Invitrogen™ LentiArray™ Human CRISPR Library, 96-well Plate

Catalog Numbers A42234, A42267, A42268, A42269, A42270, A42271, A42272, A42273, A42274, A42275, A42276, A42277, A42278, A42279, A42280, A42281, and A42282

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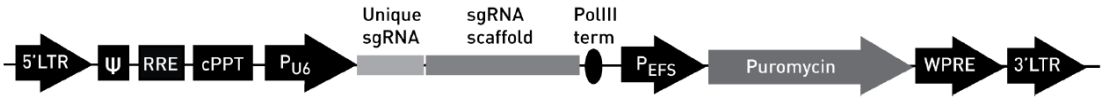
WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Invitrogen™ LentiArray™ human CRISPR libraries consist of pre-defined collections 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.

Characteristic	Description
Product	Invitrogen™ LentiArray™ Human CRISPR Library, 96-well Plate (see Table 1, for details)
Amount	2 aliquots of 50 µL/well per gene target
Viral titer	• Libraries are delivered with a range of average titer between 2×10^7–2×10^8 TU/mL by puromycin antibiotic selection. • We recommend using 1×10^8 TU/mL for starting multiplicity of infection (MOI) calculations, see "MOI determination for screens" on page 2 for additional guidance.
Lentiviral map	 • gRNA expression is driven by a U6 promoter. • Includes puromycin resistance gene to allow selection of transduced cells.
Plate layout	• Refer to the accompanying PDF file for the plate map of the specific LentiArray™ human CRISPR library and to the accompanying Excel files for gRNA target information. • First and last columns of the plates are empty.
Storage	Store at –80ºC. Avoid repeated freeze/thaw cycles, which will severely reduce functional viral titer. All components are stable for at least 1 year after receipt when stored as directed.
Biosafety precaution	LentiArray™ CRISPR Lentivirus particles have been packaged using a third generation lentiviral packaging system that has been designed to maximize its biosafety features. Although they are replication-incompetent virions, we recommend treating them as Biosafety Level 2 (BSL-2) organisms and following all published BSL-2 guidelines for the use of personal protection equipment and proper waste decontamination procedures.

Ordering information

Catalog numbers that appear as links open the web pages for those products.

Table 1 Invitrogen™ LentiArray™ human CRISPR libraries, 96-well plate

Product	Cat. No. (100 µL)
Invitrogen™ LentiArray™ Human Kinase CRISPR Library	A42234
Invitrogen™ LentiArray™ Human Phosphatase CRISPR Library	A42267
Invitrogen™ LentiArray™ Human Cancer Biology CRISPR Library	A42268
Invitrogen™ LentiArray™ Human Epigenetics CRISPR Library	A42269
Invitrogen™ LentiArray™ Human Ubiquitin CRISPR Library	A42270
Invitrogen™ LentiArray™ Human Cell Cycle CRISPR Library	A42271
Invitrogen™ LentiArray™ Human Membrane Trafficking CRISPR Library	A42272
Invitrogen™ LentiArray™ Human Transcription Factor CRISPR Library	A42273
Invitrogen™ LentiArray™ Human Nuclear Hormone Receptor CRISPR Library	A42274
Invitrogen™ LentiArray™ Human Apoptosis CRISPR Library	A42275
Invitrogen™ LentiArray™ Human Drug Transporter CRISPR Library	A42276
Invitrogen™ LentiArray™ Human Ion Channel CRISPR Library	A42277
Invitrogen™ LentiArray™ Human Cell Surface CRISPR Library	A42278
Invitrogen™ LentiArray™ Human Protease CRISPR Library	A42279
Invitrogen™ LentiArray™ Human Tumor Suppressor CRISPR Library	A42280
Invitrogen™ LentiArray™ Human DNA Damage Response CRISPR Library	A42281
Invitrogen™ LentiArray™ Human GPCR CRISPR Library	A42282
Invitrogen™ LentiArray™ Human Druggable CRISPR Library	Contact your local sales office.
Invitrogen™ LentiArray™ Human Whole Genome CRISPR Library	

