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Extending ToxTracker with duplex sequencing to further understand the mode of action of genotoxic substances and their mutagenic potential.

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The standard genotoxicity testing strategy typically investigates induction of gene mutations, chromosomal aberrations, and numerical chromosome changes. ToxTracker® is an in vitro mammalian stem cell-based reporter assay that detects activation of specific cellular signalling pathways to identify direct DNA damage induction as well as indirect genotoxicity caused by oxidative stress and protein damage. The assay provides insight into the genotoxic mode of action (MOA) and can discriminate between clastogenic and aneugenic compounds. ToxTracker was shown to predict in vivo genotoxicity of compounds with a >90% sensitivity and specificity.

The TwinStrand DuplexSeq™ mutagenesis assays use a highly accurate sequencing technique that tracks both strands of the sequenced DNA molecules to limit sequencing errors. The DuplexSeq mutagenesis assays can detect and characterize mutations induced upon chemical exposure and are supported with an easy-to-use bioinformatics pipeline.

To combine the MOA information and accurate detection of gene mutations, we applied the TwinStrand DuplexSeq Mouse Mutagenesis Assay in the ToxTracker reporter cells to further unravel the MOA of genotoxic substances and determine their mutagenic potential. Providing a mutational fingerprint of compounds helps to further explore the MOA of genotoxic substances, thereby improving the in vitro genotoxicity prediction. In a pilot study, we tested the genotoxic substances N-ethyl-N-nitrosourea, benzo[a] pyrene, and potassium bromate in ToxTracker and determined their mutational fingerprint using the DuplexSeq Mouse Mutagenesis Assay.

Keywords:
Genotoxicity; mutagenesis; mode-of-action; duplex sequencing.