

Invitrogen™ LentiArray™ Human CRISPR Library, Glycerol Stocks (96-well Plates)

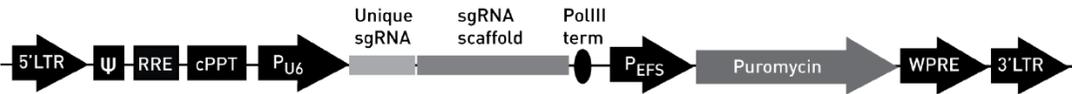
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Doc. Part No. 100044721 **Pub. No.** MAN0016074 **Rev.** B.0

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™ Human CRISPR Library, Glycerol Stocks consist of pre-defined gene sets for functional genomics screening in an arrayed format. Each library targets a subset of human genes and is provided as glycerol stocks of Stbl3™ cells in a 96-well plate format. There are up to 4 sequence-verified lentiviral gRNA constructs per gene, with cells in each well containing 1 distinct gRNA construct. 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, Glycerol (see Table 1, for details)
Content	Stbl3™ cells in LB medium with 10% glycerol in a 96-well plate format. Cells in each well of the 96-well plate contain 1 distinct gRNA construct.
Amount	50 µL LB culture per well
Lentiviral map	 <ul style="list-style-type: none"> gRNA expression is driven by a U6 promoter. Includes puromycin resistance gene to allow selection of transduced cells.
Plate layout	<ul style="list-style-type: none"> Refer to the associated PDF file for the plate map of the specific LentiArray™ Human CRISPR library and to the Excel files for gRNA target information. First and last columns of the plates are empty.
Storage	Store at -80°C. Avoid repeated freeze/thaw cycles, which will reduce the viability of the cells. All components are stable for at least six months after receipt when stored as directed.
Biosafety precaution	Follow Biosafety Level 1 (BL-1) guidelines when handling glycerol stocks of <i>E. coli</i> containing lentiviral plasmids. When handling purified lentiviral particles, strictly follow all published Biosafety Level 2 (BL-2) guidelines for the use of personal protection equipment and proper waste decontamination procedures.

Ordering information

Table 1 Invitrogen™ LentiArray™ Human CRISPR Library, Glycerol Stocks (96-well plates)

Cat. No.	Product
A32167	Invitrogen™ LentiArray™ Human Kinase CRISPR Library, Glycerol Stocks
A32168	Invitrogen™ LentiArray™ Human Phosphatase CRISPR Library, Glycerol Stocks
A32169	Invitrogen™ LentiArray™ Human Cancer Biology CRISPR Library, Glycerol Stocks
A32170	Invitrogen™ LentiArray™ Human Epigenetics CRISPR Library, Glycerol Stocks
A32171	Invitrogen™ LentiArray™ Human Ubiquitin CRISPR Library, Glycerol Stocks
A32172	Invitrogen™ LentiArray™ Human Cell Cycle CRISPR Library, Glycerol Stocks
A32173	Invitrogen™ LentiArray™ Human Membrane Trafficking CRISPR Library, Glycerol Stocks
A32174	Invitrogen™ LentiArray™ Human Transcription Factor CRISPR Library, Glycerol Stocks
A32175	Invitrogen™ LentiArray™ Human Nuclear Hormone Receptor CRISPR Library, Glycerol Stocks
A32176	Invitrogen™ LentiArray™ Human Apoptosis CRISPR Library, Glycerol Stocks
A32177	Invitrogen™ LentiArray™ Human Drug Transporter CRISPR Library, Glycerol Stocks
A32178	Invitrogen™ LentiArray™ Human Ion Channel CRISPR Library, Glycerol Stocks
A32179	Invitrogen™ LentiArray™ Human Cell Surface CRISPR Library, Glycerol Stocks
A32180	Invitrogen™ LentiArray™ Human Protease CRISPR Library, Glycerol Stocks
A32181	Invitrogen™ LentiArray™ Human Tumor Suppressor CRISPR Library, Glycerol Stocks
A32182	Invitrogen™ LentiArray™ Human DNA Damage Response CRISPR Library, Glycerol Stocks
A32183	Invitrogen™ LentiArray™ Human GPCR CRISPR Library, Glycerol Stocks
A32184	Invitrogen™ LentiArray™ Human Drugable CRISPR Library, Glycerol Stocks
A32185	Invitrogen™ LentiArray™ Human Whole Genome CRISPR Library, Glycerol Stocks

Methods

Culture cells from the glycerol stock

Each well of the 96-well plate contains 50 µL of Stbl3™ cells in LB medium with 10% glycerol and 100 µg/mL ampicillin. Follow the procedure below to culture the cells from glycerol stocks.

