pCDF cDNA Cloning and Expression Lentivectors

Cat. #s CD100A-1 – CD111B-1

User Manual

Store kit at -20°C on receipt

(ver. 5-061226)

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

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I. Introduction and Background

A. Purpose of this Manual

This manual provides details and information necessary to generate expression constructs of your gene of interest in the pCDF lentivectors. Specifically, it provides critical instructions on amplification and cloning the cDNA into the pCDF Vectors, and verifying final expression constructs. This manual does not include information on packaging the pCDF expression constructs into pseudotyped viral particles or transducing your target cells of choice with these particles. This information is available in the user manual Lentivector Expression Systems: Guide to Packaging and Transduction of Target Cells, which is available on the SBI website (www.systembio.com). Before using the reagents and material supplied with this system, please read the entire manual.

B. Advantages of the Lentivector Expression System

Lentiviral expression vectors are the most effective vehicles for delivering and expression of a gene of interest to almost any mammalian cell—including non-dividing cells and model organisms (C.A. Machida, 2003; M. Federico, 2003; W. C. Heiser, 2004). As with standard plasmid vectors, it is possible to introduce lentivector expression constructs in plasmid form into the cells with low-to-medium efficiency using conventional transfection protocols. However, by packaging the lentivector construct into viral particles, you can obtain highly efficient transduction of expression constructs—even with the most difficult to transfect cells, such as primary, stem, and differentiated cells. The expression construct transduced in target cells is integrated into genomic DNA and provides stable, long-term expression of the target gene.

The lentiviral cDNA expression system consists of three main components:

(1) The lentiviral expression vector (*e.g.*, pCDF1-MCS2-EF1-Puro)

(2) The lentiviral packaging plasmids (*e.g.*, pPACKF1™ Packaging Plasmid mix)

(3) A pseudoviral particle producer cell line (*e.g.*, 293TN cells)

The expression lentivector contains the genetic elements responsible for packaging, transduction, stable integration of the viral expression construct into genomic DNA, and expression of the target gene sequence. The packaging vector provides all the proteins essential for transcription and packaging of an RNA copy of the expression construct into recombinant viral particles. To produce a high titer of viral particles, expression and packaging vectors are transiently co-

transfected into producer mammalian cells (*e.g.*, HEK 293 cells). For a detailed description of SBI's Lentivector expression system, please refer to the Lentivector Expression Systems user manual.

SBI's novel pCDF Vectors are derived from feline immunodeficiency virus (FIV; Poeschla, 2003; for Safety Guidelines when working with these vectors, see section G). These pCDF Vectors, developed at SBI, are self-inactivating as a result of a deletion in the U3 region of 3' ΔLTR (see Appendix for Vector Features). Upon integration into the genome, the 5' LTR promoter is inactivated, which prevents formation of replication-competent viral particles.

When expressed, the hybrid CMV/FIV 5' LTR drives high level transcription of the viral construct and produces a transcript that contains all the necessary functional elements (*i.e.*, Psi, RRE, and cPPT) for efficient packaging. When this construct is expressed in HEK 293 cells that also express viral coat proteins (*i.e.*, a packaging cell line), the pCDF transcripts are efficiently packaged into pseudoviral particles. After isolation, these pseudoviral particles containing the RNA version of the pCDF expression cassette can be efficiently transduced into any mammalian target cells. Following transduction into the target cells, this expression cassette is reverse transcribed and integrated into the genome of the target cell. The pCDF Vectors also contain a bacterial origin of replication and ampicillin resistance (Amp^R) gene for propagation and selection in *E. coli*. The pCDF1-MCS2-EF1-Puro Vector (Cat. # CD110B-1) contains a puromycin resistance gene, under the control of a constitutive EF1 promoter and a WPRE regulatory element, to enable selection of target cells stably expressing the cDNA template. The pCDF1-MCS2-EF1-copGFP Vector (Cat. # CD111B-1) contains a copGFP gene under the control of a EF1 promoter and WPRE element. CopGFP is a novel fluorescent protein ,derived from copepod plankton (Panalina sp.), which is similar to EGFP but has a brighter color This gene serves as a reporter for the transfected or transduced cells.

