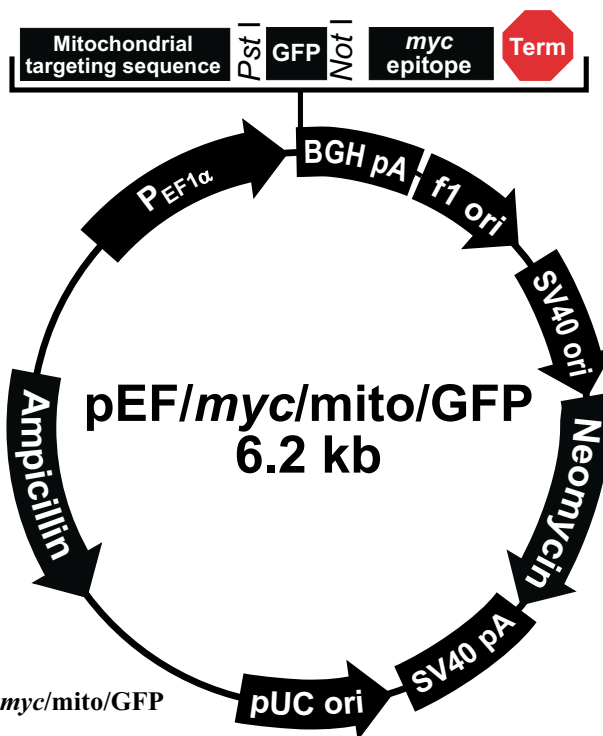


## pEF/myc/mito/GFP Map

### Map

The figure below summarizes the features of pEF/myc/mito/GFP. The complete nucleotide sequence for pEF/myc/mito/GFP is available for downloading from our Web site ([www.invitrogen.com](http://www.invitrogen.com)) or by contacting Technical Service (see page 26).



**Comments for pEF/myc/mito/GFP**  
6217 nucleotides

EF-1 $\alpha$  promoter: bases 6-1192

EF Forward priming site: bases 1133-1153

Mitochondrial targeting sequence: bases 1196-1282

GFP ORF: bases 1289-2005

*myc* epitope: bases 2015-2044

BGH Reverse priming site: 2080-2097

BGH polyadenylation sequence: bases 2079-2293

f1 origin: bases 2356-2769

SV40 promoter/origin: bases 2834-3141

Neomycin resistance gene (ORF): bases 3149-3943

SV40 polyadenylation sequence: bases 3960-4198

pUC origin: bases 4381-5054 (Complementary strand)

Ampicillin resistance gene (ORF): 5199-6059 (Complementary strand)

## Appendix

### Features of pEF Plasmids

**Table**

The table below summarizes the features of the pShooter™ vectors. These vectors were derived from pcDNA3. Changes to the vector backbone are noted. In addition, features that are unique to one vector are noted.

<b>Feature</b>	<b>Benefit</b>
Human EF-1 $\alpha$ promoter	Permits efficient, high-level expression of your recombinant protein (Kim <i>et al.</i> , 1990; Mizushima and Nagata, 1990; Uetsuki <i>et al.</i> , 1989). For more detailed information on this promoter, please see the next page.
Mitochondrial targeting sequence pEF/myc/mito only	Allows efficient targeting to the mitochondria. Isolated from subunit VIII of human cytochrome c oxidase (Rizzuto <i>et al.</i> , 1992).
ER signal peptide pEF/myc/ER only	Directs the protein of interest to the ER for retention in the ER or secretion. This is the signal peptide from a mouse V <sub>h</sub> chain (Kabat <i>et al.</i> , 1987).
Multiple cloning site	Allows insertion of your gene.
Nuclear targeting sequence pEF/myc/nuc only	Permits efficient targeting of your protein to the nucleus. Sequence is triplicated to ensure proper localization. Isolated from SV40 large T antigen (Fisher-Fantuzzi and Vesco, 1988).
<i>c-myc</i> epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of your recombinant protein by immunofluorescence with the Anti- <i>myc</i> Antibody (Catalog no. R950-25) (Evan <i>et al.</i> , 1985).
ER retention signal pEF/myc/ER only	Permits retention of your protein in the ER (Munro and Pelham, 1987).
TAG termination codon	For efficient termination of translation.
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992).
f1 origin	Allows rescue of single-stranded DNA.
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen (i.e. COS). <i>Nco</i> I site removed by site-directed mutagenesis.
Neomycin resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982). Tn5 sequence removed and the Kozak sequence improved by PCR at the 5' end of the ORF. <i>Nco</i> I, <i>Pst</i> I, and <i>Bss</i> H II sites removed by site-directed mutagenesis.
SV40 polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA.
pUC origin	High-copy number replication and growth in <i>E. coli</i> . <i>Apa</i> L I site removed by site-directed mutagenesis.
Ampicillin resistance gene ( $\beta$ -lactamase)	Selection of vector in <i>E. coli</i> . <i>Apa</i> L I site removed by site-directed mutagenesis.

# EF-1 $\alpha$ Promoter

## Description

The diagram below shows all the features of the EF-1 $\alpha$  promoter used in the pShooter<sup>TM</sup> vectors (Mizushima and Nagata, 1990). Features are marked as per Uetsuki, *et al.*, 1989. The original sequence has been mutagenized to remove the *Pst* I, *Bgl* II, *Afl* II, and *Xho* I restriction sites. In addition, *Kpn* I, *Eco*R I, *Hind* III, *Pml* I, and *Nco* I were introduced by PCR. The EF-1 $\alpha$  promoter can be excised using *Kpn* I, *Eco*R I, or *Hind* III and *Pml* I or *Nco* I.

