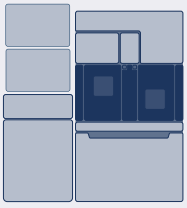
DRUG DISCOVERY

THINKCYTE

Label-Free High Throughput CRISPR-Based Screening for Morphological Phenotypes in Flow



About VisionSort

ThinkCyte's VisionSort platform combines the strengths of conventional flow cytometry fluorescence signals with a novel morphometric cellular analysis measure. The dual-mode analytical capability can be used to identify and sort phenotypically defined cell populations, label free, using machine learning approaches.

INTRODUCTION

The development of CRISPR-Cas9 genome editing technology has led to the emergence of a new generation of novel life sciences applications.[1,2] In drug discovery, researchers have harnessed the precision of selective gene knockouts by CRISPR to enable genome-wide drug screening. By mapping genotypes to phenotypes, CRISPR-based phenotypic screens can enable a better understanding of drug mechanism of actions (MOAs) and identification of novel druggable targets. However, current phenotypic CRISPR screening approaches rely heavily on microscopic imaging of target phenotypes, a process that imposes throughput limitations and restricts screening to only a handful of simple phenotypes based on binary fluorescence signals. To fully realize the potential of CRISPR-based phenotypic screening, here show application of the VisionSort platform to a pooled high throughput, label-free CRISPR screening methodology targeting morphological phenotypes. As a proof-of-concept, we used this methodology to screen for genes involved in macrophage polarization.

RESULTS

To rapidly screen for genes involved in complex phenotypes, a workflow for CRISPR-based pooled screening in flow was developed **(Figure 1)**.[3] In this implementation, macrophages derived from THP-1 cells expressing Cas9 protein were transduced with pooled CRISPR lentiviral libraries containing 7,290 sgRNAs targeting 279 kinase genes for loss-of-function gene sets to create a pooled knockout cell library. A machine learning classifier was trained to identify different macrophage polarization states. For training the machine learning model, M0 and M1 macrophages were separately prepared. After staining only the M0 macrophages with a fluorescent marker to define a ground truth phenotype, the two populations were mixed. Together with

fluorescent ground truth definitions, label-free morphological profiles for individual cells were generated by Ghost Cytometry and used to train a machine learning classifier on VisionSort. The resulting classifier was able to distinguish between the two polarized macrophage populations with an AUC score of 0.89 **(Figure 2)**.

Next, using test samples, we screened for cells exhibiting inhibition of M1 polarization following CRISPR-based gene knockouts. Cells with the target phenotype were identified and sorted using the pre-trained classifier and processed for downstream gRNA readouts. By analyzing the enrichment of gRNAs after sorting, we identified several genes, including potentially novel mediators, that influence M1 macrophage polarization **(Figure 2)**.

SUMMARY

We developed a novel high throughput CRISPR screening method and evaluated the approach using a complex morphological phenotype.[3] The approach can be adapted to screen for a wide range of simple molecular and complex phenotypes, giving researchers a new powerful tool for drug screening.

REFERENCES

1. https://pubmed.ncbi.nlm.nih.gov/28333914/
2. https://pubmed.ncbi.nlm.nih.gov/25430774/
3 https://www.biorxiv.org/content/10.1101/2023.01.26.525784v1

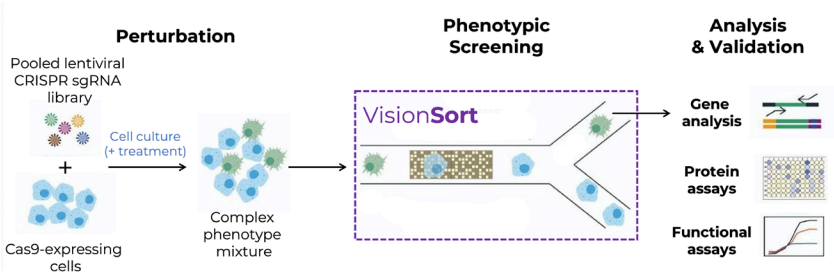


Figure 1. General workflow for pooled high-content CRISPR screening for complex phenotypes in flow. A gene-knockout cell library prepared using the CRISPR-Cas9 gene editing platform is treated with a compound or other reagents to induce the desired phenotype. A pre-trained machine learning model selectively enriches cells with the target phenotype for downstream analysis of target genes involved. In this proof-of-concept, sgRNA regions inserted in genomic DNA of target cells were amplified and sequenced by NGS to determine the enriched/depleted genes involved in macrophage polarization. For live cell analysis, proteomic, transcriptomics, and cell-based functional assays are also possible.

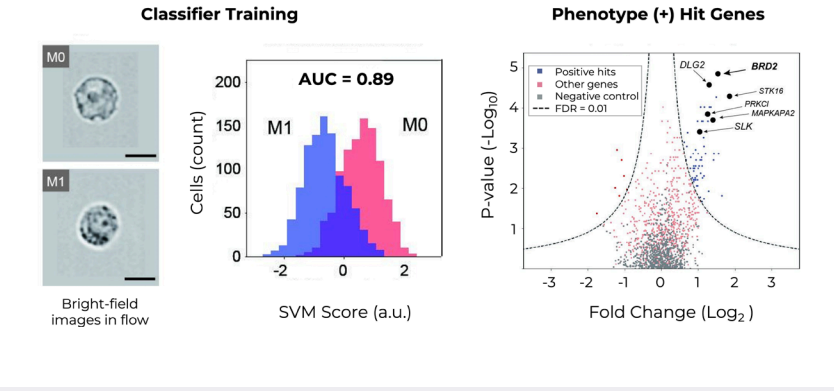


Figure 2. Validation of phenotype detection and screening. Bright-field images of THP-1 derived M0 and M1 macrophages in flow were obtained with a commercial imaging flow cytometer. Scale bars = 10 um. A mixed population of M0 and M1 polarized macrophages was used to train a machine learning classifier on VisionSort. The classifier achieved excellent performance for the detection of the target phenotype with an AUC of 0.89. sgRNAs from sorted cells were sequenced to identify enriched genes involved in the target phenotype of macrophage polarization. Volcano plot visualization of statistical significance (y-axis) and magnitude of the change (x-axis) before and after the cell sorting. Statistical significance was calculated with Mann-Whitney U test. Dashed lines: cutoff for hit genes (FDR = 0.01).

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