C. pCDF Cloning and Expression Lentivectors

The FIV derived pCDF vectors contain the following features:

- **CMV promoter—**promotes a high level of expression of your gene of interest in a wide variety of cell lines.
- **Multiple Cloning Site (MCS)—**for cloning the gene of interest in MCS located downstream of CMV promoter.
- **WPRE element**—enhances stability and translation of the CMV-driven transcripts.
- **SV40 polyadenylation signal**—enables efficient termination of transcription and processing of recombinant transcripts.

- **Optional second expression cassette**—provides expression of puromycin resistance gene or copGFP reporter under control of constitutive elongation factor 1 (EF1) promoter for selection or FACS analysis of transduced cells.
- **Hybrid CMV-5LTR promoter**—provides a high level of expression of the full-length viral transcript in producer 293 cells.
- **Genetic elements (cPPT, GAG, LTRs)**—necessary for packaging, transducing, and stably integrating the viral expression construct into genomic DNA.
- **SV40 origin**—for stable propagation of the pCDF plasmid in mammalian cells.
- **pUC origin**—for high copy replication and maintenance of the plasmid in *E.coli* cells.
- **Ampicillin resistance gene**—for selection in *E.coli* cells.

D. List of Components

pCDF cDNA Cloning and Expression Lentivectors:

pCDF1-MCS1 cDNA Expression Vector	Cat. #: CD100A-1
pCDF1-MCS2-EF1-Puro	Cat. #: CD110B-1
pCDF1-MCS2-EF1-copGFP	Cat. #: CD111B-1

Component	Conc.	Amount
pCDF cDNA Expression Vector	0.5 μg/μl	20 μg

All plasmids are shipped at a concentration of 0.5 μg/μl and an amount of 20 μg. All kits are shipped in dry ice and should be stored at -20°C upon receipt. Properly stored kits are stable for 12 months from the date received.

E. Additional Required Materials

For Cloning

- Restriction enzymes for digestion of the vectors and/or inserts (Recommended: New England BioLabs enzymes)
- High Fidelity Long-distance PCR enzymes
- T4 DNA Ligase and ligation reaction buffer (Recommended: New England BioLabs T4 DNA Ligase (400 U/μl), Cat. # M0202S. Dilute to 40 U/μl with the provided 1X reaction buffer just before use)
- High efficiency competent *E. coli* cells ($RecA^-$) (Recommended: Invitrogen One Shot OmniMAX 2 competent cells, Cat. # C8540-03)
- Petri plates containing LB Agar media with 50 μg/ml Ampicillin

For Screening Inserts and Sequencing

- Taq DNA polymerase, reaction buffer, and dNTP mix (Recommended: Clontech Titanium™ Taq DNA polymerase, Cat. # 639208)
- PCR machine
- 2-3% 1X TAE Agarose gel

For Purifying cDNA Constructs after Cloning

- Plasmid purification kit (Recommended: QIAGEN Endotoxin-free Plasmid Kit. The following kit combinations can be used for Midi scale (up to 200 μg of plasmid DNA) preparation of endotoxin-free DNA:
  - QIAfilter Plasmid Midi Kit, Cat. # 12243, and EndoFree Plasmid Maxi Kit, Cat. # 12362

- QIAfilter Plasmid Midi Kit, Cat. # 12243, and EndoFree Plasmid Buffer Set, Cat. # 19048

Please visit the QIAGEN website to download the specialized protocol that is not contained in the current user manual:

- http://www1.qiagen.com/literature/protocols/pdf/QP15.pdf

For Transfection of pCDF Constructs into Target Cells

- Transfection Reagent
  (Recommended: Invitrogen Lipofectamine 2000, Cat. # 11668-027)

