phCMV-FSR™

Fusion Stable Reporter Vectors

Genlantis
A division of Gene Therapy Systems, Inc.

Catalog #	Content	Amount
P003400	phCMV C-GFP FSR Vector	20 μg, 0.5 μg/μl
P003500	phCMV-C-Luciferase FSR Vector	20 μg, 0.5 μg/μl
P013400	phCMV N-GFP FSR Vector	20 μg, 0.5 μg/μl
P013500	phCMV N-Luciferase Vector	20 μg, 0.5 μg/μl

Shipping	Shipped on blue ice or dry ice.
Storage	Store at -20°C; stable for 1 year.

RELATED PRODUCTS	Catalog Numbers
phCMV1 Vector Kit (non tagged)	P003100
phCMV2 Vector Kit (N-terminal HA tag)	P003200
phCMV3 Vector Kit (C-terminal HA tag)	P003300
GenePORTER® 2 Transfection Reagent	T202007 (75 rxns.); T202015 (150 rxns.); T202075 (750 rxns.).
TurboCells® Chemically Competent Cells	C300020 (F¯); C301020 (F')
SmartCells™ Chemically Competent Cells	C101020 (F¯); C101120 (F')
Anti-HA Polyclonal Antibody	ABC025; ABC123 (Anti-HA-HRP)

INTRODUCTION

The phCMV Fusion Stable Reporter (FSR) Vectors are designed for high-level expression of GFP or Luciferase fusion proteins and for creating GFP or Luciferase stable cell lines. These innovative vectors contain optimized CMV promoter-intron sequences for significantly higher constitutive expression levels than other mammalian expression vectors. Two of the phCMV-FSR Vectors (P003400 and P003500) contain a stop codon at the end of the reporter gene so you can easily create a reporter gene C-terminal fusion with your gene of interest. The other two phCMV-FSR Vectors (P013400 and P013500) contain no stop codon at the end of reporter gene so an N-terminal fusion of the reporter gene with your gene of interest is easily achieved. All phCMV-FSR Vectors contain a combination Kanamycin/Neomycin antibiotic selection gene for selection in both *E. coli* and mammalian cells, and a minimized vector size for maximum transfection efficiency. The vectors can be used for either transient transfection or for generating stable cells lines expressing the reporter gene alone or in fusion with your gene of interest. The phCMV-FSR Vectors provide the following features and benefits

Feature	Benefit
Modified hCMV immediate-early promoter	High level constitutive expression promoter in a wide variety of cells and cell types.
Reporter genes	Convenient choice of GFP or Luciferase reporter genes for control expression experiments.
SV40 polyadenylation signal	Stable mRNA by providing efficient transcription termination and polyadenylation.
Kanamycin/Neomycin resistance gene	Efficient vector selection in *E. coli* (with Kanamycin sulfate) or mammalian cells (with Neomycin Sulfate)
PUC Origin of replication	High copy number replication of vector in *E. coli.*
Two multiple cloning sites (MCS)	For cloning gene of choice upstream or downstream of reporter gene (in fusion).

MATERIALS AND METHODS

A. Cloning

General notes on cloning: please note that two of the phCMV-FSR Vectors contain a stop codon at the end of the reporter gene (P003400 and P003500) so they are most suitable for cloning your gene of interest upstream of the reporter gene (i.e. C-terminal fusion). Make sure that your gene of interest does not contain a stop codon at its end if you desire to obtain a fusion with the reporter gene downstream.

If on the other hand you need to have the reporter gene upstream of your gene of interest, we recommend using the phCMV-FSR Vectors that contain no stop codon at the end of the reporter gene (P013400 and P013500). In this case, make sure that there is a stop codon at the end of your gene of interest for proper translation termination.

1. Prepare gene of interest (GOI) by adding restriction enzyme sites on both ends of GOI fragment; this can be done by PCR or by digesting GOI fragment out of a plasmid with restriction enzyme sites compatible with the phCMV-FSR Vectors.
2. Digest the phCMV-FSR with the chosen restriction enzyme(s). Make sure that enzyme sites are not too close to each other to avoid digestion interference, and that enzyme sites are not blocked by methylation.
3. Ligate the GOI fragment into the linearized phCMV-FSR Vector using the ligation enzyme supplier's recommendations.

