Mammalian expression vector pTagGFP-mito

Vector description

pTagGFP-mito is a mammalian expression vector encoding mitochondria-targeted green fluorescent protein TagGFP. The vector can be used for cyan fluorescent labeling of mitochondria. TagGFP codon usage is optimized for high expression in mammalian cells (humanized) (Haas et al., 1996). A mitochondria-targeting sequence (MTS) is fused to the TagGFP N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase (Rizzuto et al., 1989; Rizzuto et al., 1995).

pTagGFP-mito is not intended as a cloning vector; however, the vector backbone contains unique restriction sites that permit excision of MTS-TagGFP hybrid sequence. 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.

The vector backbone also contains immediate early promoter of cytomegalovirus (PCMVIE) for reporter expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC 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 (Neo) 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

The vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondria-targeted TagGFP in many cell types resulting in green fluorescent labeling of mitochondria. If required, stable transformants can be selected using G418 (Gorman, 1985).

Use

- Expression of mitochondria-targeted TagGFP in mammalian cells under the control of CMV promoter
- Source of mitochondria-targeted TagGFP coding sequence

Product

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat.#</th>
<th>Size</th>
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<tbody>
<tr>
<td>pTagGFP-mito</td>
<td>FP127</td>
<td>20 μg</td>
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Please contact your local distributor for exact prices and delivery information.
**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.

**Location of features**

- **PCMV**: 1-589
- Enhancer region: 59-465
- TATA box: 554-560
- Transcription start point: 583

**TagGFP-mito fusion**

- Start codon (ATG): 597-599
- Mitochondrial targeting sequence (MTS): 597-704
- Start of TagGFP coding sequence (ATG): 705-707
- Stop codon: 1419-1421

**SV40 early mRNA polyadenylation signals**

- Polyadenylation signals: 1575-1580 & 1604-1609
- mRNA 3’ ends: 1613 & 1625

**f1 single-strand DNA origin**: 1672-2127

**Bacterial promoter for expression of Kanf gene**

- -35 region: 2189-2194
- -10 region: 2212-2217
- Transcription start point: 2224

**SV40 origin of replication**: 2468-2603

**SV40 early promoter**

- Enhancer (72-bp tandem repeats): 2301-2372 & 2373-2444
- 21-bp repeats: 2448-2468, 2469-2489 & 2491-2511
- Early promoter element: 2524-2530
- Major transcription start points: 2520, 2558, 2564 & 2569

**Kanamycin/neomycin resistance gene**

- Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2652-2654
  - Stop codon: 3444-3446
  - G→A mutation to remove Pst I site: 2834
  - C→A (Arg to Ser) mutation to remove BssH II site: 3180

**Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals**

- Polyadenylation signals: 3682-3687 & 3695-3700

**pUC plasmid replication origin**: 4031-4674

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**References**