SINGLE CELL DISCOVERIES

Discovery-seq | High-throughput bulk RNA-sequencing

Transcriptomic phenotyping of large scale perturbation experiments

Description

Drug screening is a comprehensive process that integrates molecular biology, high-throughput imaging and data analysis. Current high-throughput screening data is generally composed of visual markers which are hard to quantify and are limited in quantity. In contrast, RNA-sequencing based screening provides transcriptome-wide quantitative information, facilitating de novo discovery of affected genes and pathways.

At Single Cell Discoveries we have developed Discovery-seq, a sensitive and cost-effective method to facilitate easy transcriptomic phenotyping of thousands of samples exposed to a variety of perturbations such as small molecules, siRNAs, CRISPR-Cas9 or antibodies. Discovery-seq has been made compatible to a high diversity of different cell culture systems and can be used to discover effects independent of cell survivability or visual detection.

Highlights of Discovery-seq

- Unbiased high-throughput screening
- Fast turnaround times
- Cost effective
- Automated processing of thousands of samples with high senstivity and reproducibility
- Suitable for any type of (dose-dependent) perturbation:
  - Small molecules, antibodies, siRNA, CRISPR, toxicity
- Possibility of multimodal readout of screening on the supernatant prior to Discovery-seq

Requirements

- Suited for 384-well and 96-well plates
- Recommended seed density of 5,000-10,000 cells
- Requires washed cell pellet (max. 10 ul volume left)

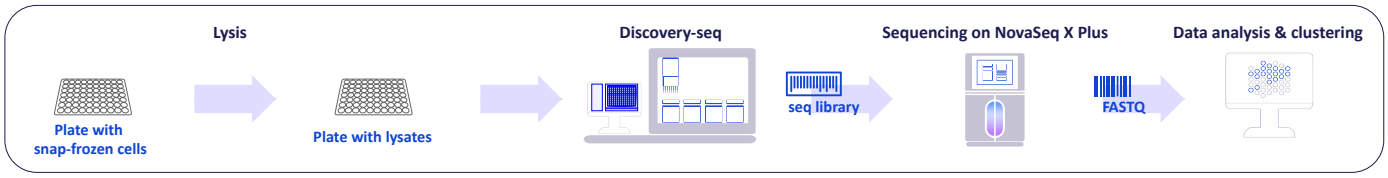
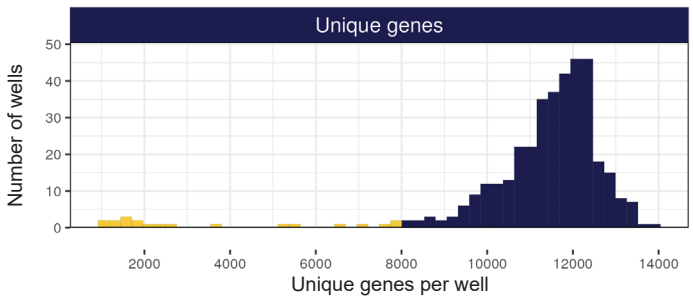


Figure 1. Workflow Discovery-seq. After high-throughput study of drug or gene perturbation, cells in 384-well or 96-well plates (seed density 5,000-10,000 cells) are washed with PBS and snap-frozen. Cell pellets are lysed and processed using Discovery-seq. Libraries are sequenced on the NovaSeq X Plus. The raw data is analyzed with our custom NextFlow pipeline.

Discovery-seq is highly sensitive

- Sequencing at 1-2M reads per well
- Recovery of around 12,000 genes per sample, ~80% of a 3' Bulk-Seq equivalent



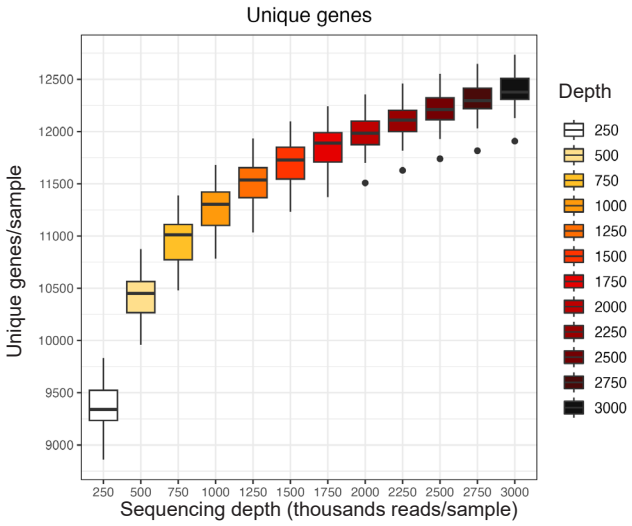


Figure 2. Discovery-seq is highly sensitive. Sequencing of Discovery-seq-processed samples at 1.2M reads per sample results in a recovery of around 12,000 genes per sample (left). Increasing sequencing depth up to 3M reads per sample marginally increases detection of unique genes (right).

