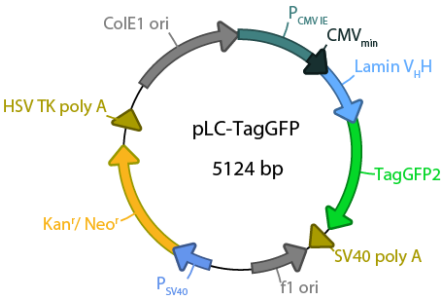
Lamin Chromobody®-TagGFP plasmid

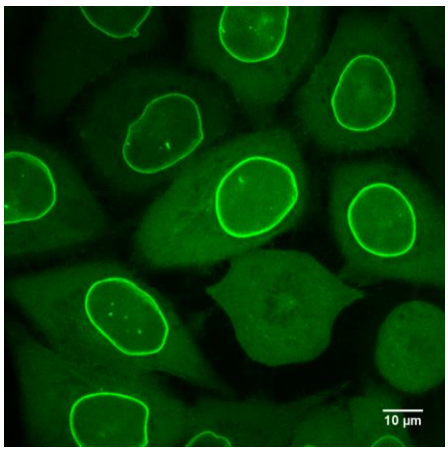
The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by ChromoTek. This vector has not been completely sequenced.



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
Lamin-VHH: 621-1004
TagGFP2: 1077-1790
Stop codon: 1788-1790
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



Product	Code	Size
pLC-TagGFP	lcg	20 µg

Vector type	mammalian expression vector
Reporter	TagGFP2
Reporter codon usage	mammalian
Promoter for Chromobody®	PCMV IE
Host cells	mammalian
Selection	prokaryotic – kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Lamin Chromobody®-TagGFP expression in mammalian cells for non-invasive live cell visualization of endogenous nuclear lamina.

Vector description

Lamin Chromobody®-TagGFP plasmid (pLC-TagGFP) is a mammalian expression vector encoding the marker of the nuclear envelope Lamin-V_HH fused to green fluorescent protein TagGFP2 (from Evrogen). The vector allows expression Lamin Chromobody®-TagGFP 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

pLC-TagGFP 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.