Actin Chromobody®-TagGFP 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

Location of features
PCMV IE: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
Actin-VH: 621-986
TagGFP2: 1050-1766
Stop codon: 1764-1766
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

Vector description
Actin Chromobody®-TagGFP plasmid (pAC-TagGFP) is a mammalian expression vector encoding the cytoskeleton marker Actin-Vi-H fused to green fluorescent protein TagGFP2 (from Evrogen). The vector allows expression Actin 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 (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 (Psv40) provides neomycin resistance gene (Neor) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan') in E. coli. Kan'/Neo' gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Expression in mammalian cells
pAC-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.

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.

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Version 2015-07-08