GeneArt® Arrayed Lentiviral CRISPR Library as Powerful High-Throughput Loss-of-Function Screening Tools

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ABSTRACT

The CRISPR/Cas9 genome editing system is a powerful tool for genetic screens to identify potential genes that are involved in various biological processes in mammalian cells. As of today, all the screening reports using CRISPR libraries have been performed only in a pooled format. We have developed a high-throughput production process to construct arrayed lentiviral CRISPR libraries with 4 sequence-verified distinct gRNA constructs per gene per well in a 96-well format. The gRNAs are designed to target primarily 5' coding exons using our proprietary gRNA design tool, attempting to maximize gene-knockout and minimize off-target effects. This arrayed format allows for the much more controlled delivery of gRNA per well, and eliminates the time-consuming deconvolution steps post-screening. The pre-made lentiviral library particles, when co-infected with lentiviral particles expressing Cas9, or alternatively in Cas9 stable expression cell lines, enable high-throughput loss-of-function screens using positive or negative selection strategies in a wide range of mammalian cell types, including primary and non-dividing cells. This new reverse genetic screening tool will enhance our ability for target discovery and validation in health and disease research and provide important information between genetic architecture and phenotypes.

INTRODUCTION

Functional characterization studies of the human genome have delivered a tremendous amount of information by using genome based loss-of-function screening in diverse models (1-2). RNA interference (RNAi) has been used as the predominant method for loss-of-function of genome screening, but it is limited by variable efficiency, frequent incompleteness of protein depletion and confounding off-target effects. The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 nuclease mediated genome engineering system enables researchers to modify genomic DNA with more precise targeting while minimizing off-target effects (3-5). Certain types of mammalian cells are difficult to transfect using lipid reagents or electroporation. To circumvent these difficulties, lentiviral vectors are commonly used as another delivery method as they can be easily titrated to manipulate transgene copy number and are stably maintained by integration into the genomic DNA during subsequent cell replication (6). Here we present the high-throughput production process to construct the arrayed lentiviral CRISPR library in a 96-well format. Furthermore, we describe the design and optimization of lentiviral CRISPR library for application in loss-of-function screenings. First, gRNAs are designed to primarily 5' coding exons of a target gene using our CRISPR gRNA design tool in order to maximize knock-out efficiency and to minimize off-target effects. Four gRNAs per gene are cloned in high-throughput 96-well format followed by lentiviral particles production with a minimum titer of 10^6 TU/ml, which is calculated by various titration methods; p24 assay, GFP expression of control lentiviral particles, and antibiotic selection for resistant cells. The subsequent lentiviral CRISPR library is amenable to screening workflows in a variety of methods. Here we show the functional validation of lentiviral CRISPR library particles with genomic cleavage efficiency and treatment with 6-Thioguanine (6-TG), which is one of anti-cancer chemotherapy drugs known as an antimetabolite. The genes that are involved in these pathways or processes could be phenotypically identified in a genetic screen for 6-TG resistance. Moreover, a human kinase loss-of-function screen using our proprietary CellSensor® NF-κB-bla ME180 cell line, which is based on the ratiometric blue/green reporter assay, easily enables identification of genomic targets associated with the NF-κB pathway.

METHODS

Lentiviral CRISPR Library Expression Constructs. The lentiviral CRISPR library expression constructs is 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.

RESULTS

1. Lentiviral CRISPR Vectors for the Arrayed Library

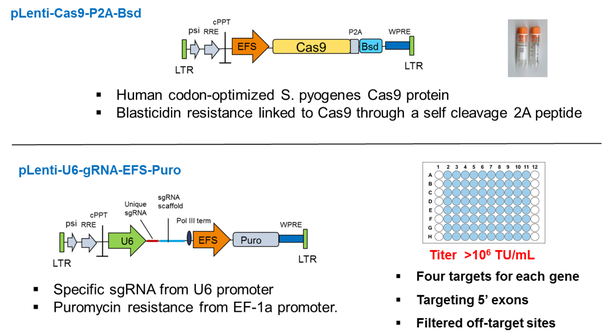
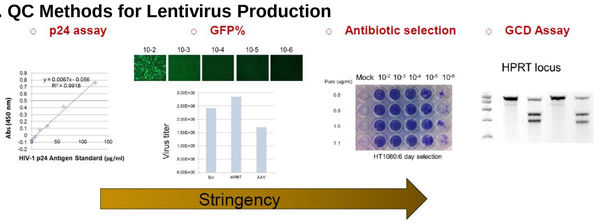


