Invitrogen™ LentiArray™ Cas9 Lentivirus

Catalog Numbers A32064 and A32069

Pub. No. MAN0016088 **Rev.** C.0

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™ Cas9 Lentivirus provides an efficient method to drive high level Cas9 nuclease expression in a wide variety of cell types. The LentiArray™ Cas9 lentivirus construct contains a human codon-optimized version of Cas9 with two nuclear localization signals that facilitate efficient delivery into the nucleus. You can use the LentiArray™ Cas9 lentivirus to create Cas9 stable cell lines for use in screening applications with LentiArray™ Human CRISPR libraries or in general editing experiments with the LentiArray™ gRNA Lentivirus. When creating a Cas9 stable cell line is not possible or desirable, you can use the LentiArray™ Cas9 lentivirus with LentiArray™ Human CRISPR libraries or LentiArray™ gRNA Lentivirus to co-infect the target cells.

Characteristic	Description
Product	Invitrogen™ LentiArray™ Cas9 Lentivirus
Amount	100 µL (Cat. No. A32064) 1 mL (Cat. No. A32069)
Viral titer	≥1 × 10^7 TU/mL (transducing unit per mL) by Blasticidin antibiotic selection (see the Certificate of Analysis for the titer of each lot)
Lentiviral map	 • Human codon-optimized *Streptococcus pyrogenes* Cas9 nuclease expression is driven by the EFS promoter. • Blasticidin resistance gene is linked to the Cas9 gene through a self-cleavage P2A peptide. • Cas9 nuclease carries nuclear localization signals on both the N- and C-termini, and also includes the V5 epitope tag.
Storage	Store at -80°C. Avoid repeated freeze/thaw cycles, which can severely reduce functional viral titer. All components are stable for at least one year after receipt when stored as directed.
Biosafety precaution	LentiArray™ Cas9 Lentivirus is 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 (BL-2) organisms and following all published BL-2 guidelines for the use of personal protection equipment and proper waste decontamination procedures.

Procedural guidelines

Getting started

To maximize the performance of the LentiArray™ Cas9 Lentivirus, determine the growth kinetics, Blasticidin sensitivity, Polybrene™ tolerance, and transduction efficiency for your cell line.

We recommend using the Invitrogen™ LentiArray™ CRISPR Positive and Negative Control Lentiviruses with GFP to optimize the transduction and antibiotic selection conditions for your cell line of interest. For more information, see the LentiArray™ CRISPR Control Lentivirus User Guide (MAN00015949), available for download at **thermofisher.com**.

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 lentiviruses to achieve the optimal degree of gene knockout.

  Note: The nature of your mammalian 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.
- 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 by 2–10-fold. Before performing any transductions, determine the Polybrene™ tolerance level for your target cells empirically. We recommend testing Polybrene™ within a concentration range of 2–8 µg/mL, then using the highest tolerated concentration (up to 8 µg/mL) in your transductions. If you observe toxicity or phenotypic changes, titrate down the Polybrene™ concentration or omit it altogether.
- Keep the volume of lentivirus to less than 30–50% of the total volume in the well. If toxicity is observed after transduction, decrease the virus volume (use a lower MOI) and/or omit Polybrene™.
- We have observed that centrifugation at 800 × g for 30–120 minutes at room temperature after the addition of lentivirus to the cells enhances viral infectivity.

- If you plan to use Blasticidin for selection, you must first determine the optimal antibiotic 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 antibiotic that is required for selection. A typical selection with Blasticidin takes 7–10 days.