B. Transformation

The following protocol for the propagation of the phCMV-FSR Vectors is optimized for use with the SmartCells™ Competent *E. coli* cells (see Related Products Table above for ordering information); follow the supplier's protocol if you use other competent *E. coli* cells.

4. Thaw one tube (50 μl) of SmartCells on ice for 10-15 min.
5. Add 1-10 μl of the ligation mix to the thawed SmartCells; mix gently and incubate on ice for 15 to 30 min.
6. Heat shock the cells at 42°C for 45 seconds.
7. Add 250 μl of SOC, mix gently and incubate at 37°C for 1 hour, in a shaking air incubator; lay tube horizontally and shake at 225 rpm.

8. Spread 50 to 100 µl of the transformation mix on LB Agar + 50 µg/ml Kanamycin.

9. Incubate plates overnight at 37°C.

10. Pick individual colonies and analyze for positive recombinants.

C. Transfection

Plasmid DNA for transfection into mammalian cells must be clean and free of phenol and sodium chloride. Transfection methods include calcium phosphate, cationic lipids, and electroporation. Genlantis offers and recommends the GenePORTER® 2 Transfection Reagent for maximized transfection efficiency and high expression levels (see Related Products Table above for ordering information).

11. Use the supplier's protocol or standard laboratory protocols (e.g. Maniatis, *et. al.*) for transfecting or electroporating cells. We recommend using a positive control when available, and a mock transfection as a negative control.

D. Stable Cell Line Selection

First, you must determine the minimum G418 sensitivity needed to kill your untransfected host cells. Because natural resistance varies among cell lines, we recommend testing a range of concentrations

12. Split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates. Allow cells to adhere overnight.

13. The next day, substitute culture medium with medium containing the following G418 concentrations: 0/50/100/200/400/600/800 µg/ml.

14. Replenish selective media every 3-4 days and observe the percentage of surviving cells.

15. The correct selective concentration of G418 is the minimum concentration that kills 100% of the cells after 2-3 weeks. Use this concentration to create stable integrants.

16. Transfect plate(s) of host cells with linearized phCMV-FSR (with or without your GOI).

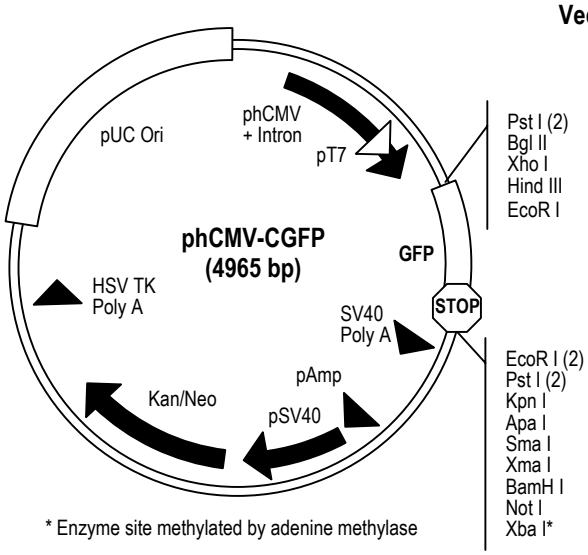
17. 24 hrs post transfection, wash cells and add fresh regular medium.

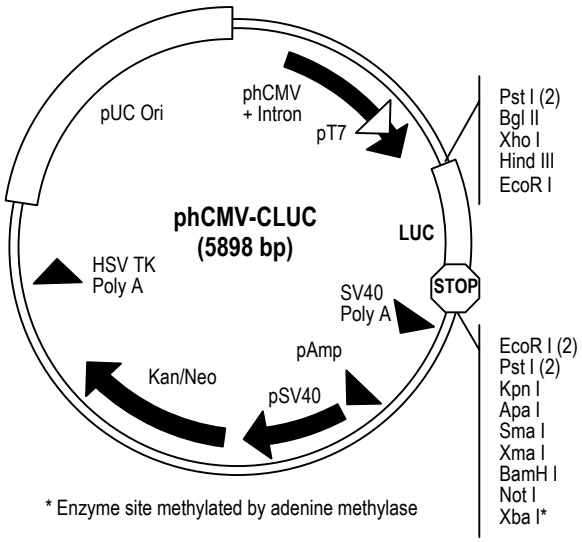
18. 48 hours post transfection, split cells into fresh medium + G418 as determined in Steps 12-15; split cells to about 25% confluency.