Procedural guidelines

Assay development before screening

- Before using the LentiArray™ human CRISPR libraries for screening, determine the growth kinetics, puromycin sensitivity, Polybrene™ tolerance, and transduction efficiency for your cell line.
- Use the Invitrogen™ LentiArray™ CRISPR Positive and Negative Control Lentivirus particles (with or without GFP) to optimize the transduction and antibiotic selection conditions for your cell line of the interest. For more information, see the *Invitrogen™ LentiArray™ CRISPR Control Lentivirus Particles User Guide* (Pub. No. MAN0015949).

Strategies for transduction using LentiArray™ libraries

- The lentivirus particles in the LentiArray™ human CRISPR libraries can be delivered into human cells stably expressing Cas9 nuclease or co-infected with Invitrogen™ LentiArray™ Cas9 Lentivirus particles (Cat. No. A32064, A32069) into the target cells for screens.
- The advantage of the co-infection approach is that it eliminates the time-consuming process of generating Cas9 stable cell lines. However, using a cell line that stably expresses the Cas9 nuclease decreases the variability of CRISPR library screens. The LentiArray™ Cas9 Lentivirus particles also provide an easy and efficient way to generate cell lines stably expressing Cas9.
- Generally, we recommend a cell line that can be maintained as an adherent culture, non-migratory, and exhibits a doubling time in the range of 18–25 hours.

Transduction conditions

- You must determine the transduction conditions and multiplicity of infection (MOI) for each cell line empirically. If co-infection is needed, we recommended using an MOI ratio of 5–10 for Cas9 to CRISPR library lentivirus particles to achieve the optimal degree of gene knock out.
- Using culture media containing lower levels of FBS (e.g. 3–5% FBS) during infection may increase the transduction efficiency for some cell types.
- Polybrene™ (hexadimethrine bromide) can enhance the transduction efficiency of lentivirus into human cells by 2–10-fold. You must determine the optimal Polybrene™ concentration for your target cells (e.g., maximal infectivity with minimal toxicity) empirically. We recommend initial testing of Polybrene™ tolerance with a concentration range (2–8 µg/mL).
- If you plan to use puromycin for selection, you must first determine the optimal puromycin concentration necessary for the selection of transduced cells. Antibiotic lot, cell type, cell growth kinetics, and cell culture conditions, including cell density, affect the amount of puromycin required for selection. A typical selection with puromycin takes 7–10 days.

MOI determination for screens

- Multiplicity of infection (MOI) is the ratio of the number of virus particles to the number of target cells and must be determined for each cell line empirically.
- The nature of your human cell line (e.g., non-dividing vs. dividing cell type) affects the optimal MOI for successful transduction and knockout of the target gene. For example, HT1080 cells are readily transducible, and an MOI of 1 gives transduction efficiencies of around 90%. In some cell types, a 10-fold higher MOI may be needed to get the same transduction efficiency.
- We recommend using the LentiArray™ CRISPR Positive and Negative Control Lentivirus particles to determine the MOI that allows for maximum editing efficiency.
- We highly recommend to include desired assay controls in addition to transduction controls that show any specific phenotypes to further optimize the assay condition.
- Once the optimal MOI has been determined for one of the GFP controls, it is highly recommended to refine that by using a customized assay development plate, that contains multiple biological and technical controls for your desired assay. It is recommend to test a small range of MOIs based on the mean titer of 1×10^8 to clearly establish the best conditions for screening. Once you identify the MOI that provides efficient transduction of your cell line utilizing the LentiArray™ controls it is recommended to test a small range of MOIs based on the mean titer of 1×10^8 TU/mL to optimize for the MOI that provides maximum editing efficiency. For example, if an MOI of 2 was found to provide good transduction efficiency (i.e. 90% or greater GFP positive cells) then utilizing your desired assay test an MOI of 1, 2, 4, 8 to determine the MOI that delivers the best editing efficiency and functional results to support downstream screening activities.

