Lentiviral Human cDNA ORF Expression System

User Manual

Revision 201604.01

RESEARCH USE ONLY.

Not for use in diagnostic procedures

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CONTENTS AND STORAGE

100ul of lentivirus > 10^9 GC/ml or >10^8 IU/ml enough for 20 10-cm cell culture plates

Please Note: The titer of lentivirus is very sensitive to the size of viral genome. Offered titer is based on that the viral genome, insert between two LTRs, is less than 5.1kb. And the gene of interest is less than 1.5kb. Every kb increases of the viral genome, the titer will be decreased by 10 times. Genome copy (GC) is measured by real-time qPCR of lentivirus; infection unit (IU) is measured by fluorescent microcopy of GFP expressing cells.

Storage buffer: PBS

DO NOT FREEZE AND THAW REPEATEDLY.

Introduction

Lentiviral vectors are valuable tools in delivering gene *in vitro* and *in vivo*. Compared to adenovirus, lentivirus has the ability to integrate foreign genes into the genome of dividing and non-dividing cells. The lentiviral cDNA ORF expression system features Myc and Flag tagged ORF expression under CMV promoter with puromycin resistance as a selection marker.

Other tags including GFP and other markers are available upon request.

Lentiviral particles can be directly applied for in vivo experiments or in vitro on cells. This Manual will outline the general process for virus titering, in vivo application on animal models and in vitro experiments on cell lines .

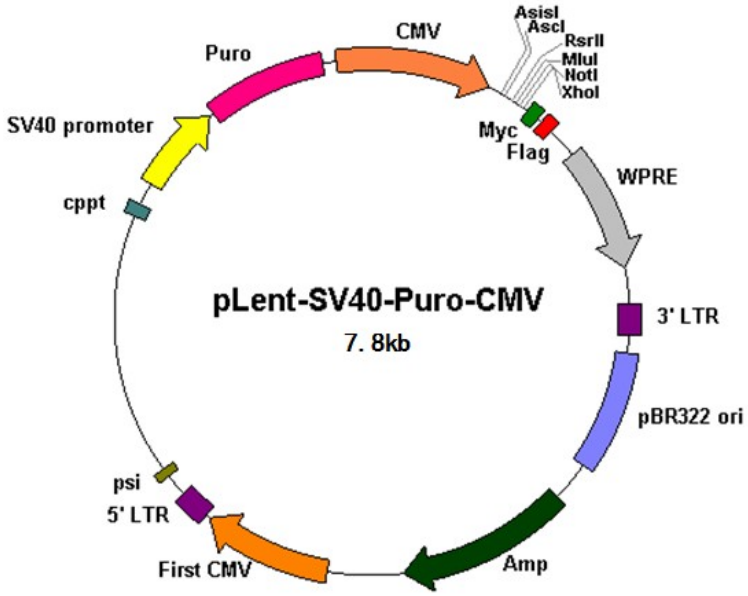
Lentiviral contruct

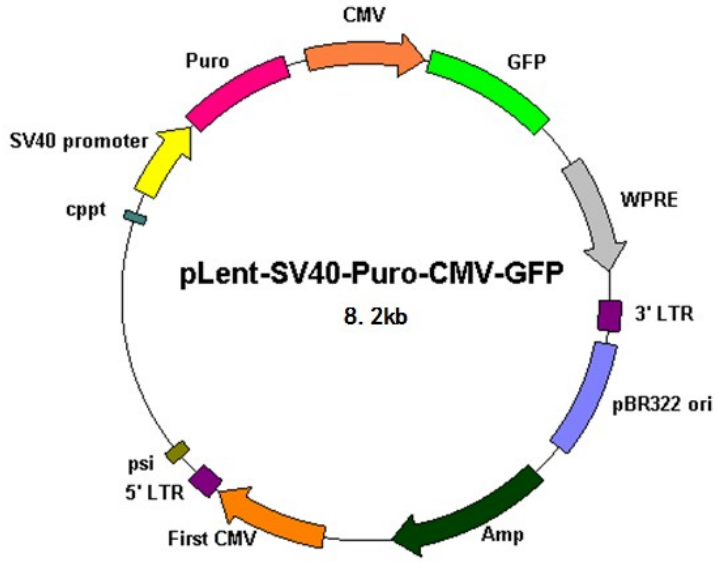
Vigene Biosciences lentiviral ORF Expression System allows for efficient gene expression both in vivo and in vitro.

Vigene Biosciences lentiviral vectors have a large cargo capacity to accommodate an insert of up to 6 kb. However, ORFs larger than 4kb will dramatically decrease the packaging efficiency.

Customers can choose three different promoters, the CMV, EF1a and PGK promoter, to drive the ORF expression. For detection and purification, the expressed proteins have different tags at either N' or C' terminus. Further two lentiviral vectors were provided for stable transfection with either GFP or puromycin selection. The following are two most popular lentiviral vectors that Vigene offers.

Please note: customers can choose from either vector for expressing human cDNA without additional cost.





Lentiviral packaging overview

All Vigene's lentiviral vectors are the third generation of lentiviral vectors with 3'LTR sefl-inactivating (3' LTR sin), and 5' CVM-LTR hybrid 5' LTR. They are packaged with the third generation packaging system.

