Lenti-Labeler™ Cell Labeling System (Plasmids & Pre-Packaged Lentivirus)

Cat# LL1xxPA/VA-1, LL2xxPA/VA-1, LL3xxPA/VA-1, LL4xxPA/VA-1

User Manual

Please refer to storage conditions in manual

Version 2
7/31/2018

Contents

Product Description

SBI's Lenti-Labeler™ Cell Labeling System is comprised of a proven set of reagents for labeling cells *in vitro* for use in downstream *in vivo* applications such as tracking/localization or visualizing effects of compounds on cell biology *in vivo*. They come in two different formats, 1) a fully propagatable, sequence-verified lentiviral plasmid DNA and 2) pre-packaged lentivirus ready to transduce your target cells of interest. Each vector contains your choice of fluorescent or luminescent markers (GFP, RFP, BFP or luciferase) and a mammalian selection marker (Puromycin or Blasticidin) for *in vitro* selection of cells. In addition, they come in two promoter formats (CMV or EF1a) for additional level of flexibility when labeling cells with differing levels of promoter activity.

List of Components (plasmid DNA)

Item	Catalog #	Amount
Lenti-Labeler™ plasmid DNA	LLXXX**PA**-1	10 µg (0.5 µg/ul)

List of Components (Packaged virus)

Item	Catalog #	Amount
Lenti-Labeler™ Packaged Virus	LLXXX**VA**-1	>2x10^6 IFUs (2 x 25 µl)

Note: For full list of Lenti-Labeler products, please see the **Appendix** section of this user manual.

Storage

1) Plasmid DNA is shipped on blue ice and should be stored at +4°C or -20°C upon receipt. Properly stored plasmid DNA is stable for 1 year from the date received.

2) Packaged virus is shipped on dry ice and should be stored at -80°C upon receipt. Properly stored virus is stable for 1 year from the date received.

General Information

Plasmid DNA:

Lenti-Labeler plasmids are provided in TE (pH 8.0) and each vial contains 10 µg of plasmid DNA at 0.5 µg/ul. It is imperative to propagate the plasmid to generate enough material for virus packaging. For transformation of the plasmid, we suggest using Stbl2 (Cat #10268019) or Stbl3 (Cat #C737303) competent cells from ThermoFisher, and growing the bacteria at +30°C in liquid culture to prevent unwanted recombination. For scale-up of plasmid required for virus packaging, we suggest endotoxin-free plasmid kits such as Qiagen EndoFree Plasmid Maxi Kit (Cat # 12362) or Macherey-Nagel NucleoBond Xtra Maxi EF Kit (Cat # 740424) for highest plasmid yields.

For virus packaging, the Lenti-Labeler plasmids are compatible with most 2nd and 3rd generation packaging mixes. For optimal titers, we recommend SBI's pPACKH1 packaging mix (Cat #LV500A-1) – presented data in the manual is based on generating lentivirus using the pPACKH1 system and concentrating the virus using SBI's PEG-It virus concentration reagent (Cat #LV810A-1).

Pre-Packaged Lentivirus Information:

Each sales unit of the pre-packaged lentivirus comes with 2 tubes, each containing 25 µl of virus. Total infectious units provided is >2x10^6 IFUs. The product analysis certificate (PAC) provided with the sales unit contains information on the actual virus concentration and IFUs provided. As a reference point, one sales unit will be enough to infect ~100,000 cells at an MOI of 20.

For infection of target cells, we recommend the use of SBI's TransDux MAX Transduction reagent (Cat #LV860A-1) containing SBI's TransDux and MAX Enhancer Reagent. It provides a level of transduction efficiency superior to that of Polybrene for most cell types.

