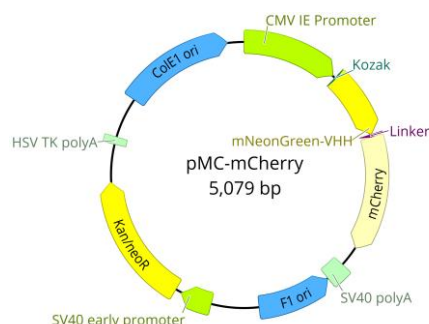


The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by ChromoTek. This vector has not been completely sequenced.



For plasmid sequence, please contact info.de@ptglab.com

## Location of features

PCMV IE: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

mNeonGreen-VHH: 616-1020

mCherry: 1036-1740

SV40 early mRNA polyA: 1866-1987

f1 replication origin: 1994-2449

SV40 early promoter: 2743-2939

Kanamycin/neomycin resistance gene

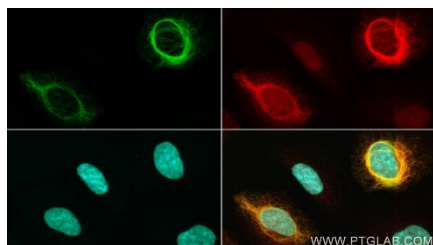
Neomycin phosphotransferase coding sequences:

2977-3768

Herpes simplex virus (HSV) thymidine kinase (TK)

polyadenylation signal: 4000-4047

ColE1 replication origin: 4300-4982



Product	Code	Size
pMC-mCherry	mcr	20 µg
Vector type	mammalian expression vector	
Reporter	mCherry	
Reporter codon usage	mammalian	
Promoter for Chromobody®	PCMV IE	
Host cells	mammalian	
Selection	prokaryotic – kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic – ColE1 ori	
Use	mNeonGreen Chromobody® - mCherry expression in mammalian cells to target mNeonGreen tagged proteins.	

## Vector description

The mNeonGreen Chromobody®- mCherry plasmid (MC-mCherry) is a mammalian expression vector encoding the mNeonGreen-VHH fused to red fluorescent protein mCherry. The vector allows expression of mNeonGreen-mCherry fusion protein in eukaryotic (mammalian) cells. Chromobody® codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996].

The vector backbone contains immediate early promoter of cytomegalovirus (PCMV IE) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, ColE1 origin of replication for propagation in *E. coli* and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA.

SV40 early promoter (P<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

## Expression in mammalian cells

MC-mCherry vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 [Gorman 1985].

## Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

Note: The plasmid DNA was isolated from dam<sup>+</sup>-methylated *E. coli*. Thus, some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam<sup>-</sup> host and make fresh DNA.

## Notice to Purchaser:

Chromobody®-related materials (the Products) are intended for research use only. The Products are covered by U.S. Pat. applications pending. By use of these Products, you accept the terms and conditions of the applicable End User License Agreement (EULA non-profit entities). 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.

**MATERIAL SAFETY DATA SHEET INFORMATION:** To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.