The viral titer at this step is usually 10^6-10^7 TU/ml. Dependent on the insert size, large inserts could have significant lower titer.

RT- qPCR protocol for titering lenti-viral particles

We utilize a RT qPCR based method to provide robust lentivirus titering analysis. The general principle is utilize the lentiviral reverse transcriptase activity of *pol* gene on lentivirus to synthesize cDNA then quantify the cDNA by using real-time PCR. Below is a outline of major steps.

1. Preparations: Make the lysis buffer / RNase inhibitor mix and qRT-PCR reaction mix.

2. Lysis of the viral samples: Addition of lysis buffer to the viral supernatant to release reverse transcriptase.

3. qRT-PCR: Addition of qRT-PCR reaction mix to the lysed samples, followed by the reverse transcription and quantitative PCR reaction.

4. Analysis: Calculation of the reverse trancritpase activity of the samples based on the obtained standard curve.

Primer sequences for real-time PCR

FWD: 5'TCCTGCTCAACTTCCTGTCGAG3'

REV: 5'CACAGGTCAAACCTCCTAGGAATG3'

Real-time PCR protocol

Detection format: Sybr Green

Reaction volume: 20 µl

Program

Step 1: Reverse transcription (rt): 42 °C for 20 min.

Step 2: Pre-incubation: 95 °C for 5 min.

Step 3: Amplification: 40 cycles of 95 °C for 5 sec

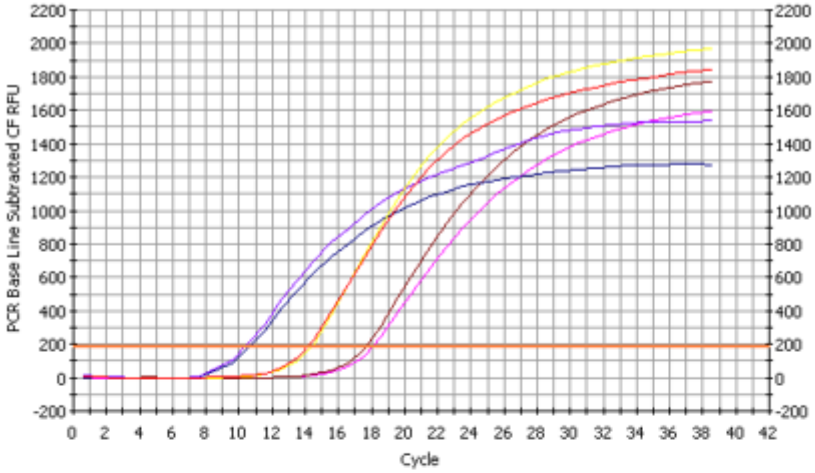
60 °C for 5 sec + detection

72 °C for 15 sec

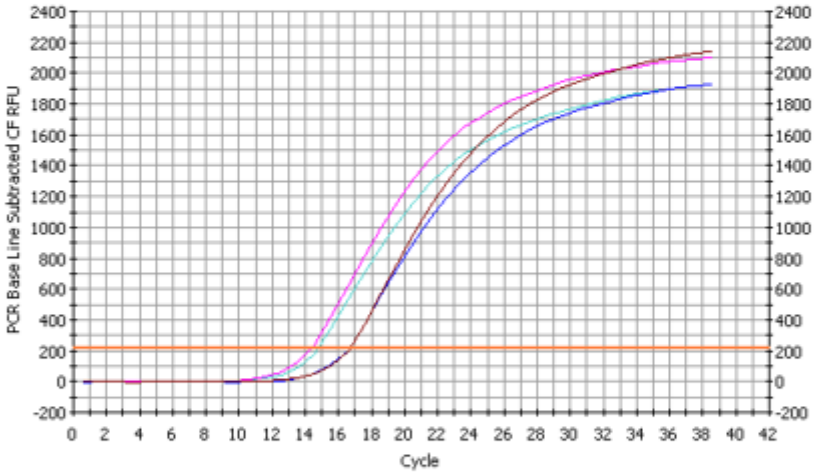
Step 4: Melting curve

Step 5: Cooling

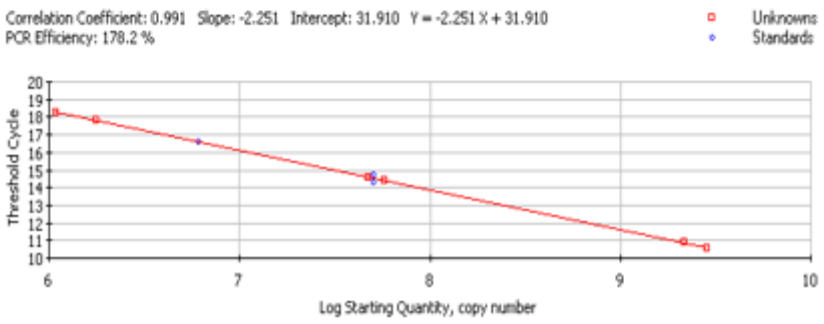
PCR Amp/Cycle Graph for Lentivirus



PCR Amp/Cycle Graph for Standard



Standard Curve Graph for Lentivirus



GFP lenti-virus quantified by Q-PCR and Transduction in HEK293 cells

Standard	Copy Number GC/ML	Samples	Copy Number GC/ML	Infection in HEK293 IU/ML
Standard I	$5.0 X10^7$	Virus containing medium	$1.4X10^7$	$2.3X10^6$
Standard II	$6.0 X10^6$	Virus Purified	$2.5X10^{10}$	$4.6X10^9$
		Virus purified 100x dilution	$5.5X10^8$	

Estimate the viral titer using the following formulation.
Viral titer (TU/ml) = number of positive cells x10x dilution.