Luciferase constructs:

Please note Lenti-Labeler constructs that contain Luciferase comes in two different configurations:

1) LL1xx and LL2xx vectors and plasmids: Firefly luciferase (FFLuc): peak emission wavelength – 560nm

2) LL3xx and LL4xx vectors and plasmids: Red-shifted luciferase (rFLuc): peak emission wavelength – 610nm

Protocol

Required Materials

1. Your Lenti-Labeler lentiviral construct (transformed and mini/midi/maxiprepped plasmid DNA, see "General Information" section for additional information)
2. HEK 293T/FT/TN cells and suitable culture media for growing these cells.
3. Tabletop low speed centrifuge (e.g. Beckman GS-6R).
4. 100mm or 150mm plate for cell culture.

Procedure

Lenti-Labeler Pseudovirus Production (100mm or 150mm plates)

The procedure below is for pseudoviral production from Lenti-Labeler plasmid DNA for 100mm plates. For production in 150mm plates, please see the red text within the instructions below. Please use equivalent surface-area adjusted volumes if you use flasks (e.g. T75, T150)

Day 1

1. Plate 3x10^6 (7-8 x10^6) 293T/FT cells in a fresh 100mm (150mm) plate in 10 mL (20 mL) of antibiotic-free DMEM medium (DMEM+FBS+Glu).

Day 2

1. The cells should be 50 to 70% confluent at day of transfection
2. Add 0.8 mL (1.6 mL) of serum-free DMEM media into an Eppendorf tube. Add 2 µg (4 µg) of transfer plasmid and 20 µL (45 µL) pPACKH1-plasmid mix to the tube and mix by pipetting.
3. Add 24 µL (55 µL) of PureFection* reagent to the tube, and vortex for 10 sec.
4. Incubate mixture at room temperature for a minimum of 15 min.
5. Add mixture drop-wise to the plate, and swirl to disperse evenly throughout.
6. Incubate plates in 37^{o}C tissue culture incubator overnight.

***PureFection reagent is a cationic-based plasmid transfection reagent available from SBI (Cat #LV750A-1). It may be substituted with other transfection reagents (e.g. Lipofectamine 2000/PLUS reagent, 3000, etc.) with similar results**

Day 3

1. Replace transfection media with fresh complete growth media w/antibiotics

Day 4 and 5

1. Collect the medium (which now contains pseudoviral particles) into 50-mL sterile, capped conical centrifuge tubes.
2. Centrifuge at 3000 x g for 15 minutes at room temperature to pellet cell debris.
3. Transfer the viral supernatant into new fresh tubes.
4. Add PEG-it at a final volume of 1:5. Example: 2 mL (5 mL) of PEG-it should be added to 10 mL (20 mL) of viral supernatant, invert 10 times to mix well. Keep everything cold from this point onwards. Store virus supernatant containing PEG-it at 4^{o}C overnight, or up to 3 days.

Day 6

1. Harvest PEG-it precipitated virus by centrifuging at 4^{o}C at 1500 x g for 30 min. Aspirate off the supernatant and resuspend the milky-white pellet in a small volume (1/100 to 1/1000 of original volume) using cold sterile PBS or cold DMEM.
2. Freeze virus aliquots at -80^{o}C.

Transduction of Target Cells (24-well transduction)

Day 1

1. Plate 50,000 cells per well in a 24 well plate in culture medium.

Day 2

1. Cells should be between 50 to 70% confluent.

2. Aspirate medium from cells.

3. Combine TransDux™ and TransDux MAX Enhancer with culture medium to a final concentration of 1x. [Example: Add 2.5 µL of TransDux™ and 100 µL of MAX Enhancer to 400 µL culture medium and then transfer to each well].

4. Add virus to each well at different MOIs or different volumes, depending on experimental aims. We recommend an initial titration of virus to achieve desired % of transduction efficiency (if using fluorescent Lenti-Labeler plasmid). In general, a 60% transduction efficiency of cells (as measured by fluorescence) in multiple field of views equates to a copy number of 1 (single integration event)

5. Incubate at 37°C for 72hrs.

Day 5

6. Look at the cells for reporter expression if the viral construct has a reporter like GFP and/or begin appropriate antibiotic selection to establish a stable cell line.

