Total DRUG-seq: Cost-efficient HTTx Platform Enabling Gene Isoforms and non-coding RNA Analysis for MoA Discovery and Risk Assessment

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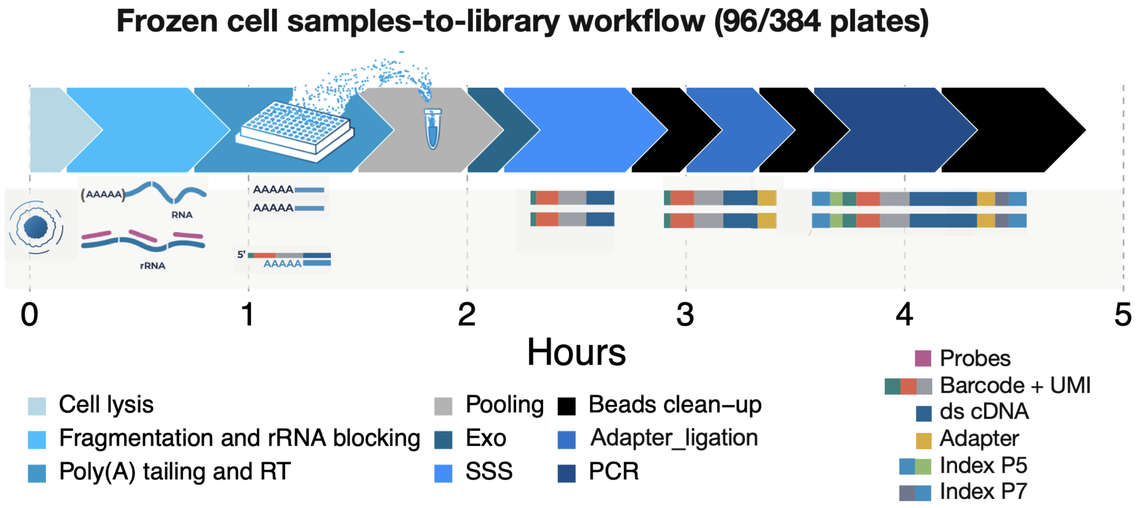
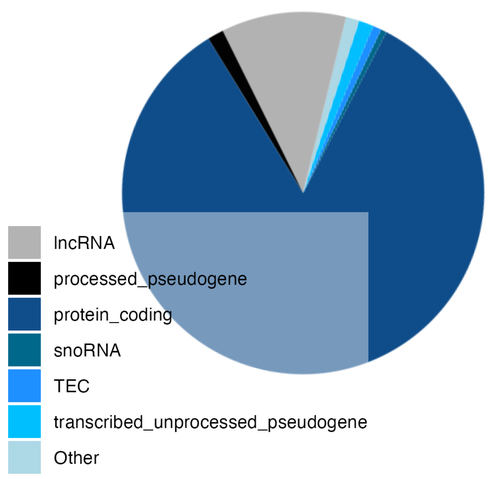
Abstract
Here, we present MERCURIUS™ Total DRUG-seq, an advanced HTTx platform that integrates early sample multiplexing and the streamlined library preparation of DRUG-seq, while enabling whole transcriptome expression analysis, including non-protein-coding transcripts with full-length gene coverage. It can capture alternative promoter usage, non-coding RNA, isoforms, and splicing events across thousands of samples, thereby offering deeper mechanistic insights into the chemical bioactivity of a compound. We used hepatocellular carcinoma cells and patient-derived fibroblasts to demonstrate the efficacy of MERCURIUS™ Total DRUG-seq in detecting alternative promoter usage and splicing events upon stimulation with TGF-β and the splicing modulator drugs, Risdiplam and Branaplam. We detect transcriptional changes and alternative splicing events that would be missed by conventional poly(A)-based transcriptomics, highlighting the utility of MERCURIUS™ Total DRUG-seq. MERCURIUS™ Total DRUG-seq enables more comprehensive transcriptomic profiling while maintaining the capacity for high-throughput library preparation at a low cost. This approach significantly expands the applicability of HTTx in predictive toxicology and provides deeper insights into MoA, compound bioactivity and molecular toxicity pathways.

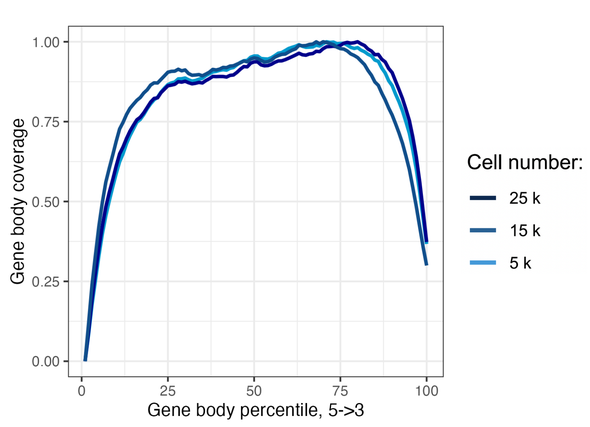
1. Overview of MERCURIUS™ Total DRUG-seq

