

pLenti Destination Vectors Application Guide

Table of Contents

Package Contents and Related Products _____	2
Related Optional Reagents _____	2
Related OriGene Products _____	2
Vector Information _____	3
Experimental Protocols _____	4
Protocol for Viral Packaging _____	4
Protocol for Transduction _____	5
Protocol for Titering Lenti-viral Particles _____	6
Frequently Asked Questions _____	6

Package Contents and Related Products

The following components are included:

- One (1) vial containing the cDNA clone as 10 µg lyophilized plasmid DNA*. Reconstitute the DNA in 100 µl of dH₂O; concentration is 100ng/µl.
- 100 picomoles of forward (V2) and reverse (LR50) DNA vector sequencing primers; dried onto the bottom of screw cap tubes. Reconstitute the primer in 10 µl of H₂O; concentration is 10 µM.
- Certificate of Analysis
- Application Guide

* OriGene plasmids are purified using ion-exchange columns for high-yield, low endotoxin preparations (PowerPrep® HP Midiprep Kit www.origene.com/other/Plasmid_Purification.)

The cDNA clone is shipped at room temperature, but should be kept at -20° C for long-term storage. If properly stored, clones are guaranteed to be stable for 12 months.

Related Optional Reagents

Restriction enzymes and buffers

ASIS I, MluI and RsrII from Fermentas

Nuclease free water

T4 DNA ligase and buffer

Competent *E. coli* cells (OriGene CC100001)

LB agar plates with 50-100 µg/ml Chloramphenicol.

LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl. Adjust pH to 7.0 with 1 N NaOH)

DNA purification reagents

Related OriGene Products

RapidShuttling Kit	http://www.origene.com/cdna/rapid-shuttling-kit
Lenti-packaging kit	http://www.origene.com/shRNA/Lenti_Packaging_Kits.aspx
TrueClone™ FL cDNA clones	http://www.origene.com/cdna
HuSH™ shRNA Plasmids	http://www.origene.com/rna
Antibodies	http://www.origene.com/antibody
Proteins from HEK293	http://www.origene.com/protein
Transfection Reagents	http://www.origene.com/cdna/transfection.msp
Anti-DDK Antibody	http://www.origene.com/antibody/4C5-AntiDDK
Anti-fluorescence protein Antibody	http://www.origene.com/Antibody/AntiTagAntibody.aspx

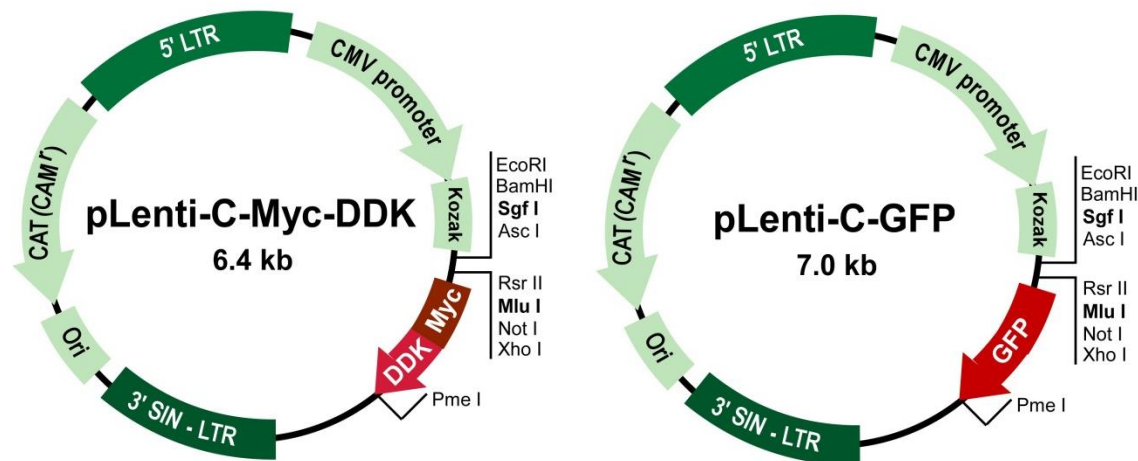
Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The plasmids may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund.

Vector Information

Eight pLenti vectors are offered by OriGene. Please see the details of the pLenti vectors at: <http://www.origene.com/cdna/TrueORF/DestinationVector.aspx> . One is tagged with C-terminal MYC/DDK tag (Cat# PS100064) for easy antibody detection and purification. OriGene recommends 4C5-AntiDDK high affinity mAb (Cat #TA50011) for this work. The other form is tagged with either GFP or RFP which can be visualized under a fluorescence microscope and also be detected by Western blotting with anti-GFP or anti-RFP specific antibody (see details at <http://www.origene.com/Antibody/AntiTagAntibody.aspx>).