19. Feed cells with selective medium every 3-4 days until resistant foci can be identified.

20. Pick and expand colonies in 96- o 48-well plates.

Vector Information

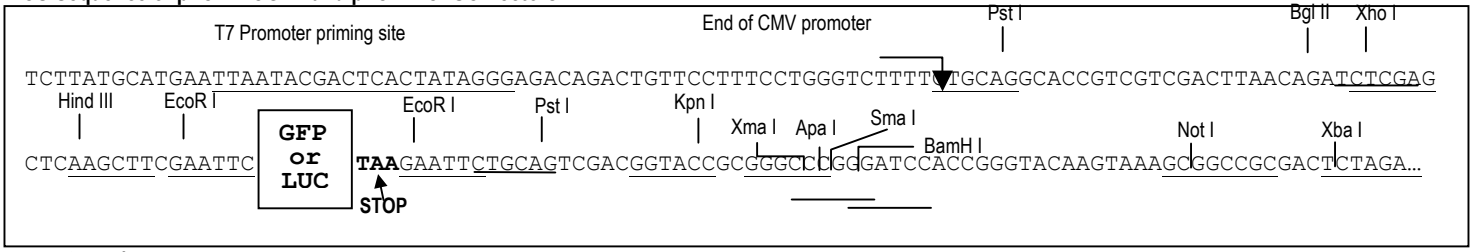


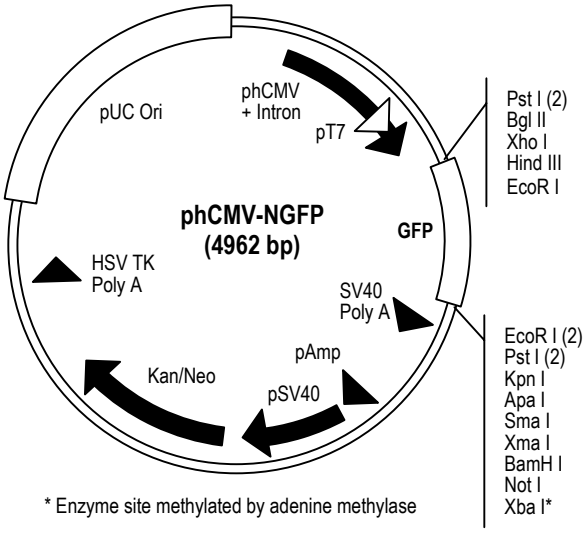


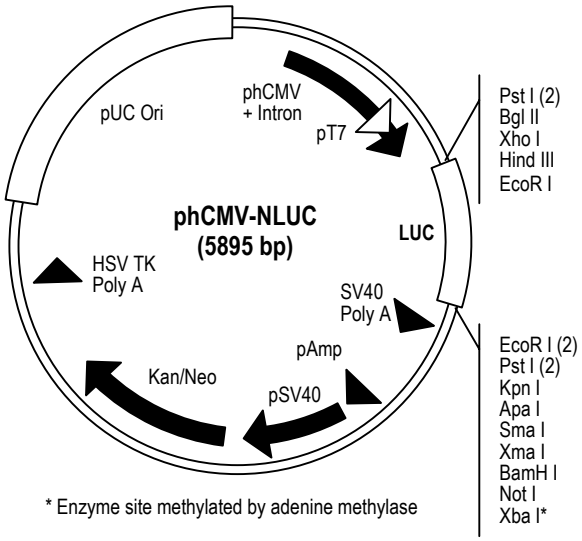
Element	Start-End	Name/Description
phCMV+intron	59-808	Human CMV promoter/enhancer & intron
pT7	759-774	T7 promoter priming for sequencing
GFP	859-1578	GFP gene
SV40 Poly A	1784-1834	SV40 polyadenylation signal sequence
pAmp	2398-2426	Ampicillin resistance gene promoter
pSV40	2510-2739	SV40 promoter
Kan/Neo	2861-3655	Kanamycin resistance gene
HSV TK Poly A	3891-3909	HSV Thymidine Kinase polyadenylation signal
pUC Ori	4240-4883	pUC origin of replication

