

# OFPSpark Lentivirus Control Plasmid

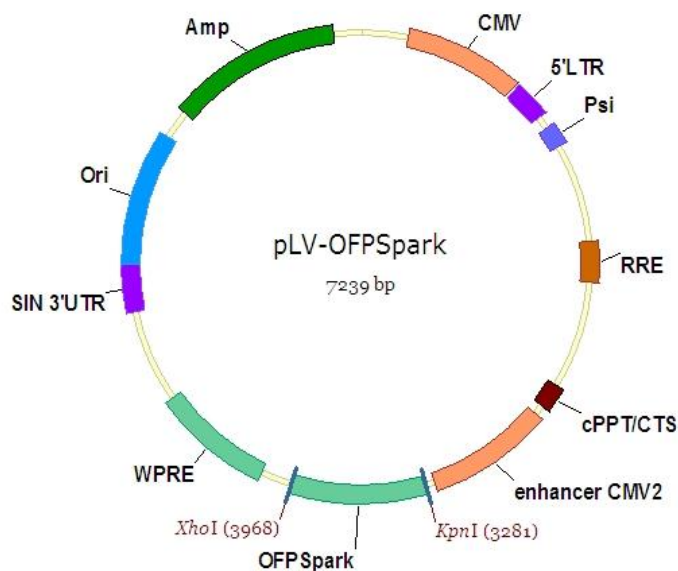


Sino Biological Inc.

Biological Solution Specialist

Catalog Number: LVCV-02

## Physical Map of Plasmid



Vector Name	pLV-OFPSpark
Vector Size	7239bp
Vector Type	Lentiviral Vector
Promoter	CMV
Antibiotic Resistance	Ampicillin

## Cloning Sites for OFPSpark Inserting

CTCGTTTAGTGAACCGTCAGAATTTTGTAAACGACTCACTATAGGGCGGCCGGGAATTCTAATACGACTCACTATAG

pLen-F sequencing Primer

GGGCCGCCACCAAGCTTGGTACCGCTAGC ATGGATAGCACTGAGAACGTCATCAAGCCCTTCATGCGCTTCAAGG  
*Kpn I* Met **OFPSpark sequence**

TGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGTGGGCGAGGGCCGCCCTACGAGGGC  
TTTCAGACCGCTAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCATTCA  
CCTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTT  
CAAGTGGGAGCGCGTGATGAAGTACGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTACAGGACGG  
CGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTGATGCAGAAGAAGACCATG  
GGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCAAGATCAAGATGAGGCTGAAG  
CTGAAGGACGGCGGCCACTACACCTCCGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGC  
GCCTACATCGTCGACATCAAGTTGGACATCACCTCCACAAACGAGGACTACACCATCGTGGAACAGTACGAACGCG  
CCGAGGGCCGCCACCACCTGTTCCAGTAA ACTCGAGTCTGCGGCCGCCGTTTAAACGGCCGGCCGCGGTCTGTAC  
stop codon *Xho I*

AAGTAGGATTCTGTCGAGGGACCTAATAACTTCGTATAGCATACATTATACGAAGTTATACATGTTTAAGGGTTCCGGT  
TC pLen-R sequencing Primer

## OFPSpark Tag Info

OFPSpark is a red (orange) fluorescent protein (excitation/emission maxima are 549 and 566 nm, respectively) derived from DsRed. Possessing high photostability and pH stability, OFPSpark is more than twice brighter than mOrange2. Fast OFPSpark maturation makes it clearly detectable in mammalian cells as early as within 8 hrs after transfection. OFPSpark can be expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with OFPSpark expression vectors produce bright fluorescence in 8 hrs after transfection. No cytotoxic effects or visible protein aggregation are observed. For its monomer structure, OFPSpark performs well in some fusions and protein labeling applications.

Manufactured By Sino Biological Inc., FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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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 :**

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pLen-F: 5' CTCGTTTAGTGAACCGTCAGAATT 3'

pLen-R : 5' GAACCGGAACCCCTTAAACATGT 3'

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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  $5,000 \times g$  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  $5000 \times g$ .
5. Store the plasmid at  $-20^{\circ}\text{C}$ .

### *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  $2.5 \times 10^6$  of 293T cells on a 10cm dish and incubate at  $37^{\circ}\text{C}$  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  $37^{\circ}\text{C}$ .



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## 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 °C for 2 weeks or aliquot and store at -80°C 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.