Workflow overview

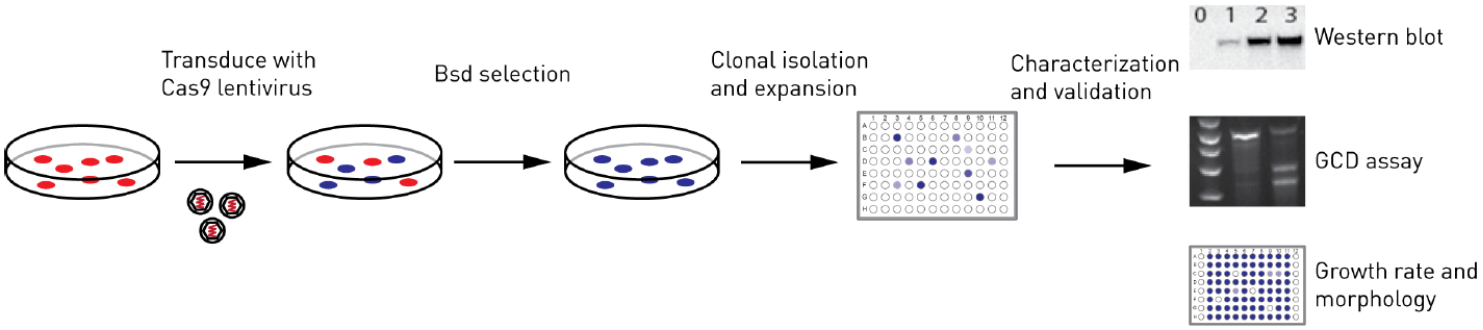

Fig. 1 Workflow for Cas9 stable cell line generation using the LentiArray™ Cas9 Lentivirus.

Methods

Blasticidin killing curve

We recommend performing a kill curve with each new cell type or Blasticidicn lot, or with changes to the cell culture conditions to determine the optimal concentration that is needed to eliminate non-transduced cells.

Day 1

Seed cells at the appropriate density in 2 mL/well of complete growth medium in a 6-well culture plate so that the culture is ~50% confluent on the day of antibiotic selection.

Day 2

Remove and replace the medium with 2 mL/well of growth medium containing varying concentrations of Blasticidin. We recommend testing selection with final Blasticidin concentrations of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 µg/mL.

Day 3–10

Change the selective medium every 3 days for up to 10 days, and examine cell viability every 2 days. Select the lowest antibiotic concentration that causes complete cell death after 7 days.

Day 11

After 10 days of selection, the percentage of cells that have survived from Blasticidin selection can be measured using crystal violet staining or PrestoBlue™ Cell Viability Reagent (Cat. No. A13261).

Transduce cells

The following procedure describes a general protocol for the transduction of adherent cells in a 6-well format.

IMPORTANT! Before starting, review "Procedural guidelines" on page 1.

Day 1

Seed the appropriate number of cells in complete growth medium into a 6-well plate to obtain ~80% confluence on the following day. Incubate the cells overnight at 37°C in a humidified 5% CO_2 incubator.

For example, seed 2×10^5 HT1080 cells in 2 mL of medium per well on a 6-well plate.

Day 2

1. Replace the spent medium in the wells with complete growth medium containing Polybrene™.

   Note: The optimal Polybrene™ amount varies with cell type and must be determined empirically (see "Transduction conditions" on page 1).
2. Remove the tube containing the LentiArray™ Cas9 Lentivirus particles and place in a 37°C water bath to thaw. When the tube contents are mostly thawed with only small ice crystals remaining, transfer the tube onto ice.
3. Before opening, centrifuge the lentivirus tube at low speef (maximum RCF at 200 × *g*) for 30 seconds to collect the content at the bottom of the tube.
4. Add the appropriate volume of Cas9 lentivirus particles in 1 mL final volume per well to achieve the desired MOI. We recommend maintaining one well of mock or uninfected cells in parallel to serve as a negative control for Blasticidin selection.
5. Gently swirl the plate to distribute the lentivirus dilutions evenly across each well, and incubate the cells at 37°C overnight in a humidified 5% CO_2 incubator.

Day 3

Remove the media containing lentivirus and replace it with 6.2 mL/well of complete culture medium.

Day 4

The following steps describe the optional Blasticidin selection of transduced cells, which takes place from Day 4 to Day 9+. A typical selection with Blasticidin takes 7–10 days. Blasticidin selection may not be necessary if you have used high MOI for transduction.

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

Day 9+

Continue selection with regular medium changes every 3–4 days until the cells in the negative control well are dead. At this point, proceed to clonal isolation and expansion.

Clonal isolation and expansion

Isolation of a clone that stably expresses Cas9 can be achieved by limiting dilution cloning (LDC) directly from the pool of stable transductants (i.e., Blasticidin-resistant cells).