For Packaging of pCDF Constructs in Pseudoviral Particles

- In order to package your pCDF cDNA constructs into VSV-G pseudotyped viral particles, you will need to purchase the pPACKF1 Lentivector Packaging Kit (Cat. # LV100A-1). The protocol for packaging and transduction of packaged pseudoviral particles is provided in the User Manual for the Lentivector Expression System.
- 293 Producer Cell Line
  (Recommended: SBI 293TN Cell Line, Cat. # LV900A-1 or ATCC 293 Cells, Cat. # CRL-11268)
- Transfection Reagent
  (Recommended: Invitrogen Lipofectamine, Cat. # 18324-111 and
- Plus Reagent, Cat. # 11514-015)

F. Safety Guidelines

SBI's Expression lentivectors together with the pPACK packaging plasmids comprise the third-generation lentiviral expression system. The original FIV expression system was developed by Eric M. Poeschla, David J. Looney, and Flossie Wong-Staal at UCSD (Poeschla, 1998; Poeschla 2003). The feline immunodeficiency virus (FIV) was originally isolated from cat blood. Despite common close exposure of humans to FIV through contact with domestic cats (including bites, scratches, etc.), no human infection or disease has ever been associated with FIV (Poeschla, 2003).

Both FIV-based and HIV-based lentivector systems are designed to maximize their biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The RSV promoter (in HIV-based vectors) and CMV promoter (in FIV-based vectors) upstream of 5'LTR in the lentivector allow efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 that are used in this system.

- Number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (*gag, pol, rev*), and the corresponding proteins are expressed from different plasmids (for HIV-based packaging plasmids) lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector, to prevent generation of recombinant replication-competent virus.
- None of the HIV-1 genes (*gag, pol, rev)* will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Pseudoviral particles will carry only a copy of your expression construct.

Despite the above safety features, use of SBI's lentivectors falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus, or the possibility of insertional mutagenesis. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm. It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and always follow standard microbiological practices, which include:

- Wear gloves and lab coat all the time when conducting the procedure.
- Always work with pseudoviral particles in a Class II laminar flow hood.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.
- Please keep in mind that pCDF vectors are integrated into genomic DNA and could have a risk of insertional mutagenesis.

II. Protocol

The following section provides general guidelines for the cloning of cDNA, amplified by PCR, into pCDF vectors.

A. cDNA Amplification

Full-length cDNA fragments can be recloned from another plasmid or amplified by PCR. PCR-based cloning is the most convenient way for full-length cDNA cloning in pCDF vectors. The cDNA lentivector does not contain an ATG initiation codon. A translation initiation sequence must be incorporated in the insert cDNA if the cDNA fragment to be cloned does not already have an ATG codon. We also recommend including a Kozak sequence (*i.e.* GCCACC) before the ATG for optimal translation. For amplification of the target cDNA fragment, design a 5'-primer (containing a Kozak sequence and ATG codon) and 3'-primer with unique restriction sites present in the MCS of the pCDF vector but not present in the cDNA sequence. Amplify the cDNA fragment by high fidelity long-distance PCR using about 200 ng of plasmid template DNA and a minimum number of cycles (usually 12-15 cycles), purify, digest the amplified product with end-specific restriction enzyme(s) and purify the digested PCR product in a 1.2% agarose gel to prevent contamination with the original plasmid used for amplification.

B. Preparation of Digested pCDF Vector

Digest pCDF vector with the corresponding restriction enzymes used for preparation of cDNA fragments, and verify complete digestion of the vector by agarose gel electrophoresis. We suggest that you perform only preparative gel purification of the digested vector if more than one restriction enzyme is used. If you use a single restriction enzyme, dephosphorylation and gel purification of vector is necessary to reduce the background in the vector ligation step.

C. Cloning of cDNA into pCDF Vector

The optimal insert-to-vector molar ratio may be different for different inserts. Always try at least two different ratios (*e.g.*, 10:1 and 30:1) for each experiment. Also make sure to include one negative control reaction, which contains only the digested vector.