Workflow for library screen

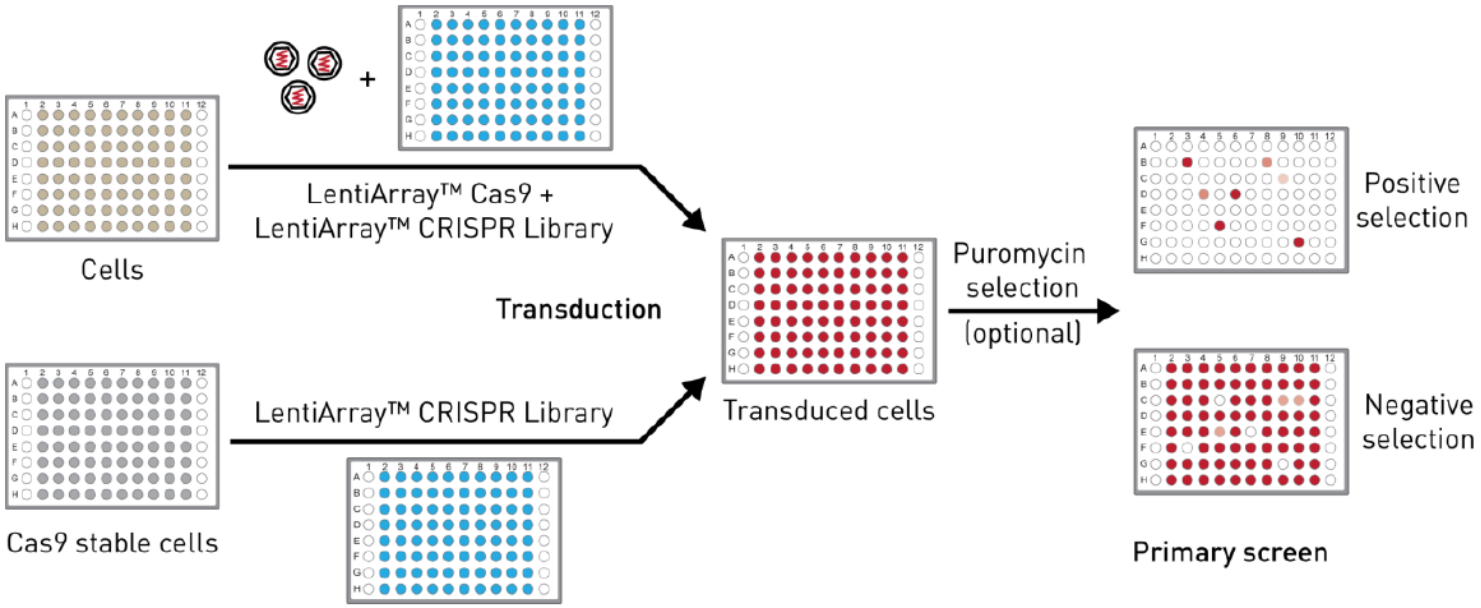


Fig. 1 Workflow for a library screen using the LentiArray™ Human CRISPR Library, 96-well Plate

Note: If MOI is greater or equal to 5, puromycin selection is optional.

Methods

Example transduction protocol in a 96-well plate with HT1080 cells

You can perform library screens in 96-well or 384-well plate formats using Cas9 stable cells or by co-infecting cells with LentiArray™ Cas9 Lentivirus and LentiArray™ human CRISPR library lentivirus particles.

The following procedure describes the suggested transduction protocol using the LentiArray™ human CRISPR lentivirus particles in a 96-well plate format.

IMPORTANT! Before starting, review "Procedural guidelines" on page 2.