OPTIONAL – Virus Titering

1. Aspirate off medium. Wash each well with PBS (at this point the plate can be frozen at -80°C).

2. Add 100µl of Lysis Buffer (SBI's UltraRapid Global Titering Kit**) to each well.

3. Titer virus according to protocol given in the UltraRapid Global Titering Kit (SBI Cat# LV961A-1).

****UltraRapid Global Titering Kit (Cat #LV961A-1) is a qPCR-based system that quantifies level of pseudoviral integration events in target cells based on presence of a specific sequence in the Lenti-Labeler plasmid. Use of other titering approaches (e.g. FACS or antibiotic selection) may be used to ascertain functional titer of virus**

Representative Data

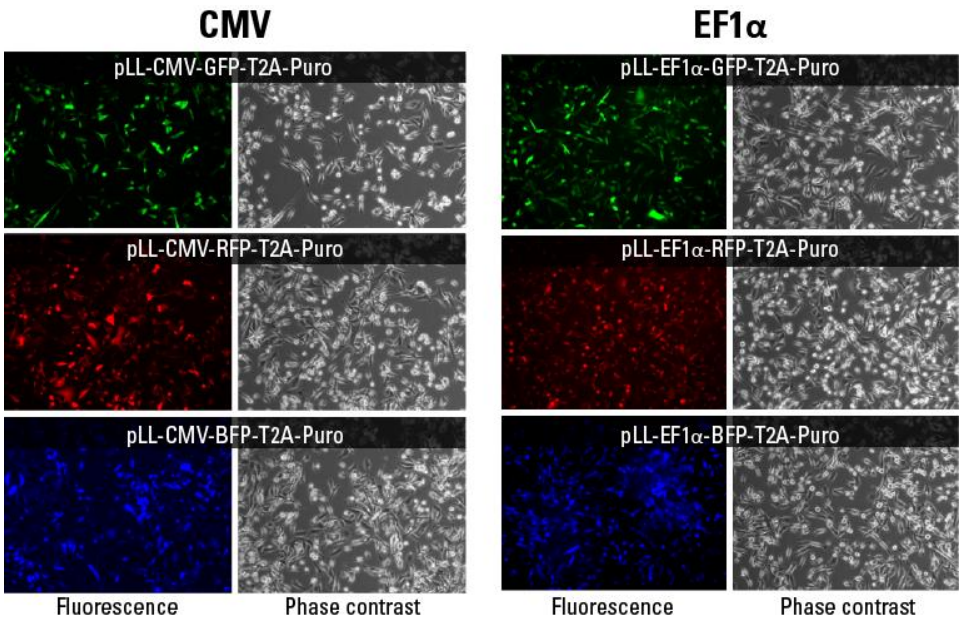

Figure 1. SBI Lenti-Labeler constructs reliably and efficiently label cells. Comparison of the number of fluorescently-labeled cells to the total number of cells seen in the corresponding phase contrast images reveals the high labeling efficiency of SBI's Lenti-Labeler constructs. HT1080 cells were infected using lentivirus generated from Lenti-Labeler constructs and virus supernatant (GFP and RFP) or concentrated virus (BFP) was added to cells. Cells were imaged 72 hrs after infection.

Next Steps and Related Products

Application	Related Products	Website links
Other Lentiviral Production Products		
Larger-scale lentiviral production	LentiSuite Basic and Deluxe Kit:	https://www.systembio.com/lentiviral-technology/delivery-systems/lentisuite/overview
Lentiviral production	HEK293TN Producer Cell Line	https://www.systembio.com/lentiviral-technology/delivery-systems/293tn-producer-cell-line/overview
Viral Titering	Global Ultra-Rapid Titering Kit	https://www.systembio.com/lentiviral-technology/delivery-systems/ultrarapid/overview
Lentiviral Production Controls	Positive Control Transduction Viruses	https://www.systembio.com/lentiviral-technology/delivery-systems/positive-transduction-controls/overview
Non-integrating Virus Packaging	Non-integrating Lentiviral System (pPACK-ID)	https://www.systembio.com/lentiviral-technology/delivery-systems/non-integrating
SBI Lentivectors		
Gene Delivery & Expression	SBI's 3rd Generation Lentivector Collection	https://www.systembio.com/lentiviral-technology/expression-vectors