Fig 1. Lentiviral CRISPR vectors for the arrayed library generation. The arrayed library screening approach provides controlled delivery of each gRNA per well, eliminating a time-consuming deconvolution step.

2. QC Methods for Lentivirus Production



Lentiviral Particles	QC methods	Target titer
Cas9 particles	Antibiotic selection, GCD	>10^7 TU/ml
HPRT1 control Particles	GFP%, GCD	>10^8 TU/ml
Scramble control Particles	GFP%, GCD	>10^8 TU/ml
Arrayed library	GFP% of controls, GCD, Antibiotic for selected plates	>10^6 TU/ml
Pooled library	Antibiotic selection, NGS	>10^8 TU/ml

Fig 2. QC procedures for lentiviral particles production. p24 assay can be used to monitor the purification and biochemical behavior of lentiviral particles. Alternatively, HPRT1 and Scramble gRNA with GFP-Puromycin fusion cassette lentiviral particles are available to measure titer by GFP expression. Antibiotic selection is a common method for viral titration. Genome editing efficiency can be measured by GeneArt® Genomic Cleavage Detection kit for functional validation.

3. High Gene Targeting Efficiency by Lentiviral Transduction

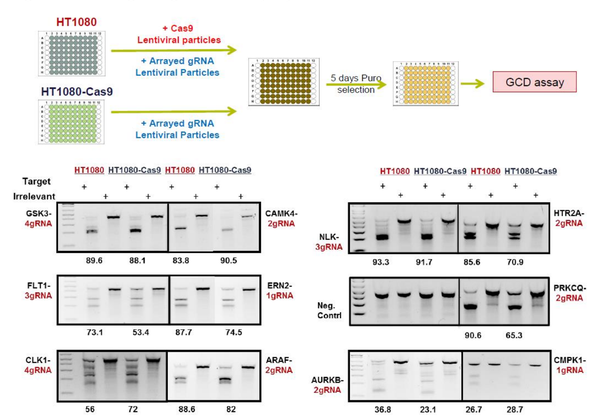


Fig 3. Lentiviral transduction to validate the gene targeting efficiency by co-infection with Cas9 lentiviral particles or alternatively in Cas9 stable expression cell line. High genome editing efficiency was observed in both single infection and co-infection as measured by GeneArt® Genomic Cleavage Detection (GCD) kit.

4. Screening Workflow of Arrayed Lentiviral CRISPR Library Particles

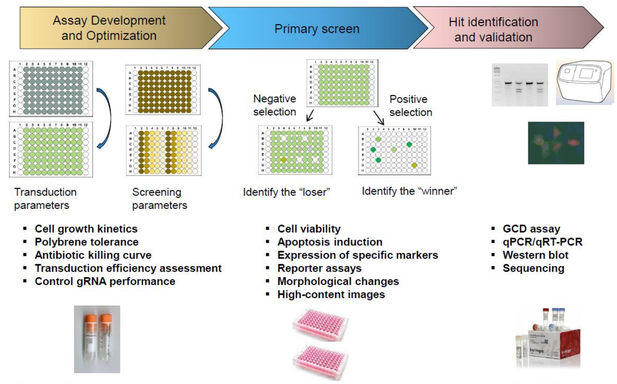


Fig 4. The standard screening process using the arrayed lentiviral CRISPR library includes assay development and optimization, primary screening, and hit identification and validation. We provide all the product to support the whole screening processes.