Day 1

Seed the appropriate number of cells in 100 µL/well of complete growth medium in a 96-well plate to obtain approximately 50% confluence on the following day. Incubate the cells at 37°C overnight in a humidified 5% CO_2 incubator.

Note: Growth rates vary by cell type and culture condition, and must be determined empirically before starting the screen. When using HT1080 cells, we usually seed 5,000 cells in 100 µL culture medium per well in a 96-well plate.

Day 2

1. Prepare desired media containing low serum and Polybrene™ prior to thawing virus.
2. Remove the 96-well plate containing your LentiArray™ human CRISPR lentivirus particles and place in a 37°C water bath to thaw, but do not immerse in water. When the viral particles are mostly thawed with only small ice crystals remaining in the wells, transfer the plate onto ice.
3. Before removing the seal, centrifuge the lentivirus plate at low speed (maximum RCF at 200 × *g*) to collect the contents at the bottom of the well.
4. Add the appropriate amount of virus particles to the cells at a suitable multiplicity of infection (MOI) (see "Procedural guidelines" on page 2). Gently swirl the plate to evenly distribute the virus across the well.
5. Incubate the cells at 37°C overnight in a humidified 5% CO_2 incubator.

   Note: Centrifugation at 800 × *g* at room temperature for 30–120 minutes after adding the virus to the cells, also known as *Spinfection*, can enhance the viral infectivity.

Day 3

1. To minimize toxicity from virus and Polybrene™, aspirate medium containing virus, and add 100 µL uL/well of complete growth medium 24 hours post-transduction.
2. Incubate the cells at 37°C overnight in a humidified 5% CO_2 incubator.

Day 4

You can perform the screen assay without puromycin selection if you have used high MOI for transduction.

The following steps describe the optional puromycin selection of transduced cells, which takes place from Day 4 to Day 9+. A typical selection with puromycin takes 7–10 days.

1. Remove the medium containing viral particles and add fresh medium containing the appropriate amount of puromycin for the selection of transduced cells (see "Procedural guidelines" on page 2).
2. Replace the spent medium with fresh medium containing puromycin every 3–4 days.

Day 9+

After selection is complete, perform the desired phenotypic assays (e.g., cell survival, surface protein expression, high-content imaging of cells, reporter assay etc.).

Related products

Product	Cat. No.
Invitrogen™ LentiArray™ Cas9 Lentivirus, 100 µL	A32064
Invitrogen™ LentiArray™ Cas9 Lentivirus, 1 mL	A32069
Invitrogen™ LentiArray™ CRISPR Positive Control Lentivirus, Human HPRT, 100 µL	A32056
Invitrogen™ LentiArray™ CRISPR Positive Control Lentivirus, Human HPRT, 1 mL	A32829
Invitrogen™ LentiArray™ CRISPR Positive Control Lentivirus with EmGFP, Human HPRT, 100 µL	A32060
Invitrogen™ LentiArray™ CRISPR Positive Control Lentivirus with EmGFP, Human HPRT, 1 mL	A32830
Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus, Human Non-Targeting, 100 µL	A32062
Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus, Human Non-Targeting, 1 mL	A32327
Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus with EmGFP, Human Non-Targeting, 100 µL	A32063
Invitrogen™ LentiArray™ CRISPR Negative Control Lentivirus with EmGFP, Human Non-Targeting, 1 mL	A32831
Invitrogen™ LentiArray™ gRNA (individual tubes)	A32042
Invitrogen™ LentiArray™ Custom CRISPR Library	A32045[1]
GeneArt™ Genomic Cleavage Detection Kit	A24372
V5 Epitope Tag Antibody	R96025
Blasticidin S HCl (10 mg/mL)	A1113903
Puromycin Dihydrochloride (10 mg/mL)	A1113802

[1] For ordering information, send an email to GEMServices@thermofisher.com.

Limited product warranty

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Revision	Date	Description
C.0	07 October 2021	• Updated catalog numbers, and corresponding information applicable to updated catalog numbers. • Initial release with revision history table. • Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty.

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