1. Thaw the LentiArray™ Human CRISPR Library, Glycerol plate at 37°C for 30–45 minutes, and record the barcode.
2. Before removing the sealing film, centrifuge the glycerol plate at low speed (200 × g) for 1 minute to collect the contents at the bottom of the wells.
3. Re-suspend any cells that may have settled at the bottom of the well.
4. Take a 10 µL of inoculum from each well of the 96-well plate containing the bacterial glycerol stock and transfer into the corresponding well of 96-deep-well culture plates containing 1.5 mL/well of Terrific Broth (TB) medium with 100 µg/mL carbenicillin.

Note: Make sure that the A1 position of all plates is aligned in the same orientation for correct construct transfer.

5. Re-seal the source plates and store at –80°C.

6. Seal each culture plate with air porous tape, label, and incubate at 37°C in a 225 rpm shaking incubator for 18–20 hours.

Note: Keep the incubation period as short as possible to minimize the chances of aberrant lentiviral plasmid recombination.

Purify lentiviral plasmids

Plasmid DNA for transfection into eukaryotic cells must be very clean and free from contamination with phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency.

Stbl3™ *E. coli* is wild type for endonuclease 1 (*endA1+*). When performing plasmid DNA isolation with commercially available kits, ensure that the lysis buffer (often called resuspension buffer) contains 10 mM EDTA to inactivate the endonuclease to avoid DNA nicking and vector degradation.

We recommend isolating plasmid DNA using the PureLink™ 96 HQ Mini Plasmid DNA Purification Kit (Cat. No. K210096). For detailed instructions, refer to the PureLink™ 96 HQ Mini Plasmid DNA Purification Kit User Guide (MAN0000400), available for download at thermofisher.com.

Quantify lentiviral plasmid DNA

We recommend quantifying the purified lentiviral plasmid DNA using the Quant-iT™ dsDNA Broad-Range Assay Kit (Cat. No. Q33130). The assay kit provides concentrated assay reagent, dilution buffer, and pre-diluted DNA standards. The assay is highly selective for double-stranded DNA over RNA, and in the range of 2–1000 ng, the fluorescence signal is linear with DNA. Common contaminants, such as salts, solvents, detergents, or protein are well tolerated in the assay.

For detailed instructions on how to use the Quant-iT™ dsDNA Broad-Range Assay Kit, refer to the Quant-iT™ dsDNA Broad-Range Assay Kit User Guide (MAN0002341), available for download at thermofisher.com.

Analyze lentiviral plasmid DNA by restriction digest

We recommend that you perform restriction analysis of the purified lentiviral plasmid DNA to confirm that no aberrant recombination events have taken place. Rearrangement of propagated lentiviral plasmid vectors can lead to low viral package.

Digest aliquots of the purified lentiviral plasmid DNA with *Apa*LI and run on 1% agarose gel. The expected band sizes are 1246 bp, 1975 bp, and 4329 bp. Additional or unexpected bands indicate aberrant recombination of the lentiviral vector.

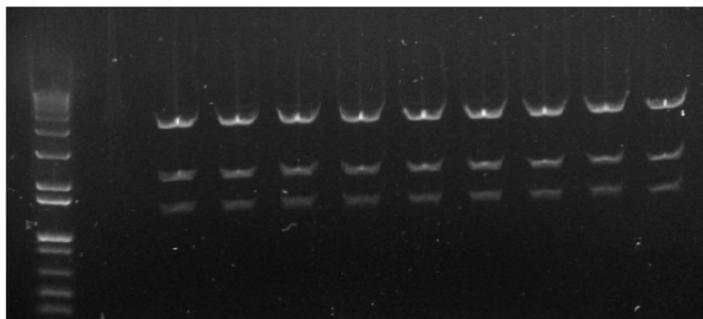


Figure 1 Representative gel image of the lentiviral plasmid DNA digested with *Apa*LI.

Downstream applications

You can use the purified lentiviral plasmid DNA for the production of lentiviral stock by co-transfecting it with ViraPower™ Packaging Mix into the 293FT cell line.

The ViraPower™ Packaging Mix (Cat. No. K497500) and the 293FT cells (Cat. No. R700–07) are available separately from Thermo Fisher Scientific. You can also purchase the ViraPower™ Packaging Mix as part of a ViraPower™ Lentiviral Support Kit (Cat. Nos. K497000, K498500), which also include Lipofectamine™ 2000 transfection reagent and a selection agent.

For more information on lentiviral production, refer to ViraPower™ Lentiviral Expression System User Guide (MAN0000273), available for download at thermofisher.com.

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	A32042
Invitrogen™ LentiArray™ Custom CRISPR Plate	A32045
GeneArt™ Genomic Cleavage Detection Kit	A24372
PureLink™ 96 HQ Mini Plasmid DNA Purification Kit	K210096
Quant-iT™ dsDNA Broad-Range Assay Kit	Q33130
ViraPower™ Bsd Lentiviral Support Kit	K497000
ViraPower™ Zeo Lentiviral Support Kit	K498500
ViraPower™ Lentiviral Packaging Mix	K497500
293FT Cell Line	R70007



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