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

Vector description

Histone Chromobody®-EGFP plasmid (pTC-EGFP) is a mammalian expression vector encoding an anti-H2A_H2B-Vi-H fused to EGFP, which 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<sub>CMV IE</sub>) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, ColE1 origin of replication for propagation in <i>E. coli</i> and T1 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>) allows neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. A bacterial promoter drives kanamycin resistance gene expression (Kan<sup>r</sup>) in <i>E. coli</i>. Kan<sup>r</sup>/Neo<sup>r</sup> 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 <i>E. coli</i>

Suitable host strains for propagation in <i>E. coli</i> include DH5alpha, HB101, XL1-Blue, and other general-purpose strains. The vector confers resistance to kanamycin (30 µg/ml) to <i>E. coli</i> hosts. Copy number in <i>E. coli</i> is about 500. Plasmid incompatibility group is pMB1/ColE1. Plasmid DNA can be isolated from <i>E. coli</i> according to standard protocols.

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