1. Ligation of cDNA to Vector

a. Dilute the gel-purified digested vector to 10 ng/μl.

b. Set up 10 μl ligation reactions for each sample and control, as follows:

1.0	μl	Digested pCDF Vector (10 ng/μl)
7.0	μl	cDNA insert (usually 15-50 ng) or Nuclease-free water
1.0	μl	10X T4 DNA Ligase Buffer
1.0	μl	T4 DNA ligase (40 U/μl)
10.0	μl	Total volume

c. Incubate the ligation reaction at 16°C for 2-5 hrs, if it is sticky-end ligation. For blunt-end ligation, an overnight incubation time is recommended.

2. Transform *E. coli* with the ligation product

Transform competent cells (with a transformation efficiency of at least 1×10^9 colonies/μg pUC19) with the whole ligation reaction (10 μl) following the protocol provided with the competent cells. Plate the transformed bacteria on LB-Ampicillin agar plates.

3. Identify Clones with the cDNA Insert

a. Depending on the ratio of colony numbers for the cDNA sample vs. the negative control sample, randomly pick 5 or more well-isolated colonies and grow each clone in 100 μl of LB Broth with 75 μg/ml ampicillin at 37°C for 2 hours with shaking.

b. Use 1 μl of each bacterial culture for screening cDNA inserts by PCR and continue to grow the culture for another 4 hours. Store the culture at 4°C.

c. Prepare a PCR Master Mix with PCR primers flanking the cDNA insert:

1 rxn	10 rxn	Composition
0.5 μl	5 μl	PCR primer 1 (10 μM)
0.5 μl	5 μl	PCR primer 2 (10 μM)
0.5 μl	5 μl	50X dNTP mix (10 mM of each)
2.5 μl	25 μl	10X PCR Reaction Buffer
19.5 μl	195 μl	Deionized water
0.5 μl	5 μl	Taq DNA polymerase (approx. 5 U/μl)
24.0 μl	240 μl	Total volume

d. Mix the master mix very well and aliquot 24 μl into each well of 96-well PCR plate or individual tubes.

e. Add 1 μl of each bacterial culture from step (b) into each well (or tube).

f. Proceed with PCR using the following program:

94°C, 4 min	1 cycle
94°C, 0.5 min, then 68°C, 1 min/1 kb*.	25 cycles
68°C, 3 min	1 cycle

* depending on the size of final PCR product, use shorter or longer time.

g. Take 5 μl of the PCR reaction and run it on a 1.2% agarose/EtBr gel in 1X TAE buffer to identify clones with correct insert.

Grow a positive clone with the cDNA insert in an appropriate amount of LB-Amp Broth, and purify the construct using an endotoxin-free plasmid purification kit (see Section I.E).

Confirm identity of the cDNA insert by sequence analysis of the construct using the one of the PCR primers. Alternatively, you may use the following sequencing primer which is located upstream of the MCS: 5'-CACGCTGTTTTGACCTCCATAGA-3'.

D. Packaging of the pCDF expression constructs into pseudoviral particles

If you are planning to create a stably transduced cell line expressing your gene of interest, you first need to package the cDNA lentiviral construct into lenti pseudoviral particles. For this purpose, you will need to purchase the pPACKF1 Lentivector Packaging Kit from SBI (see Appendix). Figure 3 schematically shows all steps which need to be performed in order to generate pseudoviral packaged cDNA expression constructs.