The fusion protein is under a CMV promoter for strong constitutive expression or EF1 promoter. The ORF insert can also be easily shuttled into over 70 destination vectors to create different tagging options via a simple “cut-and-ligate” mechanism/ OriGene offers the RapidShuttling Kits specifically for this use (origene.com/rapid-shuttling-kit). SV40 ori allows for replication in mammalian cells, the chloramphenicol resistant gene confers the selection of the plasmid in *E. coli*. Both 5' and 3' truncated LTR can be used for viral RNA transcription and packaging of viral particles.



Multiple cloning site of pLenti-C-Myc-DDK

pLenti-C-Myc-DDK

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                                     Kozac
                                     Consensus
          EcoR I      BamH I      RBS      Sgf I      Asc I
CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCT

          Rsr II      Mlu I      Not I      Xho I      Myc.Tag
CAAGCTTAACTAGCTAGCGGACCG  ACG CGT  ACG CGG CCG CTC GAG CAG AAA CTC ATC TCA GAA GAG
                          T  R    T  R  P  L  E  Q  K  L  I  S  E  E
                                     DDK.Tag      Pme I
GAT CTG GCA GCA AAT GAT ATC CTG GAT TAC AAG GAT GAC GAC GAT AAG  GTT TAA ACGGCCGGCC
D  L  A  A  N  D  I  L  D  Y  K  D  D  D  D  K  V  Stop

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Multiple cloning site of pLenti-C-mGFP

pLenti-C-mGFP

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                                     Kozac
                                     Consensus
          EcoR I      BamH I      RBS      Sgf I      Asc I
CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCT

          Rsr II      Mlu I      Not I      Xho I      mGFP Tag
CAAGCTTAACTAGTTAGCGGACCG  ACG CGT  ACG CGG CCG CTC GAG ATG AGC GGG GGC --- --- ---
                          T  R    T  R  P  L  E  M  S  G  G  -  -  -
                                     Pme I
--- --- GGA CTC AGA  GTT TAA ACGGCCGGCCGGCCGG
-  -  -  G  L  R  V  Stop

```

Experimental Protocols

Protocol for Viral Packaging

OriGene's pLenti-C-Myc-DDK and pLenti-C-mGFP are both the 3rd generation of lentiviral vectors. For the commercial packaging vectors, we recommend OriGene's Lenti-vpak packaging kit, cat#,TR30022, http://www.origene.com/shRNA/Lenti_Packaging_Kits.aspx.

Day 1. Plate 2.5 x 10⁶ of 293T cells on a 10cm dish and incubate at 37°C overnight.

Day 2. Transfection,

- 1) In a labeled ependorf tube (vial 1), mix the following DNA with 500ul Opti-MEM
 - a. 5 ug of pLenti-shRNA construct or
5 ug of pLenti-ORF expression construct
 - b. 6 ug of packaging plasmids
- 2) In a separate tube (vial 2), mix 44ul of MegaTran transfection reagent with 500ul Opti-MEM.
- 3) Transfer the DNA solution from vial 1 into vial 2 containing MegaTran. Vortex it and incubate 15-30 min at room temperature.
- 4) Add the mixture of DNA and MegaTran directly to the 10cm dish of 293T cells.

Day 3. After 12-18 hrs incubation, change the culture medium.

Day 4. Harvest the first batch of viral supernatant from the culture and store it at 4oC. Add fresh culture medium to the cell culture.

Day 5. Harvest the second batch of viral supernatant then combine it with the first batch. Spin 3000rpm/min and filter through a 0.45 micron filter to remove cellular debris.

The viral titer at this step is usually 10^6 - 10^7 TU/ml**. The viral supernatant is now ready for the majority of transduction applications. If necessary, further concentration can be applied.

** Large ORF inserts will decrease the viral titer.

Protocol for Transduction

1. Day 1, plate target cells in three 10 cm plates at a density that will produce approximately 60% confluency in 24 hours. Note: other size formats can also be used depending on the nature of your experiment. Adjust the reagent amount accordingly.
2. Day 2, Remove the growth media from the plates prepared the day before. To plate 1, add 4.5 mL of fresh growth medium and 0.5 mL of Lentiviral particles; To plate 2, add 4.0 mL of growth medium and 1 mL of Lentiviral particles; To plate 3, add 2.5 mL of growth medium and 2.5 mL of Lentiviral particles (for a low titer viral preparation, the amount of virus added can be increased to 5 mL). Mix the solution by gentle swirling.
3. Add 5 μ l polybrene (1,000x, 8 mg/mL) to each plate. Mix by gentle swirling.
4. Incubate the cells at 37 °C with 5% CO₂ for 4 hours. Remove the transduction medium and add 10 mL of fresh growth medium. Incubate the cells for three more days.

The transduced cells are ready for downstream analyses such as RNA and protein detection.

Protocol for Titering Lenti-viral Particles

This protocol is based on the GFP or Myc-DDK tag fusion protein expression to determine the transduction unit (TU/ml) of lenti-viral particles in HEK293T cells.

Day 1. Seed HEK293T $1 - 4 \times 10^4$ cells/ well in a 96-well plate.

Day 2. Carry out transduction according to the transduction protocol with series dilutions of viral particles. The viral particles should be diluted 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.

Perform immo-fluorescent staining with DDK antibody (4C5, <http://www.origene.com/Antibody/AntiTagAntibody.aspx>) if pLenti-c-Myc-DDK used.

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.

Frequently Asked Questions

Is there any safety issue with this pLenti vector?

Answer: The pLenti vector is a third generation lentiviral vector and it is the safest lenti-viral vector because both LTRs are truncated. Please contact the biosafety office at your institution prior to use of the pLenti vector for permission and for further institution-specific instructions. BL2/(+) conditions should be used at all times when handling lentivirus. All decontamination steps should be performed using 70% ethanol/1% SDS. Gloves should be worn at all times when handling lentiviral preparations, transfected cells or the combined transfection reagent and lentiviral DNA.

What is unique about the 3rd generation of Lentiviral vectors?

Answer: The 3rd generation lentiviral vectors are safer than the 2nd generation vectors. The 3rd generation packaging systems express gag and pol from one packaging vector

and rev from another. The 3rd generation packaging systems DO NOT express tat (Trans-Activator of Transcription).

What cell line should be used in order to produce lentivirus?

Answer: HEK293T cells are commonly used to produce lentivirus. The HEK293T cell line for producing lentiviral particles can be obtained from ATCC (www.atcc.org).

How do I propagate the pLenti vector in *E. coli*?

Answer: The lenti-viral vector can be amplified using high-efficiency, competent *E. coli* cells ($\geq 1 \times 10^8$ CFU/ μ g DNA) following the manufacturer's transformation protocol. Plate the transformants on LB-agar plates supplemented with 34 μ g/ml chloramphenicol.

Can I use the pLenti vector for stable selection in mammalian cells?

Answer: Only a subset of the pLenti vectors have mammalian selectable markers and those without a mammalian selection marker can not be used for mammalian selection. You can make stable cell lines using the pLenti-C-Myc-DDK-IRES-Puro vector. You might also be able to get stable cells by GFP sorting using the pLenti-C-Myc-DDK-IRES-GFP vector.

How do I clone an insert into the pLenti vector?

Answer: The multiple-cloning site of the pLenti vector is compatible with OriGene's PrecisionShuttling system, a simple cut-and-ligation process. Please refer to the corresponding protocols in the TrueORF application guide.

What is the size limit for the ORF that is to be cloned into the pLenti vector?

Answer: In general, lentiviral vectors have the capacity to accommodate an insert of 9 kb. However, ORFs larger than 4kb will dramatically decrease the packaging efficiency.

Can pLenti vectors be used in direct transfections as opposed to making virus?

Answer: OriGene's pLenti vectors can also be used in transient transfections to achieve expression of the transgene. This usually involves lower levels of protein production due to diminished transfection efficiency.

What is the difference between a lentivirus and a retrovirus?

Answer: Lenti viruses are a subtype of retrovirus. The main difference between lentiviruses and standard retroviruses from an experimental standpoint is lentiviruses are capable of infecting both non-dividing and actively dividing cell types whereas standard retroviruses can only infect mitotically active cell types. Both lentiviruses and standard retroviruses use the gag, pol, and env genes for packaging. However, the isoforms of these proteins used by retroviruses and lentiviruses are different and lentiviral vectors may not be efficiently packaged by each other's packaging systems.

Can I use a second generation packaging system with the pLenti vectors?

Answer: Yes, a second generation packaging system should work with OriGene's third generation pLenti vectors although we have not explicitly tested this. You can use OriGene's third generation packaging kit, cat# TR30002 for pLenti vectors.