Case study: Discovery-seq uncovers dose-dependent gene expression in response to drug perturbation

Method

HEK293T cells were cultured in 384-well plates at cell density of 5000 per well for 24 hours and subsequently exposed to a concentration range of several drug inhibitors for 24 hours (N = 14 replicates per condition). The effect of trichostatin A (HDAC inhibitor) is highlighted below. After perturbation, cells were washed twice with PBS. The supernatant was aspirated completely before snap-freezing and storing the plates at -80 C. For Discovery-seq, the plates were thawed and processed with the Discovery-seq protocol. Libraries were sequenced on the NovaSeq X Plus. The raw data was analyzed using our custom NextFlow pipeline.

Results

The data depicted below illustrates how Discovery-seq yields unbiased whole transcriptome data revealing expected mechanism of action for different dosages of trichostatin A (HDAC inhibitor). Differential gene expression analysis shows a complex drug response allowing us to –in an unsupervised manner- find genes that show the most biologically interesting response to a compound.

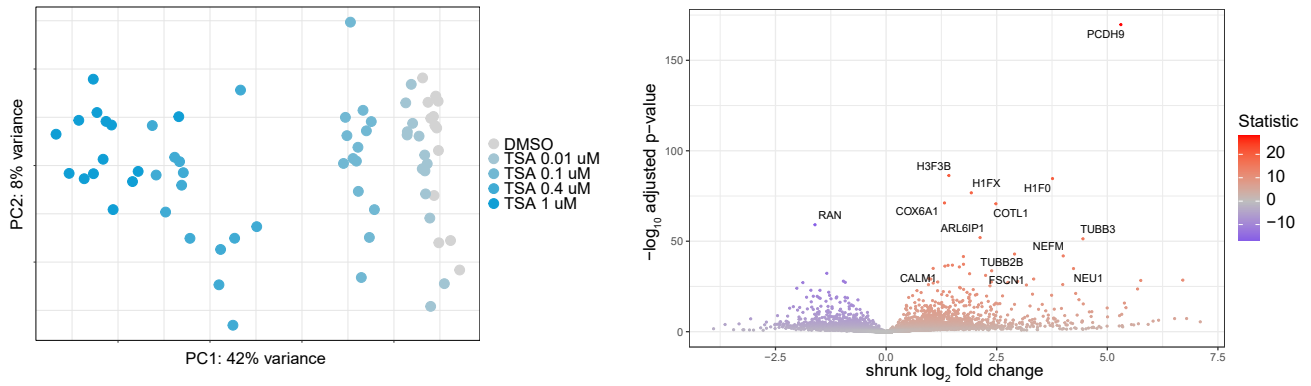


Figure 3. Discovery-seq shows distinct gene signatures for different drug concentrations and clear differential gene expression. HEK293T cells were exposed to a concentration range of (0.01 - 1 uM) trichostatin A (TSA) and control DMSO (N=14 replicates per condition) for 24h. Cells were washed, snap-frozen and processed with Discovery-seq. Libraries were sequenced on the NovaSeq x Plus. Principal component analysis shows effects of different TSA concentrations (left). With Discovery-seq we clearly observed differentially expressed genes between TSA treated and DMSO-treated cells (right).

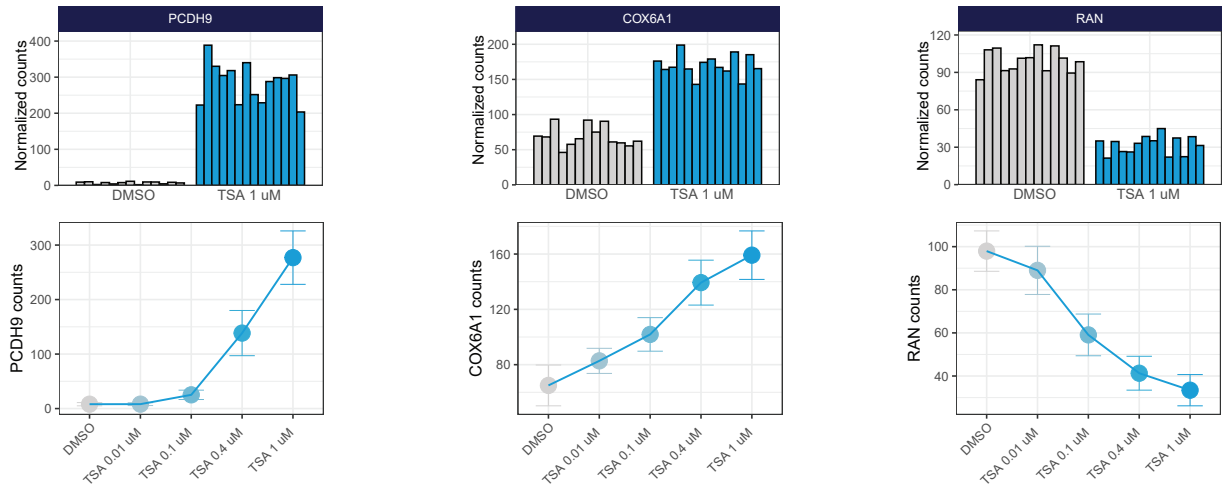


Figure 4. Discovery-seq yields highly reproducible results and reveals dose-dependent responses. Expression of differentially expressed genes PCDH9, COX6A1 and RAN (1 uM trichostatin A (TSA)-treated cells vs. DMSO-treated control cells) were plotted across replicates, which show high reproducibility between replicates (top). Plotting of these genes across the different doses reveal clear dose-dependent perturbation of gene expression (bottom).