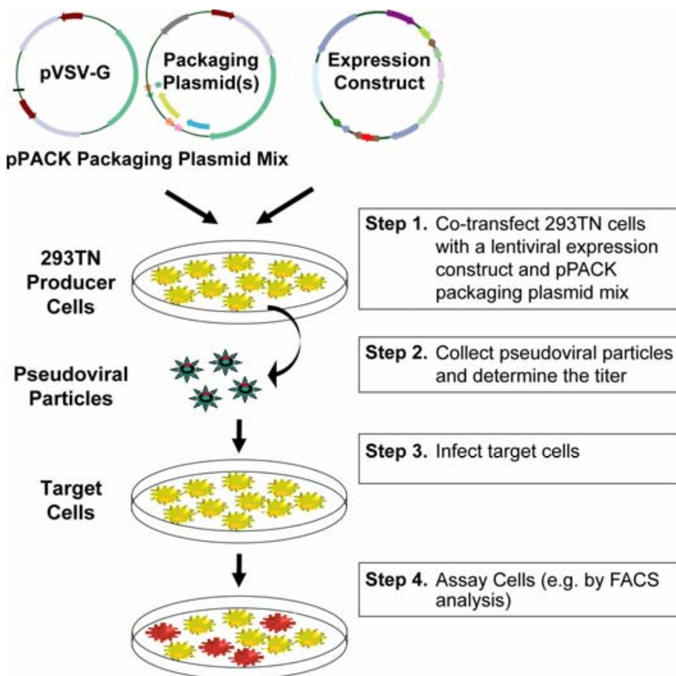

Fig. 3. Schematic presentation of the packaging procedure for lentivector expression constructs and making of stable cell lines.

The Lentivector Expression System User Manual includes the procedural information for packaging and transducing the expression constructs. This user manual is also available on the SBI web site (www.systembio.com). Although you can create stable transfectants with the lentiviral construct using standard transfection and selection protocols, transduction of the lentiviral cDNA construct using packaged pseudoviral particles is the most efficient way to deliver cDNA constructs in a wide range of cells, including dividing, non-dividing, and hard-to-transfect cells.

III. Troubleshooting

A. Large number of colonies on negative control plate

If you see that the colony number on the negative control plates (no insert) is equal or more than on the plate with the cDNA sample, there is probably undigested plasmid contamination. Check your digestion conditions, and repeat digestion with an increased concentration of restriction enzyme(s) or use a longer reaction time. For best results, gel-purify and dephosphorylate the vector after single enzyme digestion. Also, check the sequences of the PCR primers in order to be sure that the necessary restriction sites are present.

B. No or low number of colonies on plate with cDNA sample

The efficiency of cDNA cloning in pCDF vector depends on many factors, including size, purity, integrity, modification of insert, selection of restriction sites, etc. If your cDNA sample ligation resulted in only a few colonies, please continue with PCR screening first. If none of these few colonies has the right insert, or you did not get any colonies at all, it may be caused by:

1. Inappropriate ratio of insert-to-vector

Not enough or too much insert could inhibit the ligation reaction. Try a different ratio of insert-to-vector to optimize the ligation reaction. Sometimes, the yield of the ligation reaction may also be improved by increasing both the insert and vector amounts.

2. Low ligation efficiency

a. Inactive ligase and /or ligase reaction buffer	Test your ligase and reaction buffer for activity using different vector and insert. Replace the reagents if they are proven inactive.
b. Ligation inhibitors are present	EDTA and high salt may inhibit the ligation reaction.

3. Low transformation efficiency

a. Low quality or poor handling of competent cells	Handle the competent cells gently. Many cells do not allow re-freezing after thawed. Quality of competent cells may be tested by transforming a circular plasmid to determine cells' competency. Use competent cells with a transformation efficiency of at least $1x10^9$ colonies/ µg of pUC19 plasmid.
b. Wrong antibiotic or too much antibiotic in the media.	The plates used for cloning should contain 50-100 µg/ml ampicillin in the media.

C. No correct cDNA inserts

If the colony number for the cDNA sample is more than for the negative control sample (*i.e.* vector only), but you failed to amplify cDNA insert, it could be that:

1. Inactive Taq polymerase or reaction buffer	Test the activity of the PCR master mix by amplifying cDNA from original template. Replace the PCR reagents if they are proven inactive.
2. Wrong primer was used	Make sure you are using the correct primers for the specific orientation of cDNA insert.
3. Not enough clones were screened	Pick more colonies for screening.

