

TwinStrand DuplexSeq™ Mutagenesis Solution



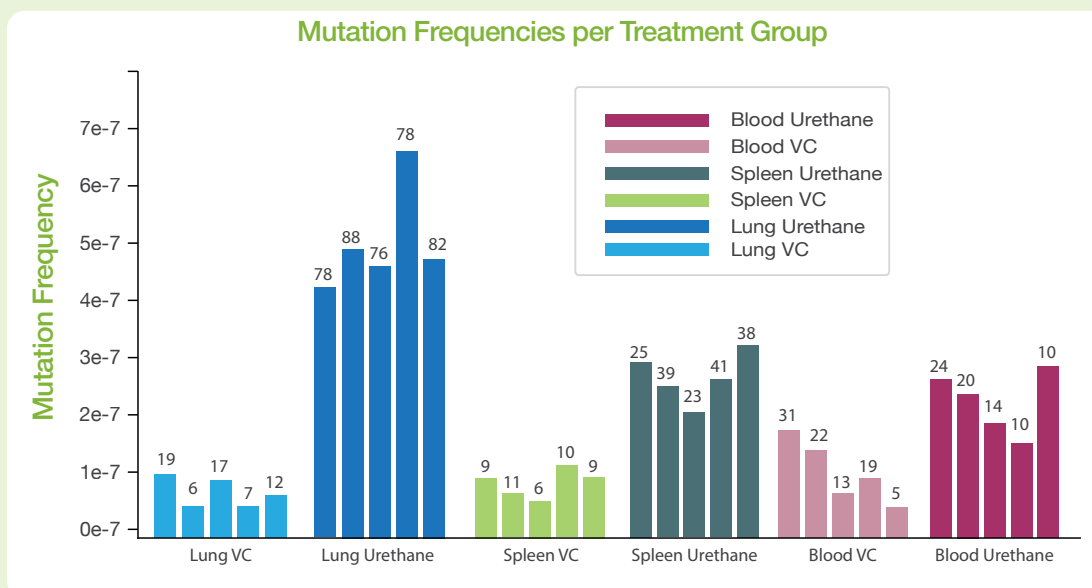
Mutagenesis is an important predictor of cancer risk in chemical and drug safety testing. Standard mutagenesis assays yield a limited amount of indirect information about a compound's mutagenic potential and/or are complex and cumbersome.

TwinStrand DuplexSeq™ Mutagenesis Assays offer a fundamentally new Next-Generation Sequencing (NGS)-based approach for mutational research that directly detect, quantify, and characterize induced mutagenesis in the DNA of exposed organisms and cells. The unprecedented richness of data unlocks powerful opportunities for novel insights and discoveries.

Duplex Sequencing for Mutagenesis

- The first NGS technology capable of directly measuring mutagenesis in DNA molecules without the need for biological selection
- Accurately & reproducibly measures mutation loads on the order of 1/10,000,000
- Equally applicable *in vitro* and to any tissue *in vivo*
- Produces comprehensive mutation data, including frequency, simple spectrum, and trinucleotide signature
- Supports the diverse needs of academic, commercial, and regulatory users
- DuplexSeq™ Mutagenesis Assays currently available in human, mouse, and rat formats

Mutation Frequencies

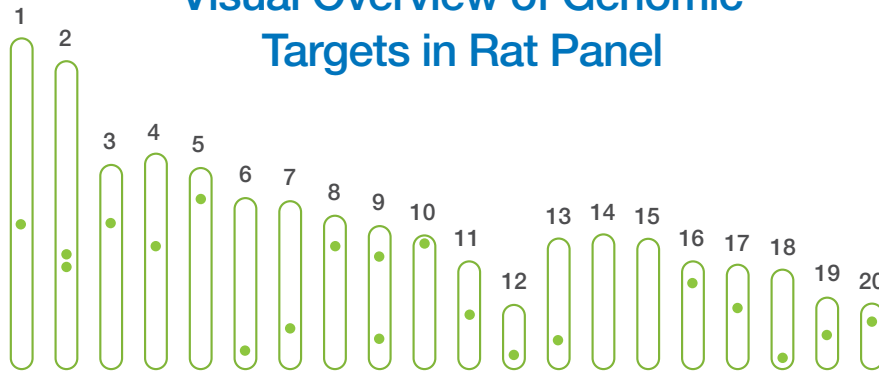


Urethane-induced mutagenesis detected by duplex sequencing in 3 mouse tissues. Mutation frequencies are highly reproducible across biological replicates ($n=5/\text{group}$), and statistically robust. The number of individual mutant nucleotides identified per sample among hundreds of millions screened is indicated above each bar. VC: vehicle control (untreated).

