Functional Genomics Screening with Invitrogen™ LentiArray™ CRISPR Libraries and CellSensor™ Assays

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OVERVIEW

Identifying and validating targets that underlie disease mechanisms and can be addressed to provide efficacious therapies remains a significant challenge in the drug discovery and development process. The understanding of RNA interference has led to the use of tools such as siRNA and shRNA to knock-down mRNA and suppress gene function. However, depending on the nature of the targets, cells, biology and end-point assays these approaches may suffer variously from their transient nature, design complexity, incomplete knock-down or off-target effects. The use of CRISPR (clustered regularly interspaced short palindromic repeat)-associated Cas9 nuclease and guide RNA (gRNA) provides a strong alternative that can produce long-lasting impact, straightforward design, knock-out of genes and increased specificity. A number of laboratories have already published reports demonstrating how pools of gRNA can be delivered to cells and "hits" can be established through enrichment or depletion of cells following a "survival" assay and identified by sequencing the introduced gRNAs in the remaining cell population. Here we demonstrate a knock-out screening approach that utilizes the Invitrogen™ LentiArray™ CRISPR library to interrogate the impact of individual gene knock-outs on the NFκB pathway as measured by a functional cell-based assay. We describe the library design concepts, the assay development, initial screening results and validation of specific identified hits. The gRNAs are designed to primarily 5' coding exons of a target gene using our CRISPR design tool to maximize knock-out efficiency and minimize off-target effects. Each gRNA is delivered as a separate lentiviral particle including an antibiotic-resistant marker and each gene is targeted by 4 gRNAs per well, delivered in a 96-well plate. We tested the approach using a library that targets the human kinome and developed a loss-of-function assay using our CellSensor™ NF-κB-bla ME180 cell line, which is based on the ratiometric blue/green reporter assay and easily enables identification of genomic targets associated with the NF-κB pathway. We elucidate the key factors in developing a robust assay including both transduction and assay optimization to achieve the highest levels of transduction efficiency and assay window. Using these optimized parameters, we screened the Invitrogen™ LentiArray™ CRISPR kinome library that targets >800 kinases and demonstrate how we followed-up on and validated a subset of the identified hits. We expect these approaches to be scaleable to the entire human genome and portable to multiple cell types and end-point assays including both high-throughput plate-based assays and high-content imaging based assays.

METHODS

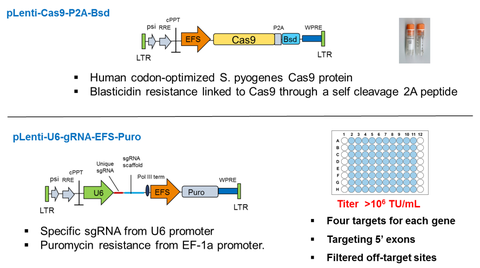
Lentiviral CRISPR Library Expression Constructs. The lentiviral CRISPR library expression constructs are generated by in-house high-throughput automation procedures followed by QC using in-house sequencing processes.

Packaging of Lentiviral CRISPR Library Particles. Lentiviral particles are generated by in-house high-throughput automation procedures. The viral titer is measured based on p24 assay, GFP expression and antibiotic-selection for QC.

CellSensor™ Assay. CellSensor™ NF-κB-bla ME180 Cas9 cells were cultured in D-MEM with GlutaMAX™ – high glucose, 10% dFBS, HEPES, NEAA, and Pen/Strep. GeneBLAzer™ Technology uses a fluorescence resonance energy transfer (FRET)-based substrate to provide reliable and sensitive detection of beta-lactamase (BLA) reporter activity in CellSensor™ cell-lines engineered with signal transduction pathway-specific response elements (RE). In the absence of BLA activity, the intact dual-fluorophore substrate molecule emits green light. Following stimulation of the pathway to induce BLA expression, the FRET substrate is cleaved, and instead of transferring energy to the green fluorophore, the blue fluorophore now simply emits blue light.

