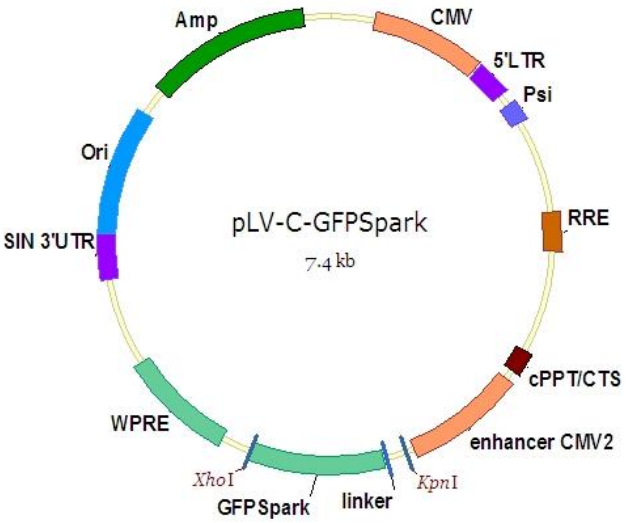
pLV-C-GFPSpark Lentivirus Control Plasmid

Catalog Number: **LVCV-35**

Physical Map of Plasmid



Vector Name	pLV-C-GFPSpark
Vector Size	7395bp
Vector Type	Lentiviral Vector
Promoter	CMV
Antibiotic Resistance	Ampicillin

Schematic of pLV-C-GFPSpark Multiple Cloning Sites

CTCGTTTAGTGAACCGTCAGAATTTTGTAATACGACTCACTATAGGGCGGCCGGGAATTCTAATACGACTCACTATAG
pLen-F sequencing Primer *EcoR I*
GGGCCGCCACCAAGCTTGGTACCGCTAGCGGATCCGTTAACCTTAAGACCGGTATGGGCTGGTCCTGCATCATCCTG
Kpn I **linker**
TTCCTCGTGGCGACCGCGACCGGGGTCCACAGCGATATCATCGATAGCGCTCCC GGG GGT GGA GGC TCT
Sma I G G G G S
GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACA
AGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGC
AAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCA
CATGAAGAAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGA
CGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATC
GACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCC
GACAAGCAGAAGAACGGCATCAAGGCTAACTTCAAGGTTCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGA
CCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCG
CCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTC
GGCATGGACGAGCTGTACAAG* TAA ACTCGAGTCTGCGGCCGCCGTTTAAACGGCCGGCCGCGGTCTGTACAAGTA
stop *Xho I* *Not I* *Pme I*
GGATTCGTCGAGGGACCTAATAACTTCGTATAGCATACATTATACGAAGTTATACATGTTTAAGGGTTCCGGTTC
pLen-R sequencing Primer

* : GFPSpark sequence

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Other Information

Lot : Please refer to the label on the tube

Shipping carrier : Each tube contains approximately 10 μg of lyophilized plasmid.

Storage : The lyophilized plasmid can be stored at ambient temperature for three months.

Sequencing primer list :

pLen-F：	5’ CTCGTTTAGTGAACCGTCAGAATT 3’
pLen-R：	5’ GAACCGGAACCCTTAAACATGT 3’

pLen-F and pLen-R are designed by Sino Biological Inc. Customers can order the primer pair from any oligonucleotide supplier.

Plasmid Resuspension protocol

1.Centrifuge at 5,000×g for 5 min.

2.Carefully open the tube and add 100 μl of sterile water to dissolve the DNA.

3.Close the tube and incubate for 10 minutes at room temperature.

4.Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.

5.Store the plasmid at -20 ℃.

E.coli strains for transformation (recommended but not limited)

Most commercially available competent cells are appropriate for the plasmid, e.g. Stbl3,TOP10, DH5α and JM109.

Lentivirus Production

Plasmid Purification and Cell Culture

1. Prepare high quality plasmid DNA.

2.18 - 24 hours prior to transfection, plate 2.5 x 106 of 293T cells on a 10cm dish and incubate at 37 ℃ overnight. Cells should reach 65-70% confluence within 24 hours.

Transfect into 293T Cells

3. Add transfer vector and packaging plasmids into the Opti-MEM. Mix by pipetting completely.

4. Add transfection reagent into the same tube. Vortex for 10 seconds.

5. Incubate the mixture at room temperature for 15 minutes.

6. Add the mixture drop-wise to the dish, and swirl to disperse evenly throughout the plate. Return the dish to the cell culture incubator at 37° C.

Harvest Viral Supernatant

7. After 12-18 hours incubation, change the culture medium and continue to incubate the plate for 48 hours.

8. Transfer the cell culture supernatant to a 15mL centrifuge tube. Centrifuge at 3000 x g for 15 mins and filter the supernatant through a syringe filter (0.45 micron). Transfer the viral supernatant into a new tube.

9. The viral particles are ready to be used. They can be stored at 4 ℃ for 2 weeks or aliquot and store at -80℃ for long-term.

Lentivirus Transduction

10. Plate 50 000 target cells per well in a 24 well plate to 50% confluence upon transduction.

11. Remove medium from wells and add appropriate amount of Lentiviral particles, culture medium, polybrene (Optional). Gently swirl the plate to mix.(Optional: Add increasing amounts of virus to different wells at varying MOIs (5, 10 and 20, etc.) to optimize the transduction).

12. 72 hours post transduction, the viral genome will be integrated into the host cell genome. Harvest the cells and perform qRT-PCR or Western blot or flow cytometer.