DNA Sequencing of Ultra-Rare Variants

Cancer is a disease of somatic evolution characterized by the natural selection of genomic mutations that facilitate enhanced cell survival and proliferation. Historically, our ability to identify early genetic patterns of clonal selection in both human and model organisms has been hampered by inadequately sensitive methods for identifying variants during the long period between their occurrence and the final outgrowth of a clinically apparent tumor.

We present the use of Duplex Sequencing of both human and mouse tissues to demonstrate the detection of subclones at allelic fractions below $1 \times 10^{-4}$ showing occurrence and the final outgrowth of a clinically apparent tumor.

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.

DNA Binding Domain Tetramerisation Motif Transactivation Motif

Aminio acid position

Common Sources of DNA Sequencing Error

- Sequencer Miscall
- PCR Misincorporation
- DNA Damage
- 8-oxoguanine
- Daminated cytosine
- Abasic Sites
- Many others...

The error rate of NGS is ≈0.1%, which creates a background that obscures rare variants. Duplex Sequencing overcomes these errors by forming consensus among PCR duplicates from the same source molecule and increases the overall accuracy of sequence data by more than 10,000x.

Duplex Sequencing™ Detects Rare Subclonal Variants that Mark Early Carcinogenesis and Preneoplastic Clonal Evolution


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Duplex Sequencing™ Technology

Duplex Sequencing™ is the only genotoxicity assay that delivers common sources of DNA Sequencing Error in normal mouse tissues mere weeks after mutagen exposure.

TP53 Mutations Correlate with Age and Those Seen in Human Cancers

Uterine Lavage Biopsy Experimental Design

TP53 biological background mutations detected using Duplex Sequencing from uterine lavage samples (top) mirror the prevalence of clonal mutations seen in all human cancers from the UMD Seshat database (lower).

Conclusions

- Duplex Sequencing™ enables detection of variants below a frequency of one-in-a-million bases, including those which have been induced by carcinogens.
- Quantification of subclones with frequencies below the error rate of traditional NGS technologies enables shorter timescale assays for detecting early cancer initiating events.
- Duplex Sequencing illustrates how similar patterns of clonal selection can be seen in multiple otherwise healthy tissues of humans as part of normal aging.
- Duplex Sequencing is a sensitive and data-rich assay for detecting both mutagenesis and carcinogenesis of any genetic locus, in any tissue, in any organism.

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