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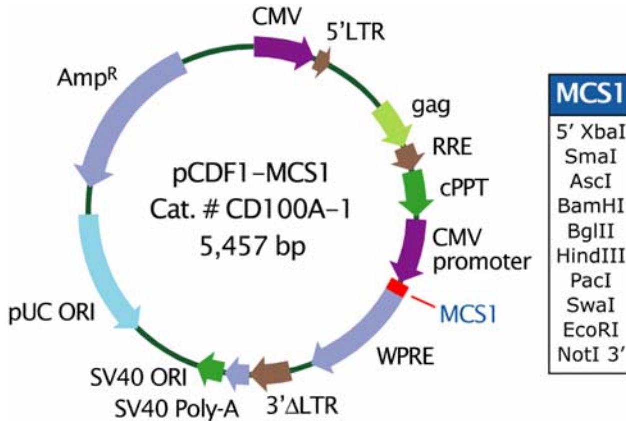
V. Appendix

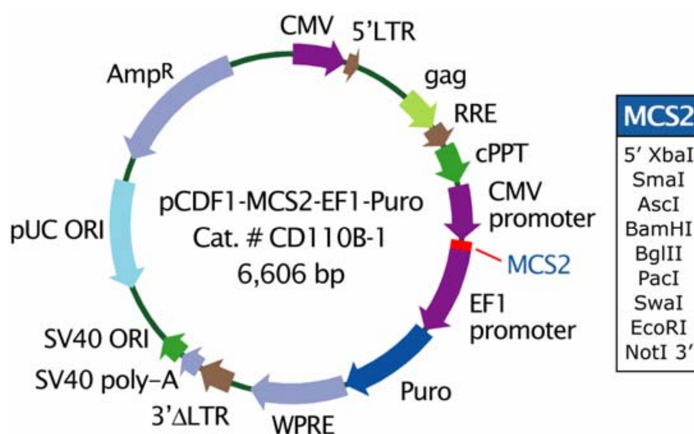
A. Map and Features for pCDF1-MCS1 Vector



Feature	Location*	Function
CMV/5'LTR	1-415	Hybrid CMV promoter-R/U5 long terminal repeat; required for viral packaging and transcription
gag	762-1011	Packaging signal
RRE	1012-1143	Rev response element binds gag and involved in packaging of viral transcripts
cPPT	1150-1391	Central polypurine tract (includes DNA Flap region) involved in nuclear translocation and integration of transduced viral genome
CMV promoter	1407-1746	Human cytomegalovirus (CMV)--constitutive promoter for transcription of cloned cDNA insert
WPRE	1817-2406	Woodchuck hepatitis virus posttranscriptional regulatory element--enhances the stability of the viral transcripts
3' ΔLTR (ΔU3)	2525-2740	Required for viral reverse transcription; self-inactivating 3' LTR with deletion in U3 region prevents formation of replication-competent viral particles after integration into genomic DNA
SV40 Poly-A	2741-2872	Transcription termination and polyadenylation
SV40 Ori	2881-3027	Allows for episomal replication of plasmid in eukaryotic cells
pUC Ori	3397-4070 (C)	Allows for high-copy replication in *E. coli*
AmpR	4215-5075 (C)	Ampicillin resistant gene for selection of the plasmid in *E. coli*

* The notation (C) refers to the complementary strand.