5. QC Controls for Lentivirus Production

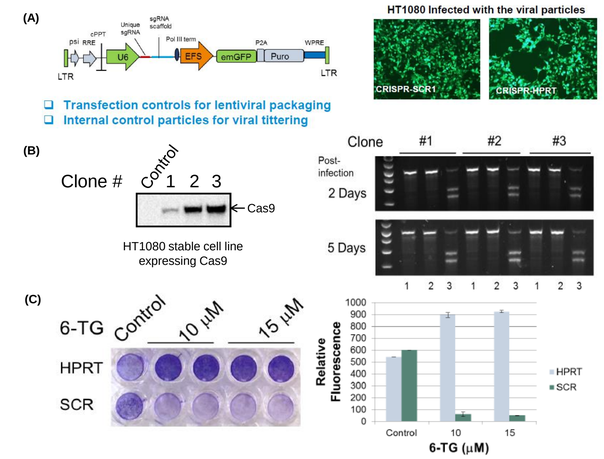


Fig 5. Functional validation of lentiviral CRISPR-HPRT1 or Scramble (SCR) with GFP-Puromycin fusion cassette particles in Cas9 stable expression cell line (A). Cas9 stable expression HT1080 cells are verified by western blot and the functional efficiency is validated by GCD assay with infection of lentiviral CRISPR-HPRT1 control particles (B). HPRT1 control lentiviral particles infected cells were treated with 6-TG to confirm the functional effect of gene knock-out. The final output was measured by Crystal Violet staining and PrestoBlue Cell Viability assay (C).

6. Lentiviral CRISPR particles for CellSensor® NF-κB-bla ME180 cell line

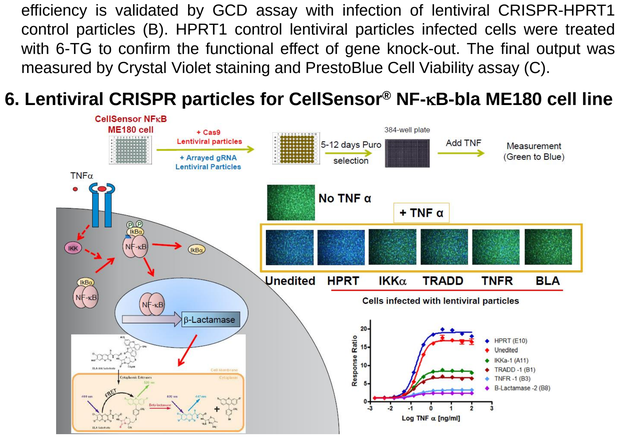


Fig 6. A subset of kinases screened in CellSensor NF-κB ME180 cells with a ratiometric reporter assay. After stimulation with TNFα, the ratio of blue/green fluorescence increased in unedited cells. Cells infected with lentiviral particles carrying gRNA that effectively disrupted the NF-κB pathway remained green with low ratio of blue/green fluorescence.

7. Workflow Needs and Solutions for Genome Editing

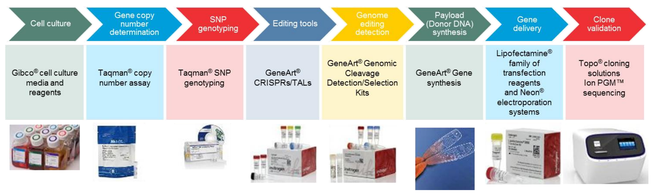


Fig 7. A collection of optimized and validated end to end solutions. We offer entire workflow needs and solutions that will support each step in order to optimize conditions and validate the functional efficiency of genome editing.

CONCLUSIONS

- Invitrogen GeneArt® arrayed lentiviral CRISPR libraries are powerful high-throughput loss-of-function screening tools for target identification.
- The gRNA libraries targeting various human gene sets, such as kinases and GPCR are available as pre-made, ready to use lentiviral particles that are arrayed in 96-well plate format.
- Lentiviral CRISPR library contains 4 sequence-verified distinct gRNA constructs per gene per well.
- The average titer of lentiviral CRISPR library particles is > 10^6 TU/ml.
- Invitrogen CellSensor® cell lines, combing with lentiviral CRISPR library particles offer simple high-throughput workflow for target screenings.

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