Ghost Cytometry for Functional Genomics Screening

THINKCYTE

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Abstract

Functional genomics requires a rapid and unbiased screening tool to determine cells that have the proper functional outcome after genetic manipulation. Ghost Cytometry (GC) offers such a tool that can rapidly screen cells for signaling events such as nuclear translocation. Standard FACS with cell sorting requires staining the cells with fluorescent NFkB labels may render the cells unsuitable for downstream culture. Thus, the need for a label-free method of isolating genetically redirected cells is needed to discriminate those cells that are functionally desirable while not having any additional labels that can interfere with downstream applications. We show here that GC corresponds well to NFkB translocation to the nucleus after Lipopolysaccharide (LPS) stimulation using standard fluorescent staining techniques. Ghost Cytometry with its label free and AI technology offers a method to ensure the sorting of genetically modified cells that have the correct phenotype and suitable for downstream application.

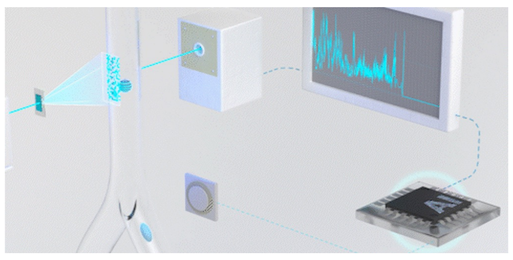
Introduction

Conventional methods of high – content screening can take up to 10,000 plate and take between 6-12 months. Ghost Cytometry simplifies the process with pooled phenotypic screening. The cells are altered and pooled and assayed by GC for their phenotypic expression with the cells of interest being sorted for downstream analysis. In the present study, we use a lentivirus RNA library and select for those cells that block NFkB translocation to the nucleus in response to LPS stimulation.

Methods

Functional genomics requires a rapid and unbiased screening tool to determine cells that have the proper functional outcome after genetic manipulation. Ghost Cytometry (GC) offers such a tool that can rapidly screen cells for signaling events such as nuclear translocation. Standard FACS with cell sorting requires staining the cells with fluorescent NFkB labels may render the cells unsuitable for downstream culture. Thus, the need for a label-free method of isolating genetically redirected cells is needed to discriminate those cells that are functionally desirable while not having any additional labels that can interfere with downstream applications. We show here that GC corresponds well to NFkB translocation to the nucleus after Lipopolysaccharide (LPS) stimulation using standard fluorescent staining techniques. Ghost Cytometry with its label free and AI technology offers a method to ensure the sorting of genetically modified cells that have the correct phenotype and suitable for downstream application.

VisionSort
POWERED BY
Ghost Cytometry



Label free Ghost Motion Imaging Signals

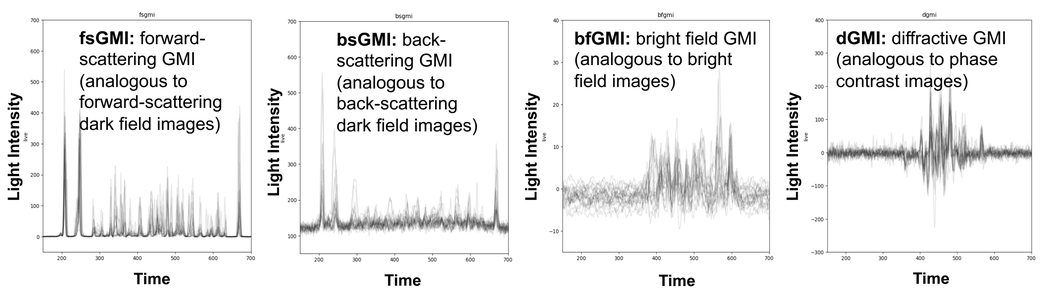


Figure 1: ThinkCyte® ImageSort™ – Combining Ghost Cytometry with Traditional Fluorescence Cytometry. ImageSort™ utilizes standard, fluorescent flow cytometry with ThinkCyte® Ghost Cytometry. Ghost Cytometry utilizes structured illumination where the laser lite is diffused through a pinhole structure to reduce the laser intensity and increase the signal to noise ratio. The signals are read by four modalities: forward scatter, back scatter, bright field and diffractive Ghost Motion Imaging (GMI) to create waveforms for each mode. The artificial intelligence interprets the waveforms and uses specific peaks that are predictive of cell populations. Two general modes can be used in Ghost Cytometry: Supervised learning where cells of interest are defined by existing fluorescently labeled biomarkers for Ground Truth Labels