The MERCURIUS™ Total DRUG-seq protocol enables RNA-extraction free library preparation in < 5 h for up to 384 samples

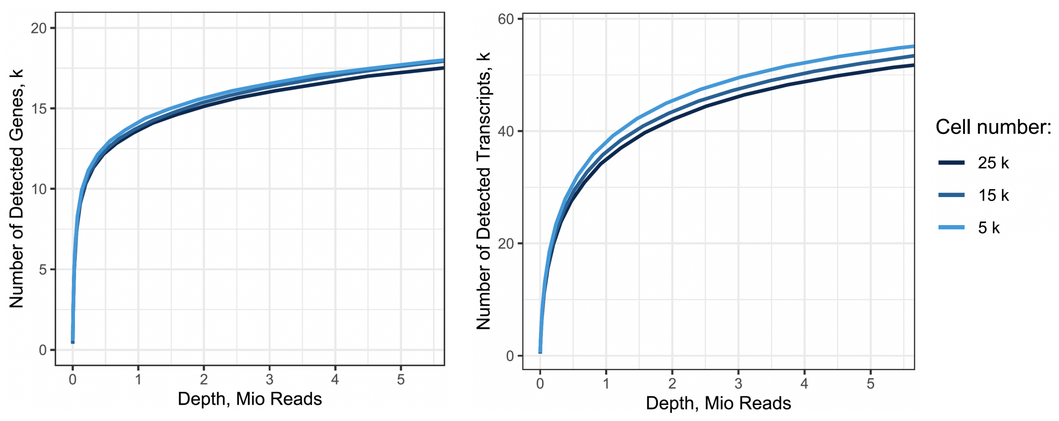


Total, full-length gene body coverage allows the detection of coding and non-coding RNA





Lower cell inputs are favored and enable the detection of 15K gens and 40K transcripts at a depth of 1Mio reads



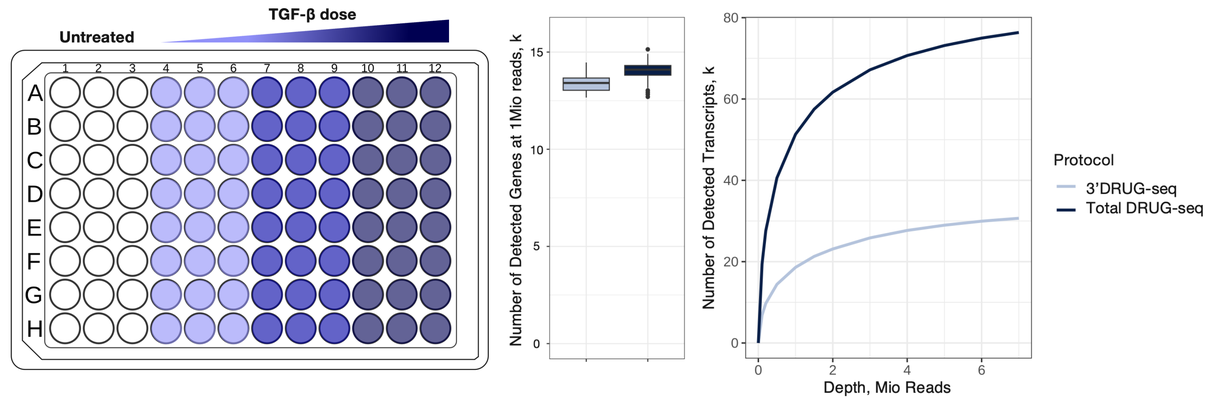
4. Conclusion

MERCURIUS™ Total DRUG-seq is an **early multiplexing** protocol that provides **full-length** gene body coverage from **extraction-free RNA,** enabling **isoform detection**, alternative promoter usage, and splicing event analysis, while maintaining **high sensitivity** with as few as **2'000 cells**.

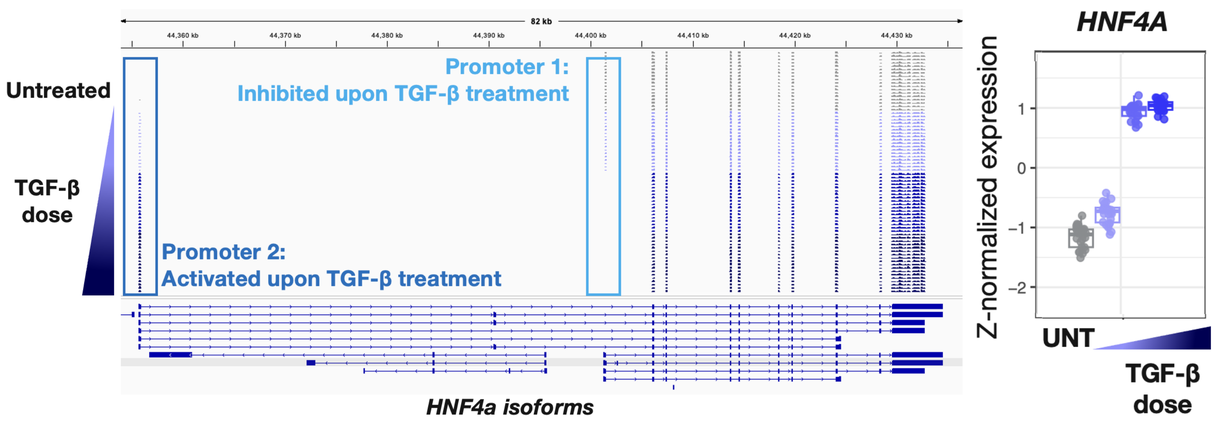
MERCURIUS™ Total DRUG-seq is available as **kit and service.**