Element	Start-End	Name/Description
phCMV+intron	59-808	Human CMV promoter/enhancer & intron
pT7	759-774	T7 promoter priming for sequencing
LUC	859-2511	Luciferase gene
SV40 Poly A	2717-2767	SV40 polyadenylation signal sequence
pAmp	3331-3359	Ampicillin resistance gene promoter
pSV40	3443-3672	SV40 promoter
Kan/Neo	3794-4588	Kanamycin resistance gene
HSV TK Poly A	4824-4842	HSV Thymidine Kinase polyadenylation signal
pUC Ori	5173-5816	pUC origin of replication

MCS Sequence of phCMV-CGFP and phCMV-CLUC Vectors



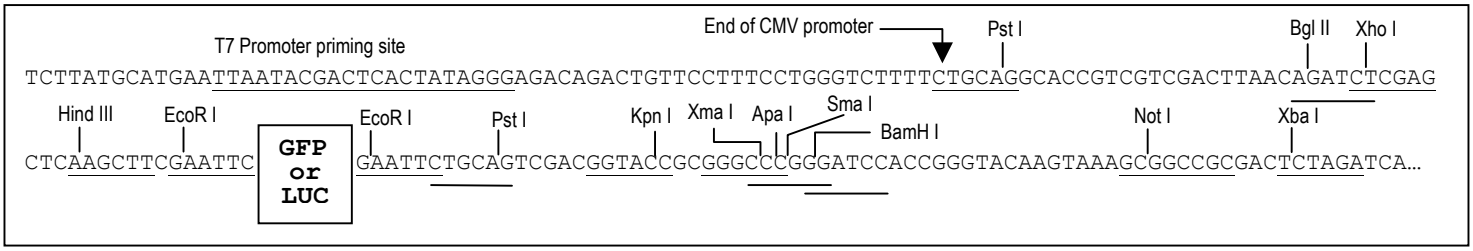




Element	Start-End	Name/Description
phCMV+intron	59-808	Human CMV promoter/enhancer & intron
pT7	759-774	T7 promoter priming for sequencing
GFP	859-1575	GFP gene
SV40 Poly A	1784-1831	SV40 polyadenylation signal sequence
pAmp	2398-2423	Ampicillin resistance gene promoter
pSV40	2507-2739	SV40 promoter
Kan/Neo	2858-3652	Kanamycin resistance gene
HSV TK Poly A	3888-3906	HSV Thymidine Kinase polyadenylation signal
pUC Ori	4237-4880	pUC origin of replication

Element	Start-End	Name/Description
phCMV+intron	59-808	Human CMV promoter/enhancer & intron
pT7	759-774	T7 promoter priming for sequencing
LUC	859-2508	Luciferase gene
SV40 Poly A	2714-2764	SV40 polyadenylation signal sequence
pAmp	3328-3356	Ampicillin resistance gene promoter
pSV40	3440-3669	SV40 promoter
Kan/Neo	3791-4585	Kanamycin resistance gene
HSV TK Poly A	4821-4839	HSV Thymidine Kinase polyadenylation signal
pUC Ori	5170-5813	pUC origin of replication

MCS Sequence of phCMV-NGFP and phCMV-NLUC Vectors



LIMITED LICENSE: The purchase price paid for the phCMV-FSR™ Vectors grants end users a non-transferable, non-exclusive license to use the kit and/or its components for **internal research use only** as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis, a division of Gene Therapy Systems, Inc. (GTS) -- separate licenses are available for non-research use or applications. p**hCMV-FSR Vectors are not to be used for human diagnostic or included/used in any drug intended for human use.** Care and attention should be exercised in handling the kit components by following appropriate research laboratory practices.
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