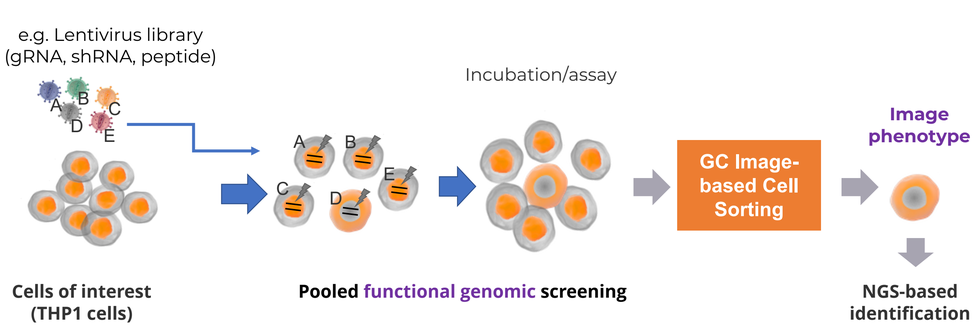


Figure 2: Protocol for Image-based Phenotypic Screening in Flow Format

Results

Supervised Learning to Train Ghost Cytometry Model

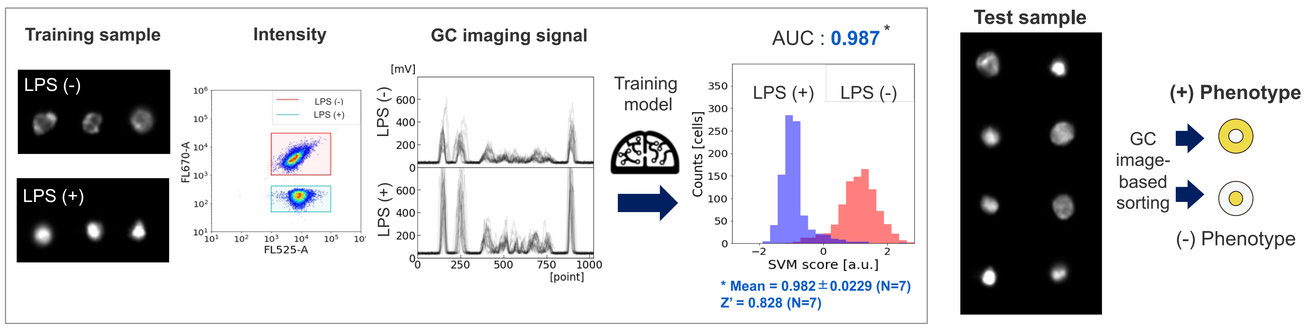


Figure 3: Supervised Learning for NFkB Translocation for Ghost Cytometry Provide a Reliable, Label-free Assay to Assay a Pooled Mixture of Gene Knockout Cells. Ghost Cytometry was trained using LPS treated THP1 cells resulting in the translocation of labelled NFkB into the cell nucleus.

After the Ghost Cytometry Model is Created – Cells Carrying Genes that Inhibit LPS-induced nuclear translocation of NF-kB

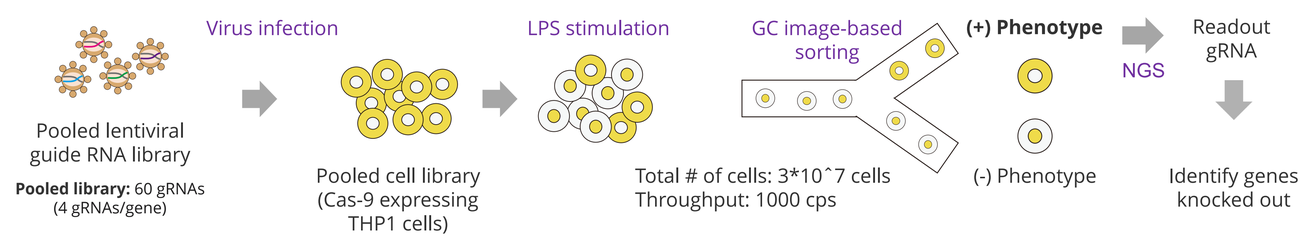


Figure 4: Ghost Cytometry – Based Pooled CRISPR Screening. Once the AI model was created, cells containing the RNA library were assayed and used to determine which gene constructs interrupted LPS induced NFkB translocation to the nucleus.