2. Alternative promoter usage in HUH7 cells upon TGF-β stimulation

60K isoforms detected at 2Mio reads for MERCURIUS™ Total DRUG-seq for HUH7 treated with 0.1, 1 or 10 ng/ml TGF-β

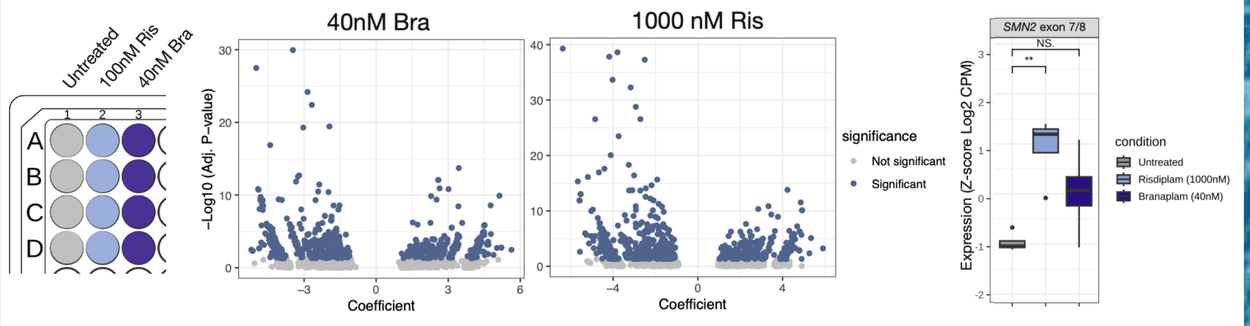


Example of a promoter switch mediated by increased *HNF4A* expression following TGF-β stimulation

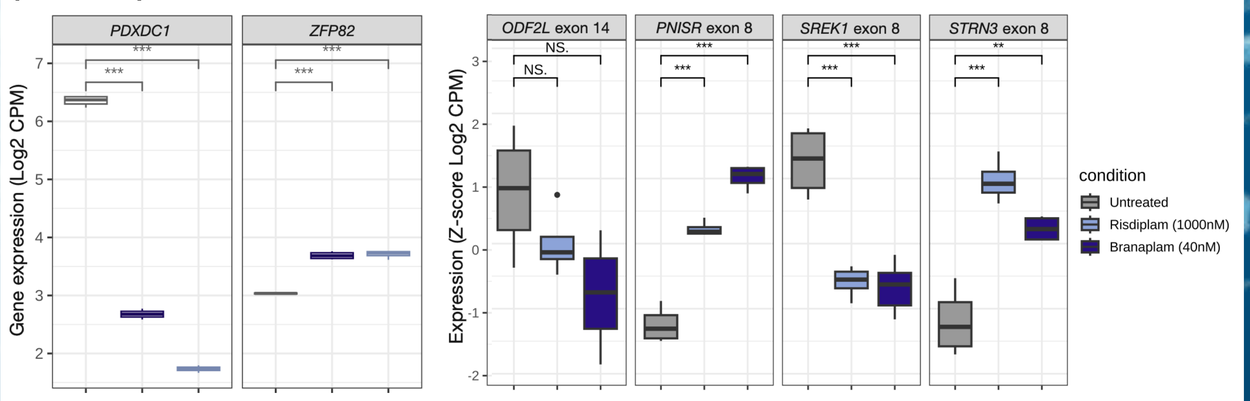


3. Alternative splicing and isoform detection in SMN1-depleted cells upon Branaplam and Risdiplam treatment

Differential exon usage detected with diffSpliceDGE in *SMN1*-depleted cells treated with 1µM Risdiplam or 40nM Branaplam



Altered gene expression (*ZFP82, PDXDC1*), alternative splicing (*STRN3, SREK1*), exon skipping (*ODF2L*) and intron retention (*PNISR*)[1]



[1] Ottesen et al. (2023) Diverse targets of SMN2-directed splicing-modulating small molecule therapeutics for spinal muscular atrophy. *Nucl Ac Res*

Presentation:
When? Tue. 16.Sept, 11:20h
Where? N. Skalkotas Hall

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