# Linking high-throughput transcriptomics and imaging for functional drug response profiling

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## Introduction

Drug discovery and toxicological risk assessment require scalable, cost-effective profiling platforms capable of generating biologically rich datasets to power AI/ML-driven decision-making.
Imaging-based phenotypic screening (Cell Painting) enables robust and economical high-throughput profiling, however, its limited molecular resolution constrains direct interpretation of pathway- and target-level mechanisms of action (MoA).
Low-cost and scalable transcriptomics profiling, enabled by Total DRUG-seq addresses this gap, delivering thousands of independent gene-level readouts that provide direct mechanistic insight and support multiomics integration.

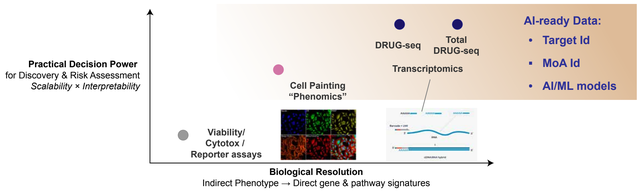
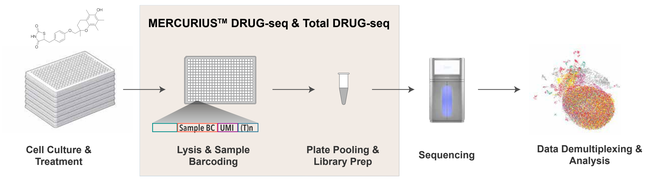



**Figure 1** | Perturbation rofiling technologies mapped by mechanistic resolution and decision power

## Scaled Transcriptomics with Total DRUG-seq

MERCURIUS™ Total DRUG-seq is a highly scalable, RNA extraction-free library preparation method enabling to prepare single library for up to 384 samples in a single day. It produces whole-transcriptome data with paired-end sequencing capability—capturing the full complexity of transcriptome-level RNA expression.




| Technology | Type | Coverage | Detection @1M/reads | Sample-to-library (384) |
|---|---|---|---|---|
| DRUG-seq | polyA(+) | 3' | >13'000 genes | 6.5 hours |
| Total DRUG-seq | polyA(+)&(-) | full length | >13'000 genes + 65'000 transcripts | 5.0 hours |

**Figure 2** | Total DRUG-seq workflow for RNA extraction-free early multiplexing library preparation

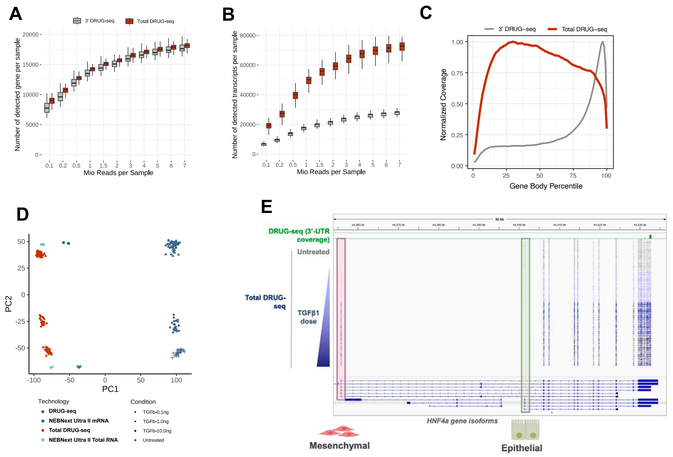



**Figure 3** | Benchmarking Total DRUG-seq on Huh7 cells upon TGF-β activation.

Total DRUG-seq demonstrates increased sensitivity compared to standard 3′ DRUG-seq (Fig. 3A) and enables robust detection of gene isoforms due to full-length transcript coverage (Fig. 3B, 3D). The resulting expression profiles closely match those generated by conventional RNA-seq library preparation methods, including NEBNext Ultra (Fig. 3D). Application of Total DRUG-seq across 96 samples reveals shifts in HNF4α isoform expression associated with TGF-β–induced epithelial-to-mesenchymal transition (EMT).