B. Map and Features for pCDF1-MCS2-EF1-Puro Vector



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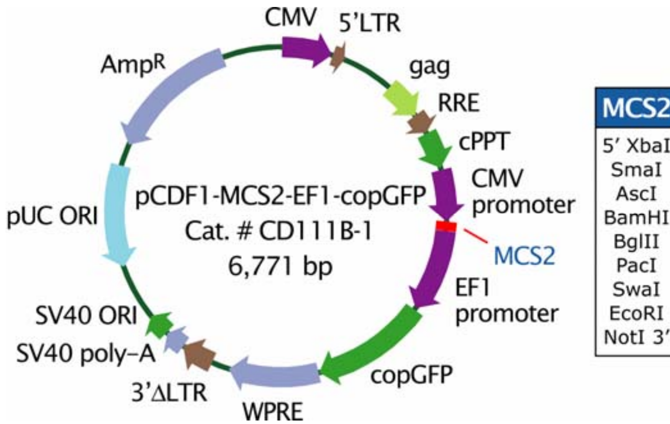
cPPT	1150-1391	Central polypurine tract (includes DNA Flap region) involved in nuclear translocation and integration of transduced viral genome
CMV promoter	1407-1746	Human cytomegalovirus (CMV)--constitutive promoter for transcription of cloned cDNA insert
EF1	1807-2352	Elongation factor 1α promoter--constitutive promoter for transcription of Reporter gene (Puromycin resistance or copGFP)
Puro	2358-2957	Puromycin-resistant marker for selection of the transfected/transduced cells
WPRE	2694-3553	Woodchuck hepatitis virus posttranscriptional regulatory element--enhances the stability of the viral transcripts
3' ΔLTR (ΔU3)	3674-3889	Required for viral reverse transcription; self-inactivating 3' LTR with deletion in U3 region prevents formation of replication-competent viral particles after integration into genomic DNA
SV40 Poly-A	3890-4021	Transcription termination and polyadenylation
SV40 Ori	4030-4176	Allows for episomal replication of plasmid in eukaryotic cells
pUC Ori	4546-5219 (C)	Allows for high-copy replication in *E. coli*
AmpR	5364-6224 (C)	Ampicillin resistant gene for selection of the plasmid in *E. coli*

* The notation (C) refers to the complementary strand.

C. Map and Features for pCDF1-MCS2-EF1-copGFP Vector



Feature	Location*	Function
CMV/5'LTR	1-415	Hybrid CMV promoter-R/U5 long terminal repeat; required for viral packaging and transcription
gag	762-1011	Packaging signal
RRE	1012-1143	Rev response element binds gag and involved in packaging of viral transcripts
cPPT	1150-1391	Central polypurine tract (includes DNA Flap region) involved in nuclear translocation and integration of transduced viral genome
CMV promoter	1407-1746	Human cytomegalovirus (CMV)--constitutive promoter for transcription of cloned cDNA insert
EF1	1807-2352	Elongation factor 1α promoter--constitutive promoter for transcription of Reporter gene (Puromycin resistance or copGFP)
copGFP	2366-3124	Copepod green fluorescent protein (similar to regular EGFP, but with brighter color) as a reporter for the transfected/transduced cells
WPRE	3131-3720	Woodchuck hepatitis virus posttranscriptional regulatory element--enhances the stability of the viral transcripts
3' ΔLTR (ΔU3)	3839-4054	Required for viral reverse transcription; self-inactivating 3' LTR with deletion in U3 region prevents formation of replication-competent viral particles after integration into genomic DNA
SV40 Poly-A	4055-4186	Transcription termination and polyadenylation
SV40 Ori	4195-4341	Allows for episomal replication of plasmid in eukaryotic cells
pUC Ori	4711-5384 (C)	Allows for high-copy replication in *E. coli*
AmpR	5529-6389 (C)	Ampicillin resistant gene for selection of the plasmid in *E. coli*

* The notation (C) refers to the complementary strand.

D. Restriction Maps of Multiple Cloning Sites (MCS)

MCS1

```
                                                          XbaI
                                                          ~~~~
1701                                                      TCTA
                                                          AGAT
                 AscI          BglII       PacI
               ~~~~~~~~~       ~~~~~~    ~~~~~~~~
            SmaI                                   SwaI
           ~~~~~~                                ~~~~~~~~~
        XbaI            BamHI        HindIII             EcoRI
        ~~              ~~~~~~~      ~~~~~~~              ~~~~
1751    GAGCCCGGGC GCGCCGGATC CAGATCTAAG CTTAATTAAT TTAAATGAAT
        CTCGGGCCCG CGCGGCCTAG GTCTAGATTC GAATTAATTA AATTTACTTA
            NotI
          ~~~~~~~~
        EcoRI
        ~~
1801    TCGCGGCCGC
        AGCGCCGGCG
```

