

Human CD146 / MCAM ORF mammalian expression plasmid, N-Flag tag



Sino Biological Inc.
Biological Solution Specialist

Catalog Number: HG10115-NF

General Information

Gene : melanoma cell adhesion molecule
Official Symbol : MCAM
Synonym : MCAM, CD146, MUC18
Source : Human
cDNA Size: 1941bp
RefSeq : NM_006500.2
Plasmid: pCMV3-Flag-MCAM

Description

Lot : Please refer to the label on the tube

Sequence Description :

Identical with the Gene Bank Ref. ID sequence except for the point mutations: 213C/T, 1818 A/G not causing the amino acid variation.

Restriction site: HindIII + XbaI (6kb + 1.96kb)

Vector : pCMV3-SP-N-FLAG

Shipping carrier :

Each tube contains approximately 10 µg of lyophilized plasmid.

Storage :

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

Quality control :

The plasmid is confirmed by full-length sequencing with primers in the sequencing primer list.

Sequencing primer list :

pCMV3-F: 5' CAGGTGTCCACTCCCAGGTCCAAG 3'

pcDNA3-R : 5' GGCAACTAGAAGGCACAGTCGAGG 3'

Or

Forward T7 : 5' TAATACGACTCACTATAGGG 3'

ReverseBGH : 5' TAGAAGGCACAGTCGAGG 3'

pCMV3-F and pcDNA3-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°C .

The plasmid is ready for:

- Restriction enzyme digestion
- PCR amplification
- *E. coli* transformation
- DNA sequencing

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

Most commercially available competent cells are appropriate for the plasmid, e.g. TOP10, DH5α and TOP10F'.

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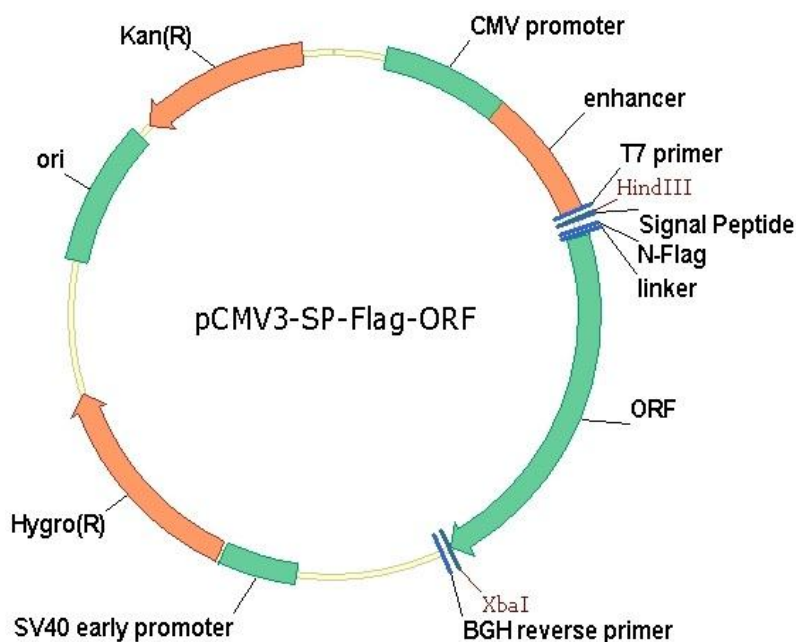
Vector Information

All of the pCMV vectors are designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human enhanced cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells.
- Hygromycin resistance gene for selection of mammalian cell lines.
- A Kozak consensus sequence to enhance mammalian expression.

Vector Name	pCMV3-SP-N-FLAG
Vector Size	6143bp
Vector Type	Mammalian Expression Vector
Expression Method	Constitutive, Stable / Transient
Promoter	CMV
Antibiotic Resistance	Kanamycin
Selection In Mammalian Cells	Hygromycin
Protein Tag	FLAG

Physical Map of Plasmid :



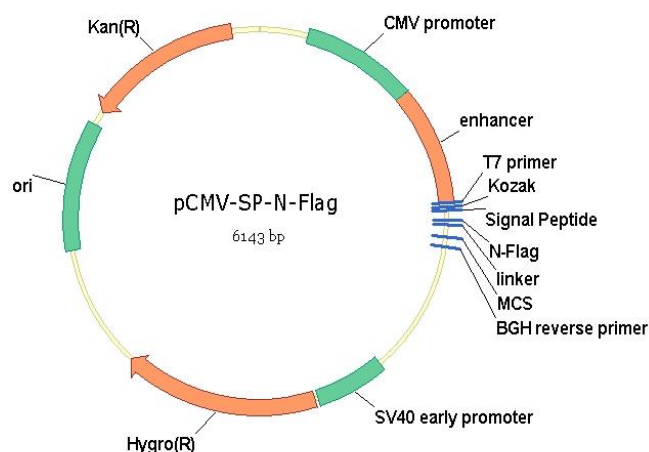
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Physical Map



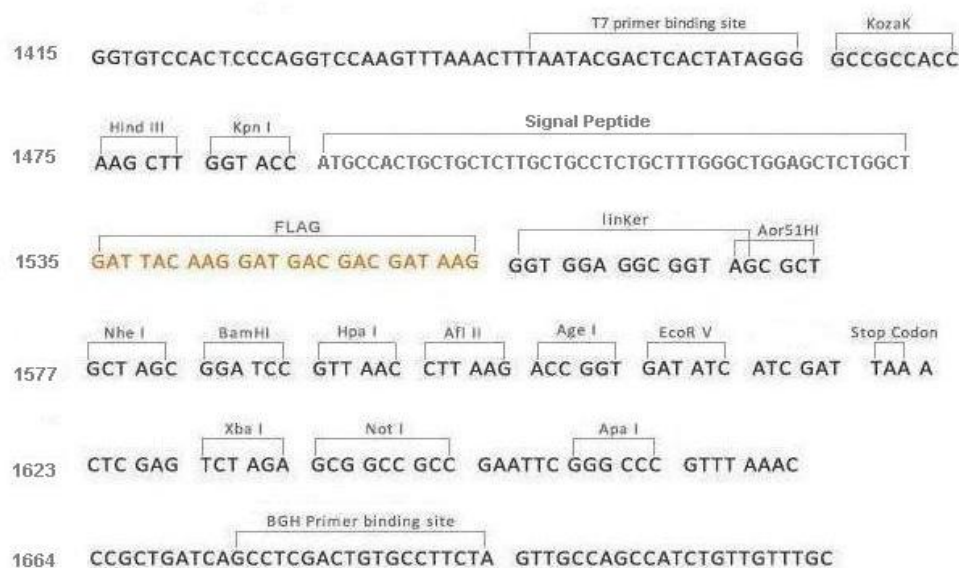
Comments for pCMV3-SP-N-FLAG:

CMV promoter: bases 250-837
enhancer: bases 838-1445
SV40 early promoter: bases 2384-2753
Hygromycin ORF: bases 2771-3793
pUC origin: bases 4439-5112
Kanamycin ORF: bases 5186-6001

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Promoter	CMV
Antibiotic Resistance	Kanamycin
Selection In Mammalian Cells	Hygromycin
Protein Tag	FLAG
Sequencing Primer	Forward:T7(TAATACGACTCACTATAGGG) Reverse:BGH(TAGAAGGCACAGTCGAGG)

Schematic of pCMV3-SP-N-FLAG Multiple Cloning Sites



pCMV3-SP-N-Flag is recommended for constructing the N-FLAG tag secretory and membrane proteins expression vector which containing a naïve signal peptide. An universal signal peptide is used to instead the naïve signal peptide.