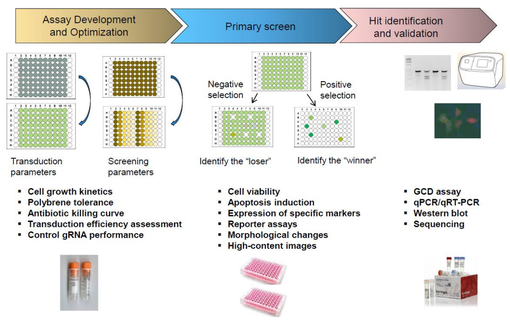
RESULTS

1. Lentiviral Vectors for the Invitrogen™ LentiArray™ CRISPR Libraries



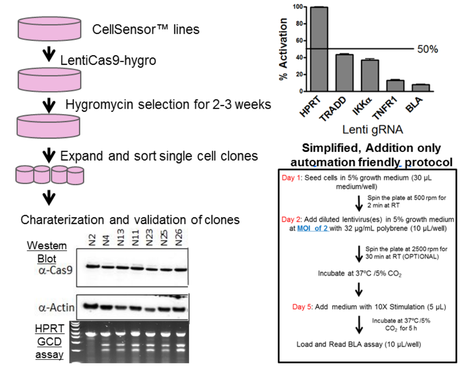
The lentiviral vectors used to express Cas9 nuclease and CRISPR gRNAs are illustrated above. The LentiArray library screening approach provides controlled delivery of gRNA targeting each gene in a separate well, eliminating a time-consuming deconvolution step and enabling functional cell-based screens of many types.

2. Screening Workflow of the Invitrogen™ LentiArray™ CRISPR Libraries



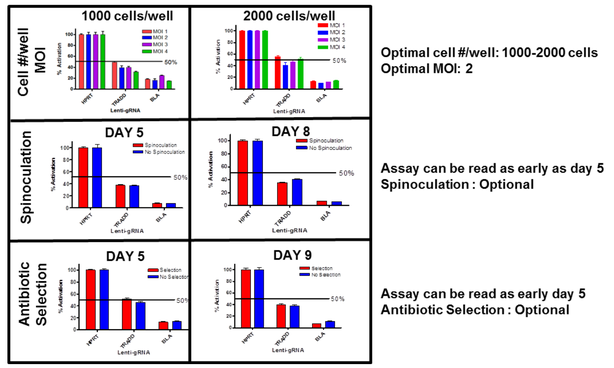
The standard screening process using the LentiArray CRISPR libraries includes assay development and optimization, primary screening, and hit identification and validation.

3. Improved Automated Workflow for LentiArray™ CRISPR Particles Screening Using a Stable CellSensor™ NF-κB-bla ME180 Cas9 Cell Line

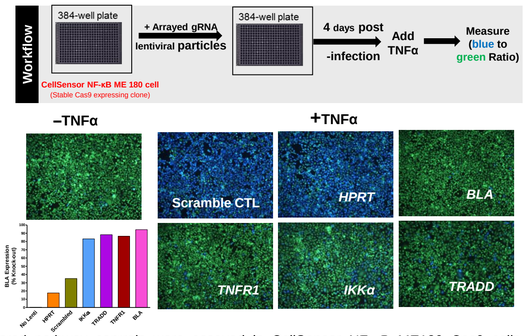


Improved automation compatible workflow using a CellSensor NF-κB-bla ME180 Cas9 cell line with a ratiometric reporter assay. After stimulation with TNFα, the ratio of blue/green fluorescence increased in un-edited cells (cells appeared blue). Cells infected with lentiviral particles carrying gRNA that effectively disrupted the NF-κB pathway remained green with a low blue/green ratio.

4. Assay Development

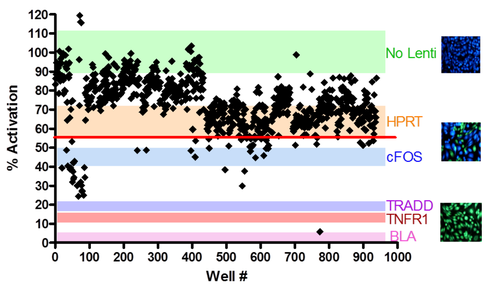


5. Assay Performance

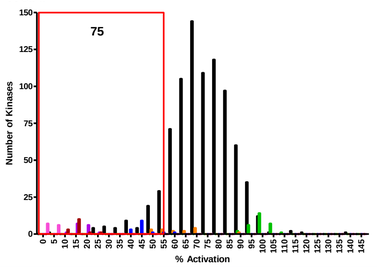


Validated pathway controls were assayed in CellSensor NF-κB ME180 Cas9 cells with a ratiometric reporter assay. After stimulation with TNFα, the ratio of blue/green fluorescence increased in un-edited cells (cells appeared blue). Cells infected with lentiviral particles carrying gRNA that effectively disrupted the NF-κB pathway remained green with a low blue/green ratio.