## Multi-Omics Profiling of 90 Compounds from JUMP-MoA Library

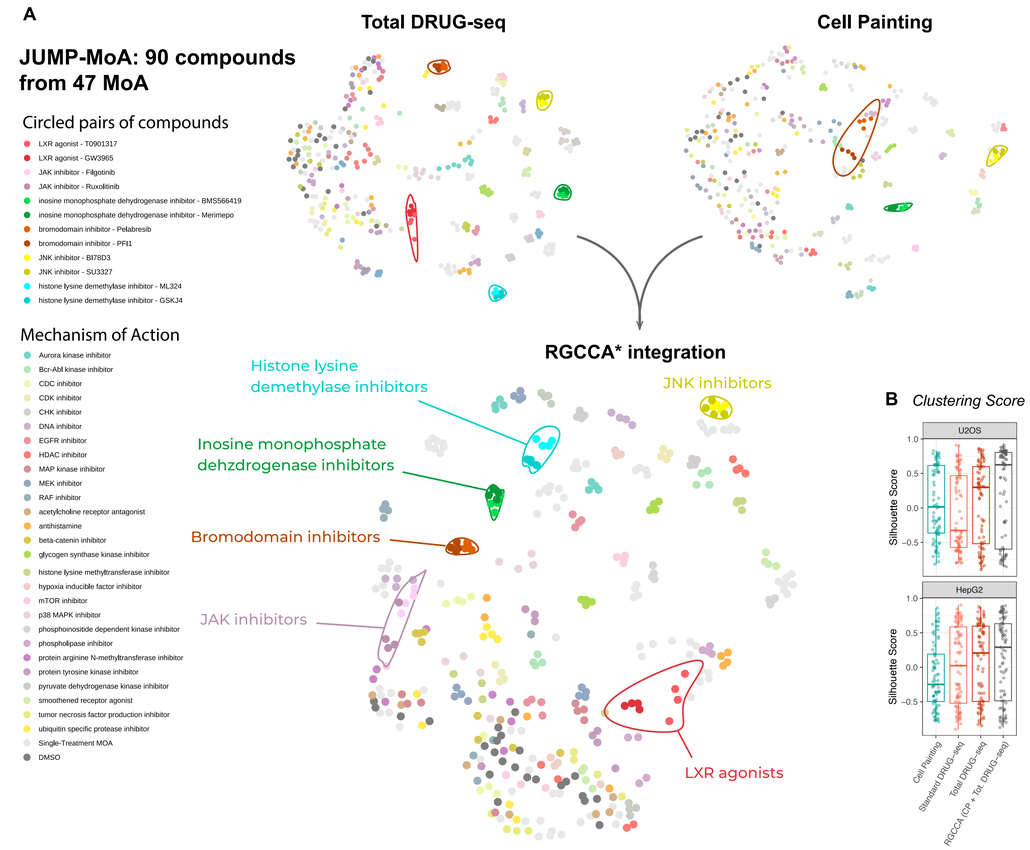



**Figure 4** | Multi-omics integration of Cell Painting and DRUG-seq: UMAP embedding and Silhouette-based clustering quality

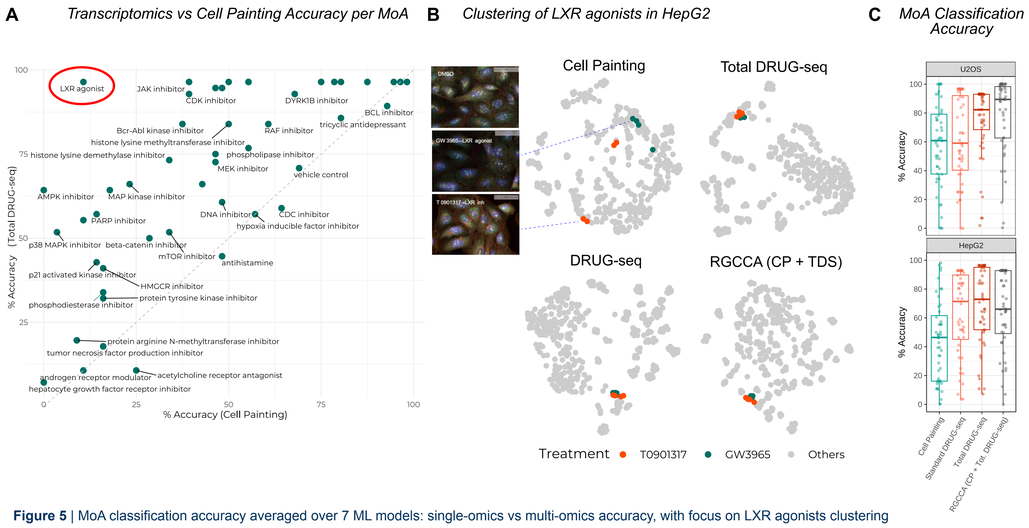



**Figure 5** | MoA classification accuracy averaged over 7 ML models: single-omics vs multi-omics accuracy, with focus on LXR agonists clustering