Ghost Cytometry Sorted Cells Containing Knockouts for TLR4 Signaling Pathway Genes

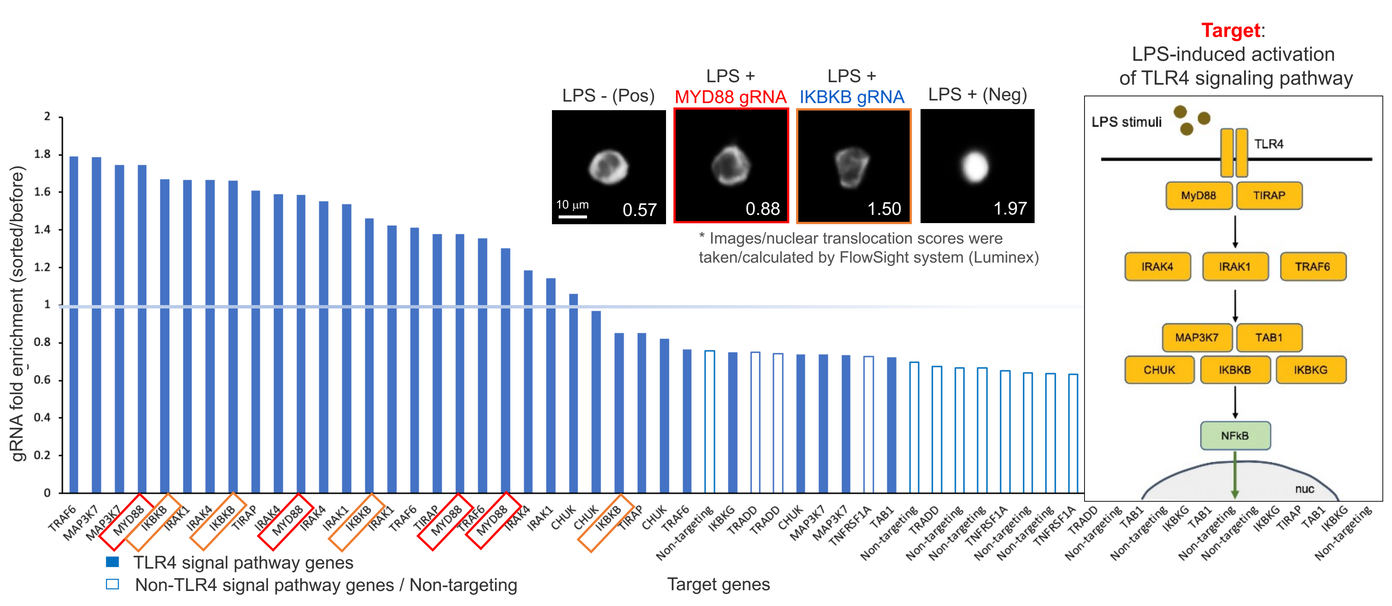


Figure 5: Image-based Pooled CRISPR Screening Shows the Utility of Ghost Cytometry. The pooled library of 60 gRNAs (4 gRNAs/gene) were assay in pooled cells that were isolated by GC for those cells which indicated interrupted NFkB translocation. The cells were enriched, collected and the cells assayed for gene knockouts.

Conclusions

1. Ghost Cytometry can detect and create a model NFkB localization in the nucleus
2. VisionSort™ GC was able to create a label – free AI model that was used to screen genomic constructs
3. Cells with knockouts in the TLR4 pathway were enriched with the GC AI Model
4. Ghost Cytometry allows for genomic screening with out the need for single cell plating – significantly increasing throughput and reducing time to results