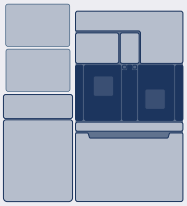
DRUG DISCOVERY

THINKCYTE

High Throughput CRISPR-Based Phenotypic Screening in Flow for Complex Intracellular Phenotypes



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]. 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 by 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 CRISPR screening methodology targeting nuclear translocation as the target phenotype. As a proof-of-concept, we show a small scale screen to identify genes involved in regulating a well-characterized nuclear translocation pathway.

RESULTS

THP-1 cells expressing Cas9 protein were transduced with pooled CRISPR lentiviral libraries containing 60 gRNAs for loss-of-function gene sets to create the pooled knockout cell library. The cell library was treated with LPS, a TLR4 agonist known to induce nuclear translocation of NF-kB[2] **(Figure 1)**. Cells exhibiting nuclear NF-kB and cells showing cytoplasmic NF-kB were mixed and used to generate a machine learning classifier on VisionSort. The resulting classifier was able to distinguish between the two populations with an AUC score of 0.987 **(Figure 2)**.

Next, using test samples, cells exhibiting cytoplasmic NF-kB were identified and sorted using the classifier and processed for downstream gRNA readouts. Enrichment of gRNAs relating to genes known to be involved in the TLR4 signaling pathway including MYD88, MAP3K7, IRAK4 and IKBKB was observed, demonstrating the utility of the approach **(Figure 3)**.

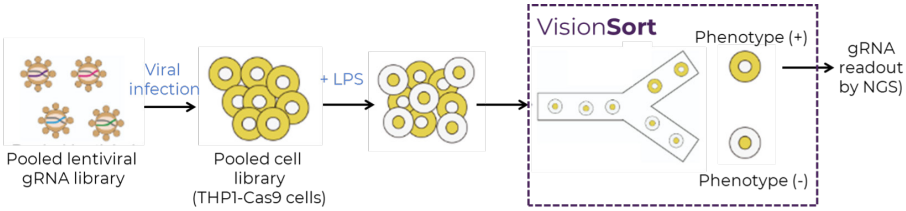


Figure 1. Workflow for pooled phenotypic CRISPR screening with VisionSort. A gene-knockout cell library was prepared via the CRISPR-Cas9 system with a lentiviral gRNA library containing 60 gRNAs. Cells were stimulated with LPS to induce nuclear translocation NF-kB and then analyzed and sorted with VisionSort. Cells exhibiting cytoplasmic NF-kB (phenotype positive) as the target phenotype were collected from the pool and processed for downstream readouts of gRNAs by NGS.

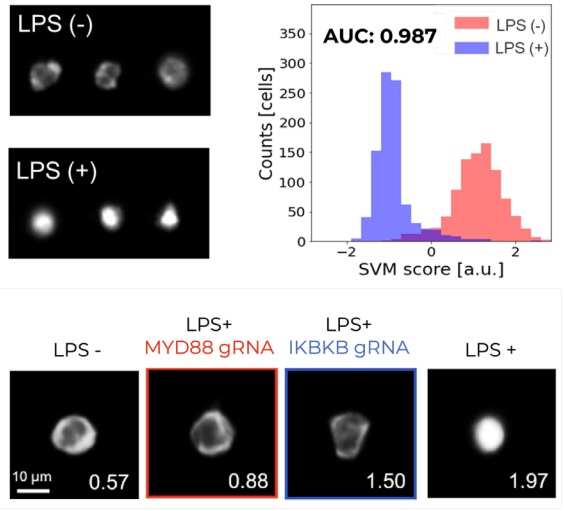


Figure 2. Validation of phenotype detection. Nuclear translocation of NF-kB in THP-1 cells induced by LPS stimulation was visualized and confirmed using fluorescence microscopy. A mixed population of cells was used for training the classifier. The classifier achieved outstanding performance for the detection of the target phenotype with an AUC of 0.987.

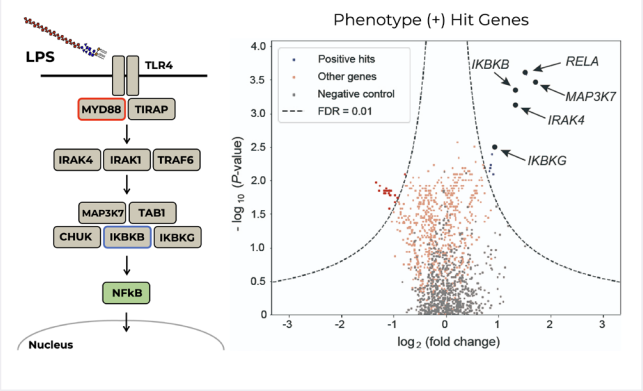


Figure 3. Biological validation of phenotype detection. gRNA from sorted cells were sequenced to identify enriched genes involved in the target phenotype (nuclear translocation of NF-kB (green box)). As confirmation of the approach, genes known to be involved in the TLR4 signaling pathway upstream of NF-kB nuclear translocation (e.g., MYD88 (red box), IKBKB (blue box)) were enriched in the phenotype positive fraction. Volcano plot visualization of statistical significance (y-axis) and magnitude of the change (x-axis) in gGNA enrichment before and after cell sorting. Statistical significance was calculated with Mann-Whitney U test. Dashed lines: cutoff for 'hit' gene calls (FDR = 0.01). Images and nuclear translocation scores were taken and calculated using the FlowSight system (Amnis).

SUMMARY

We used VisionSort to perform a novel high throughput CRISPR screening approach and show validation using a simple, well-characterized phenotype[3]. The approach can be adapted to screen for more complex phenotypes requiring intracellular spatial resolution, giving researchers a powerful new tool for drug screening.

REFERENCES

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