* *Haghighi et al. 2022*

## MoA and Tox-linked Gene Signature Analysis

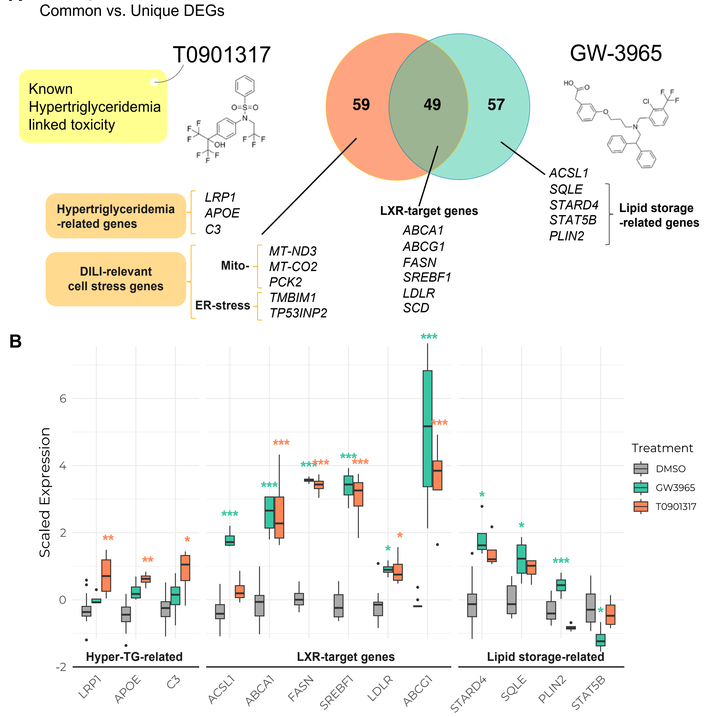



**Figure 6** | LXR agonist signature genes: common pathways and presumable toxicity-linked hallmarks

Among the 6 pairs of treatments from the same MoA highlighted in the RGCCA-integrated multi-omics data (Fig. 4A, "Circled Pairs of Compounds"), 5/6 also cluster in Total DRUG-seq data, while only 3/6 cluster in Cell Painting data. Silhouette scores further show that Total DRUG-seq outperforms Cell Painting, and combining both assays improves clustering — mostly in U2OS, but also in HepG2 — highlighting the complementarity of both assays (Fig. 4B, boxplot on the right). Transcriptomics better grouped compounds affecting signaling pathways, such as Wnt modulators and checkpoint kinase inhibitors. In contrast, Cell Painting was more sensitive to epigenetic and chromatin modifiers.

MoA classification generally showed higher accuracy for transcriptomics than for Cell Painting (Fig. 5A–C), especially in HepG2, where both Total DRUG-seq and standard DRUG-seq outperformed Cell Painting. RGCCA-integrated data was the top performer in U2OS, reaching >90% accuracy, but did not improve transcriptomic accuracy in HepG2, where top accuracy was observed for Total DRUG-seq (73%). LXR agonists showed the largest accuracy gain in Total DRUG-seq compared to Cell Painting (Fig. 5A). In HepG2, UMAP clustering of the LXR agonist pair showed clear grouping for transcriptomics and RGCCA-integrated data, whereas Cell Painting did not achieve a clear separation (Fig. 5B).

Analysis of misregulated genes revealed LXR agonism markers shared across treatments, along with treatment-specific features (Fig. 6A). Shared DE genes included the expected LXR-related genes (*ABCA1, ABCG1, FASN, SREBF1, LDLR*). T0901317's higher toxicity is known to be linked with hypertriglyceridemia, captured by known relevant genes as well as DILI associated genes (Fig. 6B, *LRP1, APOE*, and *C3*).

## Conclusions

- Total DRUG-seq enables scalable, high-throughput transcriptomics, generating thousands of independent gene-level readouts per compound to support AI/ML-driven drug discovery and toxicological risk assessment.
- Across multiple cellular models and experimental conditions, Total DRUG-seq delivers information that complements Cell Painting, as demonstrated by both unsupervised clustering and supervised classification analyses.
- Using LXR agonists as a case study, we show that Total DRUG-seq detects subtle, toxicity-related transcriptional changes that are not captured by imaging-based assays, highlighting its value for early safety assessment and multi-omics integration