Mutagenesis Panel Overview



Visual Overview of Genomic Targets in Rat Panel

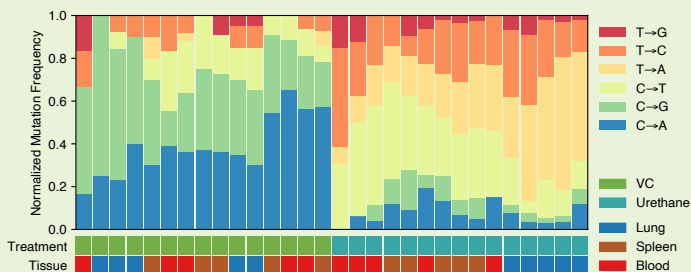


Location of genomic targets in the TwinStrand Duplex Sequencing™ rat mutagenesis panel. Mouse and human panels are similarly constructed.

The TwinStrand Duplex Sequencing mutagenesis assays use a 48kb panel targeting 20 representative regions spread throughout the genome. The regions are balanced to provide an unbiased sampling of sequence contexts throughout the genome (GC content, genic/non-genic, coding/non-coding, etc). These sites have no known role in cancer and are unlikely to be significantly influenced by positive or negative selection. Currently available in human, mouse, and rat formats.

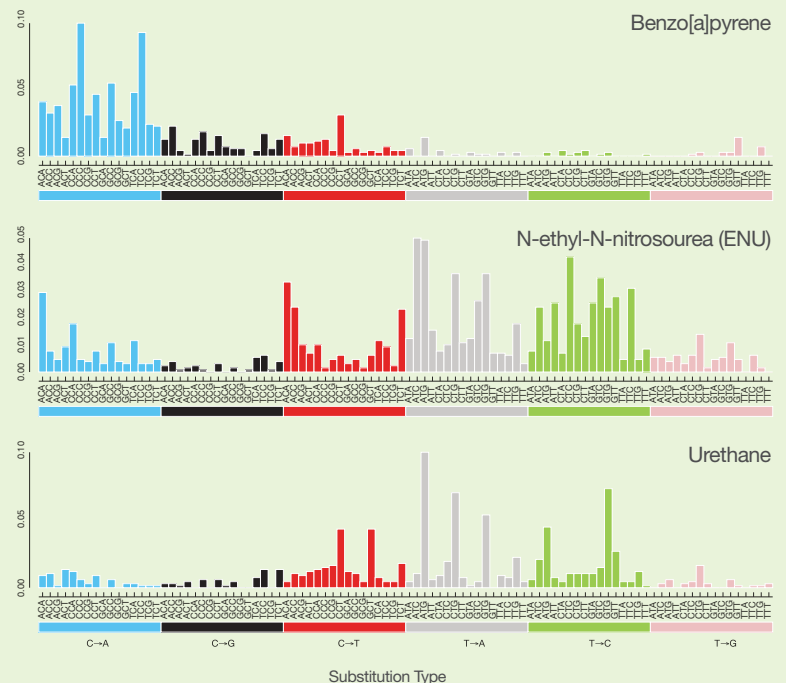
Duplex Sequencing Mutational Spectra

Simple Mutation Spectra



Simple mutation spectra detected by duplex sequencing of urethane-exposed mouse tissue. Unsupervised hierarchical clustering perfectly assigned all of the 30 tissues to the correct treatment groups (vehicle control on left, urethane on right).

Trinucleotide Mutation Signatures



Highly distinct trinucleotide signatures generated by duplex sequencing of normal (non-tumor) mouse tissues exposed to three mutagens: urethane, benzo[a]pyrene, and N-ethyl-N-nitrosourea. Figures generated using ultra-low frequency mutations present on the order of 1-in-1 million.

Valentine et al 2020, PNAS, PMID 33318186