1. Harvest the Blasticidin-resistant cells and serially dilute them to a final concentration of 5 cells/mL (0.5 cells/100 µL) into complete growth medium with Blasticidin.
2. Using a multi-channel pipettor, dispense 100 µL of the diluted cells into each well of a 96-well plate.
3. Incubate the cells at 37°C in a humidified 5% CO_2 incubator, and replace the spent medium with fresh growth medium containing Blasticidin every 3–4 days.
4. After 10–12 days of incubation, visually inspect the wells of the 96-well plate under a microscope to identify which wells show evidence of colonies that are derived from a single cell (i.e., monoclonal cells).
5. Transfer the monoclonal cells from each well identified in Step 4 on page 3 into separate wells of a 24-well plate in complete growth medium with Blasticidin. Incubate the cells at 37°C in a humidified 5% CO_2 incubator.
6. Scale up the volume of cells every 2–5 days by transferring each clonal population into the next larger plate or vessel (i.e., from 24-well plates to 6-well plates or T-25 flasks, and then to T-75 flasks).
7. Cryopreserve at least five vials of cells from each clone as a back-up, then characterize the isolated clones for cell morphology, growth kinetics, Cas9 protein expression, and gene editing efficiency to select the best clone for future studies.

Analyze cell morphology and cell growth rate

Examine the morphology of the selected clones by microscopy to ensure that there are no gross morphological changes from the parental cell population (e.g., granularity around the nucleus, cytoplasmic vacuolation, detachment from substrate).

Note: You may observe that cell morphology changes due to long period of culture during clonal isolation.

Determine the proliferation rate of the parental and Cas9 stable cells by seeding the cells in a 96-well plate and measuring the population-doubling time by various methods such as PrestoBlue™ Cell Viability Reagent (Cat. No. A13261), trypan blue staining, or the MTT assay (e.g. Vybrant™ MTT Cell Proliferation Assay Kit, Cat. No. V13154).

Verify Cas9 protein expression

We recommend that you verify Cas9 protein expression by Western blot analysis using antibodies against the Cas9 protein or the V5 epitope.

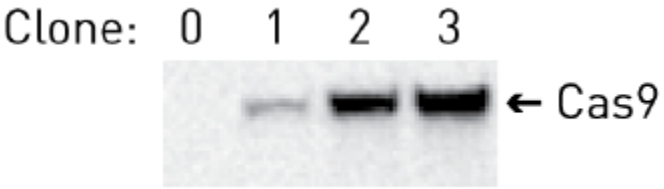

Fig. 2 Cas9 protein from HT1080 cells that were stably transduced with LentiArray™ Cas9 Lentivirus particles was detected by Western blot analysis using an anti-Cas9 antibody.

Verify gene editing efficiency

We recommend using the GeneArt™ Genomic Cleavage Detection Kit (Cat. No. A24372) to verify gene editing efficiency in the Cas9 stable cell lines with the LentiArray™ CRISPR Positive Control Lentivirus against HPRT (Cat. No. A32056). The genomic cleavage detection (GCD) assay provides a rapid method for evaluating the efficiency of indel formation following an editing experiment. Pick the clones that show the highest cleavage efficiency to use in your experiments. Note that the clone that shows the highest cleavage efficiency may not always be the clone with the highest Cas9 expression.

For more information and protocols, see the GeneArt™ Genomic Cleavage Detection Kit User Guide (MAN0009849), available for download at **thermofisher.com**.

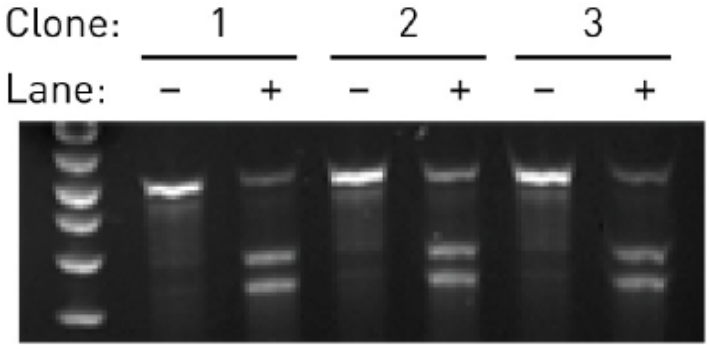

Fig. 3 Cas9-stable HT1080 cells were transduced with LentiArray™ CRISPR Negative and Positive Control Lentivirus expressing non-targeting (–) or human HPRT1 (+) gRNA sequences, respectively. The cells were harvested 2 days post-transduction, and analyzed using the GeneArt™ Genomic Cleavage Detection Kit. The transductions with the positive control gRNA (+) show ~80% cleavage efficiency in the GCD assay.

Related products

Product	Cat. No.
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
GeneArt™ Genomic Cleavage Detection Kit	A24372
Blasticidin S HCl (10 mg/mL)	A1113903

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.