcytosine. Urethane shows the most consistent pattern of mutation frequency and spectrum across different tissues and times.

Conclusions

- Duplex Sequencing™ enables anyone to detect genotoxin-induced variants below a frequency of one-in-a-million bases.
- We show a robust ability to detect, and precisely quantify, the effect of mutagen exposure on the genomic DNA of five tissue types from two mouse models against three mutagen treatments.
- Duplex Sequencing is a sensitive and data-rich assay for detecting mutagenesis of any genetic locus, in any tissue, in any organism.
- Duplex Sequencing-produced trinucleotide base substitution spectra enables the discovery of links between mutagenic exposure and human genetic disease. These spectra can also be used to infer the etiology of a mutagenic compound.

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