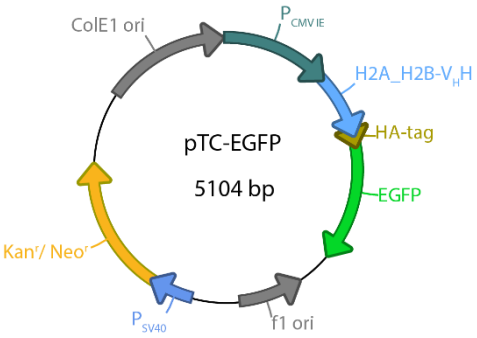
Histone Chromobody-EGFP plasmid

The plasmid map 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

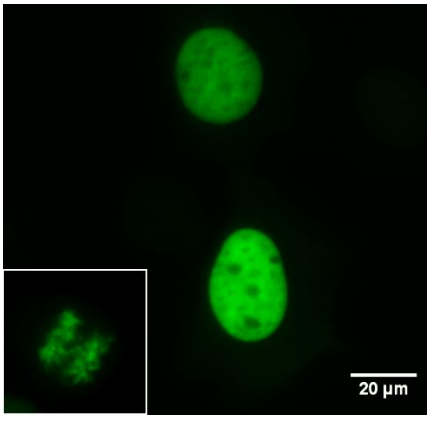


Product	Code	Size
pTC-EGFP	tcg	20 µg plasmid DNA (1 g/L)

Vector type	mammalian expression vector, pEGFP-N1 (Clontech) backbone
Chromobody®	anti-H2A_H2B-V_HH fused to EGFP
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	Histone Chromobody®-EGFP expression in mammalian cells for live-cell visualization of chromatin

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Chromobody coding region:
- H2A_H2B-V_HH: 624-998
- HA-tag: 1005-1031
- EGFP: 1050-1769
- Stop codon: 1767-1769
Sequencing primer locations:
- pEGFPN1for (GTC GTA ACA ACT CCG CCC): 495-512
- pEGFPC1rev (CAT TTT ATG TTT CAG GTT CAG GG): 1873-1851
Kanamycin/neomycin resistance gene



U2OS cells were transiently transfected with the pTC-EGFP plasmid (tcg). The cells were imaged live 18 h after transfection. Histone Chromobody®-EGFP highlights chromatin in transfected cells (green).

Vector description

Histone Chromobody®-EGFP plasmid (pTC-EGFP) is a mammalian expression vector encoding an anti-H2A_H2B-V_HH fused to the enhanced green fluorescent protein EGFP. The vector allows expression of Histone Chromobody-EGFP 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 the 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}) allows neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. A bacterial promoter drives kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with the Herpes simplex virus thymidine kinase (HSV TK) polyadenylation signals.

Expression in mammalian cells

pTC-EGFP vector can be delivered into mammalian cells by common transfection methods, e.g. by lipofection with Lipofectamine®2000. Transfection should be performed according to the instructions of the transfection reagent manufacturer, optimized for the destination cell line. 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. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500. Plasmid incompatibility group is pMB1/ColE1. Plasmid DNA can be isolated from *E. coli* according to standard protocols.

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.