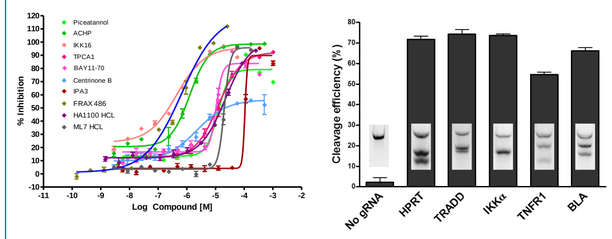
6. Screening results



A high-throughput screen of the CellSensor NF-κB ME180 Cas9 cell line with the LentiArray Human Kinase CRISPR library, containing up to four gRNAs per target for 840 kinase targets, yielded ~75 hits which knocked out the NF-κB pathway. No hits were observed that caused induction of the NF-κB pathway in ME180 Cas9 cell background. These hits identified some known NF-κB mediators such as CHUK, PLK and Rho, and some putatively novel targets to be further investigated.



7. Validation (Small molecule hits and GCD)



The known NF-κB mediators, identified to have specific small molecule inhibitors were tested on the CellSensor NF-κB ME180 Cas9 cell line to validate the targets from the primary screen. CellSensor NF-κB ME180 Cas9 cells were pre-treated with various doses of the small molecules, and then stimulated with TNFα. To explore the possibility of validating the targets using Genomic Cleavage Detection Assay, we validated the controls along the NF-κB pathway using single gRNA.

8. Invitrogen™ LentiArray™ Human CRISPR Libraries, 96-well format

Product	Cat. No.
Invitrogen™ LentiArray™ Human Kinase CRISPR Library	A31931
Invitrogen™ LentiArray™ Human Phosphatase CRISPR Library	A31932
Invitrogen™ LentiArray™ Human Cancer Biology CRISPR Library	A31933
Invitrogen™ LentiArray™ Human Epigenetics CRISPR Library	A31934
Invitrogen™ LentiArray™ Human Ubiquitin CRISPR Library	A31935
Invitrogen™ LentiArray™ Human Cell Cycle CRISPR Library	A31936
Invitrogen™ LentiArray™ Human Membrane Trafficking CRISPR Library	A31937
Invitrogen™ LentiArray™ Human Transcription Factor CRISPR Library	A31938
Invitrogen™ LentiArray™ Human Nuclear Hormone Receptor CRISPR Library	A31939
Invitrogen™ LentiArray™ Human Apoptosis CRISPR Library	A31940

Product	Cat. No.
Invitrogen™ LentiArray™ Human Drug Transporter CRISPR Library	A31941
Invitrogen™ LentiArray™ Human Ion Channel CRISPR Library	A31942
Invitrogen™ LentiArray™ Human Cell Surface CRISPR Library	A31943
Invitrogen™ LentiArray™ Human Protease CRISPR Library	A31944
Invitrogen™ LentiArray™ Human Tumor Suppressor CRISPR Library	A31945
Invitrogen™ LentiArray™ Human DNA Damage Response CRISPR Library	A31946
Invitrogen™ LentiArray™ Human GPCR CRISPR Library	A31947
Invitrogen™ LentiArray™ Human Drugable CRISPR Library	A31948
Invitrogen™ LentiArray™ Human Whole Genome CRISPR Library	A31949

LentiArray Human CRISPR libraries consist of pre-defined collection of gene families for functional genomics screening in an arrayed format. Each library targets a subset of human genes with up to 4 sequence-verified distinct lentiviral gRNA constructs per gene, pooled in a single well in a 96-well format. The gRNAs are based on the latest research on gRNA design. The gRNAs included in the LentiArray libraries are designed to knockout all known isoforms of the target genes and are selected for maximum knockout efficiency without sacrificing specificity.

CONCLUSIONS

- LentiArray Human CRISPR libraries are powerful high-throughput functional genomic screening tools for target identification.
- The gRNA libraries targeting various human gene sets are available as pre-made, ready to use lentiviral particles that are arrayed in 96-well plate format.
- CellSensor cell lines, combined with LentiArray Human CRISPR library particles offer a simple high-throughput workflow for target identification (screens may be performed by transient Cas9 introduction).
- CellSensor cell lines stably expressing Cas9, combined with LentiArray CRISPR library particles, offer an improved, automation friendly, simple high-throughput workflow for functional genomic screening.
- We successfully used CellSensor NF-κB ME180 cell line in combination with LentiArray Human Kinase CRISPR library to identify some known NF-κB mediators such as CHUK, PLK and Rho kinase, and some novel targets to be further investigated.