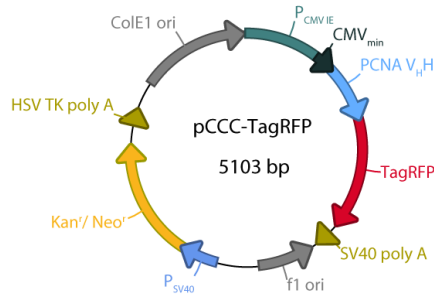


CellCycleChromobody®-TagRFP plasmid

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@chromotek.com

Location of features

PCMV IE: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

PCNA-VHH: 621-986

TagRFP: 1056-1769

Stop codon: 1767-1769

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1919-1924 & 1948-1953

mRNA 3' ends: 1957 & 1969

f1 single-strand DNA origin: 2016-2471

SV40 origin of replication: 2812-2947

SV40 early promoter Enhancer (72-bp tandem repeats): 2648-2719 & 2720-2791

21-bp repeats: 2795-2815, 2816-2836 & 2838-2858

Early promoter element: 2871-2877

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2999-3001; Stop codon: 3791-

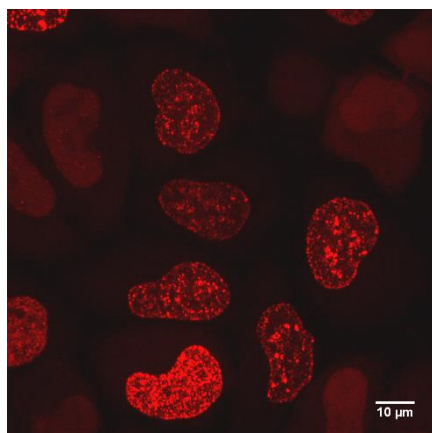
3793

Herpes simplex virus (HSV) thymidine kinase (TK)

polyadenylation signal

Polyadenylation signals: 4029-4034 & 4042-4047

ColE1 replication origin: 4325-5007



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.

| Product | Code | Size |
|--------------------------|---|-------|
| pCCC-TagRFP | ccr | 20 µg |
| Vector type | mammalian expression vector | |
| Reporter | TagRFP | |
| Reporter codon usage | mammalian | |
| Promoter for Chromobody® | P _{CMV IE} | |
| Host cells | mammalian | |
| Selection | prokaryotic – kanamycin eukaryotic - neomycin (G418) | |
| Replication | prokaryotic - pUC ori eukaryotic - SV40 ori | |
| Use | Cell Cycle Chromobody®-TagRFP expression in mammalian cells to monitor cell cycle progression | |

Vector description

Cell Cycle Chromobody®-TagRFP plasmid (pCCC-TagRFP) is a mammalian expression vector encoding the cell cycle marker PCNA-V_{HH} fused to red fluorescent protein TagRFP (from Evrogen). The vector allows expression Cell Cycle Chromobody®-TagRFP 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 (P_{CMV 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_{SV40}) provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Expression in mammalian cells

pCCC-TagRFP 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⁺-methylated *E. coli*. Therefore 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⁻ host and make fresh DNA.