MCS2

```
                                                          XbaI
                                                          ~~~~
1701                                                      TCTA
                                                          AGAT
                  AscI
               ~~~~~~~~~
            SmaI               BglII          SwaI        NotI
           ~~~~~~              ~~~~~~       ~~~~~~~~~      ~~~
        XbaI            BamHI         PacI            EcoRI
        ~~              ~~~~~~~     ~~~~~~~~~         ~~~~~~
1751    GAGCCCGGGC GCGCCGGATC CAGATCTTAA TTAATTTAAA TGAATTCGCG
        CTCGGGCCCG CGCGGCCTAG GTCTAGAATT AATTAAATTT ACTTAAGCGC
        NotI
        ~~~~~
1801    GCCGC
        CGGCG
```

E. Properties of the copGFP Fluorescent Protein

The pCDF1-MCS2-EF1-copGFP Vector contains the full-length copGFP gene with optimized human codons for high level of expression of the fluorescent protein from the CMV promoter in mammalian cells. The copGFP marker is a novel natural green monomeric GFP-like protein from copepod (*Pontellina sp.*). The copGFP protein is a non-toxic, non-aggregating protein with fast protein maturation, high stability at a wide range of pH (pH 4-12), and does not require any additional cofactors or substrates. The copGFP protein has very bright fluorescence that exceeds at least 1.3 times the brightness of EGFP, the widely used *Aequorea victoria* GFP mutant. The copGFP protein emits green fluorescence with the following characteristics:

emission wavelength max – 502 nm;
excitation wavelength max – 482 nm;
quantum yield – 0.6;
extinction coefficient – 70,000 M^{-1} cm^{-1}

Due to its exceptional properties, copGFP is an excellent fluorescent marker which can be used instead of EGFP for monitoring delivery of lentivector constructs into cells.

F. Related Products

- **pPACKF1™ Lentivector Packaging Kit (Cat. # LV100A-1)**
  Unique lentiviral vectors that produce all the necessary FIV viral proteins and the VSV-G envelope glycoprotein from vesicular stomatitis virus required to make active pseudoviral particles. 293TN cells (SBI, Cat. # LV900A-1) transiently transfected with the pPACKF1 and a pCDF cDNA expression construct produce packaged viral particles containing a pCDF cDNA construct.

- **HIV-Based pCDH cDNA Cloning and Expression Vectors**
  - **pCDH1-MCS1** (Cat. # CD500A-1)
  - **pCDH1-MCS2** (Cat. # CD501A-1)
  - **pCDH1-MCS1-EF1-Puro** (Cat. # CD510A-1)
  - **pCDH1-MCS1-EF1-copGFP** (Cat. # CD511A-1)

- **RNAi Cloning and Expression Lentivectors**
  These FIV and HIV-based single- and double-promoter shRNA and siRNA cloning vectors allow you to clone siRNA templates and efficiently transduce these siRNA constructs in a wide range of cells. For a list of currently available vectors, please visit our website at http://www.systembio.com.

- **MicroRNA Precursor Construct Collection**
  FIV-based microRNA Precursor Constructs allow you to express pre-miRNA, consisting of the stem loop structure and upstream and downstream flanking genomic sequence. For a list of currently available vectors, please visit our website at http://www.systembio.com.

- **PathNet™ Transcriptional Reporter Lentivectors**
  FIV and HIV-based transcriptional reporter vectors, allow detection of the activation of transcriptional factors (TFs) in a natural environment (nuclei). For a list of currently available vectors, please visit our website at http://www.systembio.com.

G. Technical Support

For more information about SBI products, to download manuals in PDF format, or to obtain vector sequences, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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1616 North Shoreline Blvd.
Mountain View, CA 94043

Phone: (650) 968-2200
(888) 266-5066 (Toll Free)

Fax: (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

VI. Licensing and Warranty Statement

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Use of the pCDF cDNA Cloning and Expression Vector (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

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This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

FIV Vector System

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CMV Promoter

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

CopGFP Reporter

This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein(s) intended to be used for research purposes only. Any use of the proprietary nucleic acids other than for research use is strictly prohibited. USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN. To obtain such a license, please contact Evrogen at license@evrogen.com.

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