Appendix

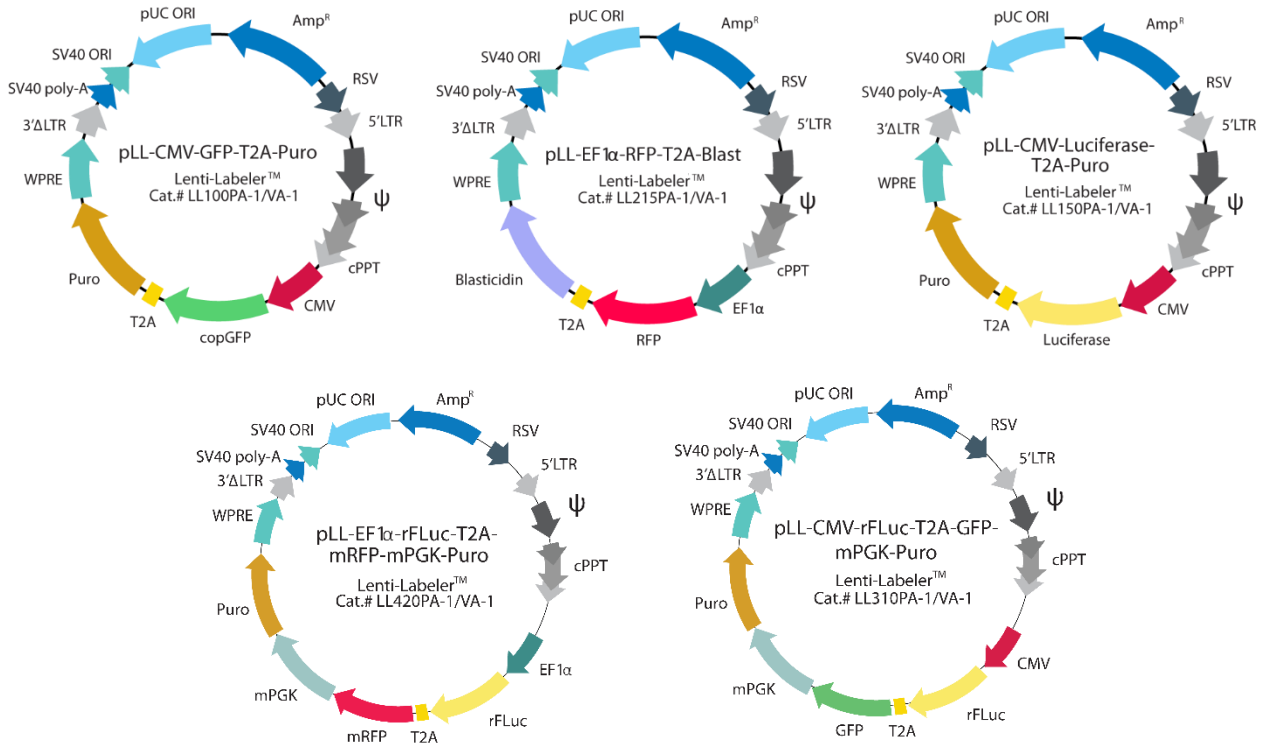

Figure 2. Representative vector maps for selected Lenti-Labeler™ lentiviral vectors, showing major elements present in the vectors

Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: **http://www.systembio.com**

For additional information or technical assistance, please call or email us at:

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Licensing and Warranty Statement

Limited Use License

Use of the Lenti-Labeler™ Cell Labeling System (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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