Fluorescent microscopy protocol for titering lenti-viral particles

This protocol is only for lentiviral vectors expression of fluorescent proteins. It determines the transduction unit (TU/ml) of lenti-viral particles based on fluorescent protein expression in HEK293T cells.

Day 1.Seed HEK293T 1 - 4 x 104 cells/ well in a 96-well plate.
Day 2. Serial dilute the viral particles to 1:100, 1:1000, $1:10^4$, and $1:10^5$ in 500ul final volume in culture medium. 100ul of the viral particles mixture should be added to each well with at least three replicates.

Day 6.Determine the titer of lenti-viral particles.
Count the fluorescent positive or GFP positive cells using fluorescent microscopy. Select the dilution which gives around 50 to 200 positive cells/well. Count the triplicates and average the number of positive cells.

Estimate the viral titer using the following formulation.
Viral titer (TU/ml) = number of positive cells x10x dilution.

Cell Transduction Protocol for Making Stable Cell Lines

*Adpated from **http://www.addgene.org/tools/protocols/plko/#E**

This protocol is for the stable cell line selection based on puromycin resistance.

Day 1:

1. Plate target cells and incubate at 37°C, 5% CO2 overnight.

Day 2:

2. Target cells should be approximately 70% confluent. Change to fresh culture media containing 8 µg/mL polybrene. Polybrene increases the efficiency of viral infection. However, polybrene is toxic to some cell lines. In these cell lines, substitute protamine sulfate for polybrene.
3. Add lentiviral particle solution from step E. For a 6 cm target plate, add between 0.05-1 mL virus (add ≥0.5 mL for a high MOI, and ≤0.1 mL for a low MOI). Scale the amount of virus added depending on the size of your target plate.
   MOI (multiplicity of infection) refers to the number of infecting viral particles per cell. We recommend that you test a range of MOIs to determine the optimal MOI for infection and gene expression in your target cell line.
4. Incubate cells at 37°C, 5% CO2 overnight.

Day 3:

5. Change to fresh media 24 hours after infection.
   If viral toxicity is observed in your cell line, you may decrease the infection time to between 4 – 20 hours. Remove the virus-containing media and replace with fresh media. Do not add puromycin until at least 24 hours after infection to allow for sufficient expression of the puromycin resistance gene.
6. To select for infected cells, add puromycin to the media at the concentration determined in step E.2.

   We recommend that you maintain one uninfected plate of cells in parallel. This plate will serve as a positive control for the puromycin selection.

Days 4+:

7. Change to fresh puromycin-containing media as needed every few days.
8. Assay infected cells. you should wait until harvesting your cells. However, you should optimize the time based on your cell line and assay.

In vivo Injection Protocols for Lentiviral Particles

Lentivirus-based gene manipulations *in vivo*, offers a number of experimental advantages for cellular neuroscience, including the large transfer capacity of the vector and the lack of any cellular or humoral response associated with injection of the virus. Stereotaxic delivery of lentiviruses to specific tissues provides an alternative to traditional mouse genetics with high spatiotemporal control over the genetic manipulation and a short time from experimental design to data collection and analysis. The caveat is that the diffusion of lentiviral particles are rather limited. The following protocols can be used as references .

- Lentiviral vectors perivitelline injection into embryos
- Stereotactic injections of lentiviral particles into brains
- Intravenous injection into neonatal mouse

Frequently Asked Questions

Is there any safety issue with this pLenti vector?
All ViGene's lentiviral vectors are the third generation of lentiviral vectors with 3'LTR sefl-inactivating (3' LTR sin), and 5' CVM-LTR hybrid 5' LTR. It is the safest lenti-viral vector. ViGene recommended customers to contact the biosafety office at your institution prior to use of the lentiviral vector for permission and follow institution-specific instructions. BL2/(+) conditions should be used at all times when handling lentivirus. All disinfection steps should be performed using 10% bleach.
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What cell line should be used in order to produce lentivirus?
HEK293T cells are commonly used to produce lentivirus.

Can I use the pLenti vector for stable selection in mammalian cells?
You may choose pLent-GFP-CMV or pLent-puro-CMV for stable transfection. Puromycin or GFP will facilitate the selection process.

What is the size limit for the ORF that is to be cloned into the pLenti vector?
In general, lentiviral vectors have the capacity to accommodate an insert of 8 kb. However, ORFs larger than 4kb will dramatically decrease the packaging efficiency.