Plasmid RD-PGA16

Quantity:	50 µg supercoiled plasmid DNA, ethanol-precipitated and air dried.
Storage and Reconstitution:	The dry pellet is shipped at room temperature. To reconstitute, spin the tube briefly and dissolve the contents in 50 µl of sterile, nuclease-free water to make a 1 µg/µl solution. Store at 4˚C.

Note: A License from Molecular Devices Corporation is required prior to purchasing or using the plasmid.

Description

The cDNA for Gα16, the alpha-subunit of the promiscuous G-protein, was cloned from human hematopoietic cell line TF-1 by RT-PCR. When expressed in transfected cells, the Gα16 protein allows many Gi/o- and Gs-coupled receptors, which normally act through the cAMP pathway, to stimulate phospholipase C and mobilize intracellular calcium.

RD-PGA16 will be provided in two constructs with two different selectable markers (hygromycin, G418) to allow co-transfection with receptors.

pCEP-Gα16 (hygromycin)

- 1.5-kb human Gα16 cDNA in pCEP4 (vector size is 10.4-kb)
- Cytomegalovirus (CMV) enhancer/promoter for strong, constitutive expression of Gα16
- Hygromycin resistance gene for selection of stable transfected cells

- Epstein Barr Virus (EBV) origin of replication (oriP) and nuclear antigen (EBNA) for episomal replication in canine and primate cell lines
- Amp^r and ColE1 origin for selection and propagation in E. coli

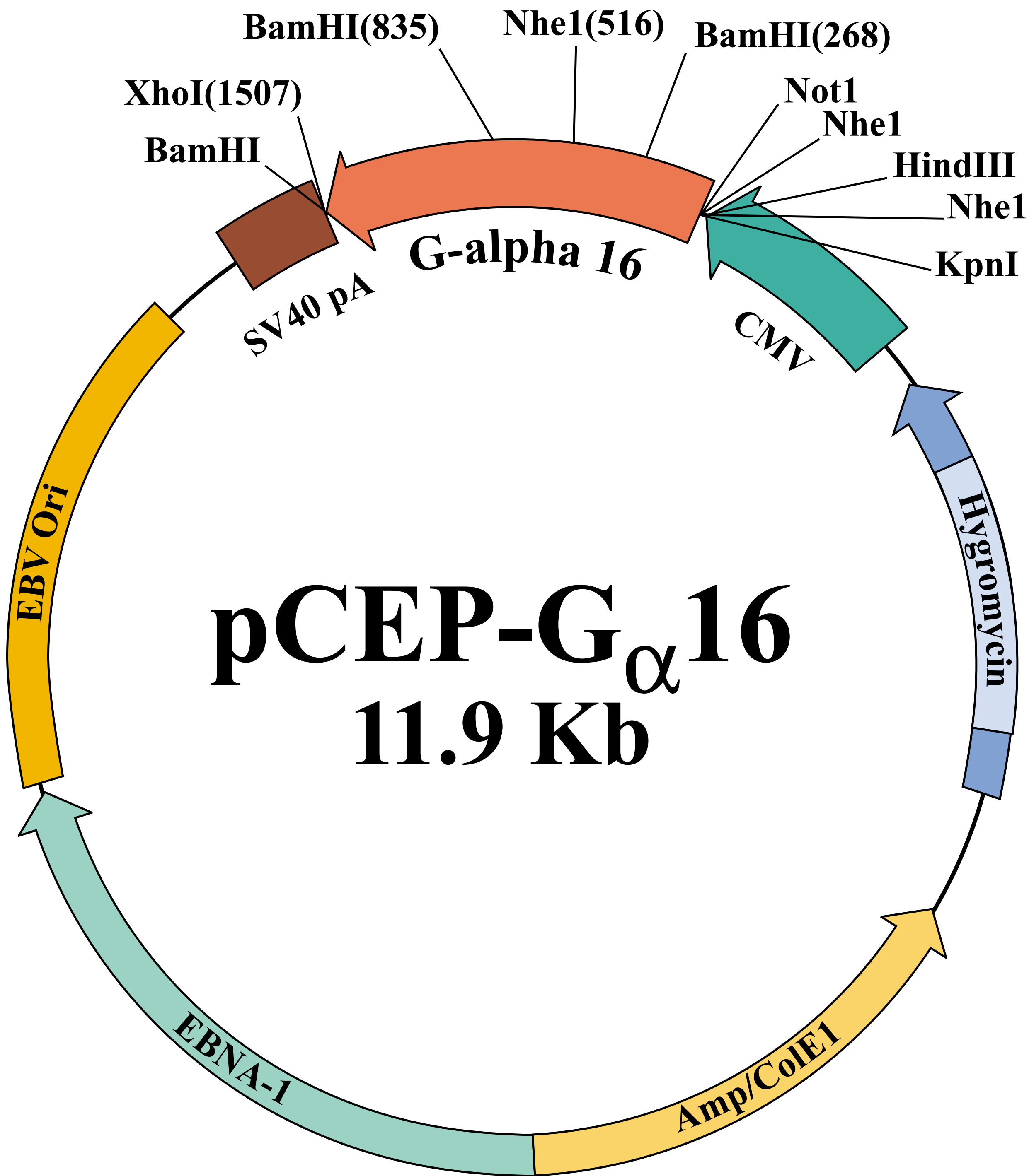
pLEC1-Gα16 (G418)

- 1.5-kb Gα16 cDNA in pLEC1 (vector size is 5.5-kb)
- Cytomegalovirus (CMV) enhancer/promoter for strong, constitutive expression of Gα16
- Neomycin resistance gene for G418 selection of stable transfected cells
- T7 and SP6 promoters allowing *in vitro* synthesis of sense and antisense Gα16 RNA transcripts
- Amp^r and ColE1 origin for selection and propagation in E. coli

Quality Control

1. Identity and purity of the plasmid DNA is confirmed by restriction analysis on agarose gel.

2. Functional testing is carried out by transient transfection of the plasmid into CHO cells stably expressing the human nociceptin receptor (Gi/o-coupled) and measuring the stimulation of calcium mobilization by nociceptin in the FLIPR™ instrument (Molecular Devices Corporation, Sunnyvale, CA).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BamHI(268)
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Nhe1
HindIII
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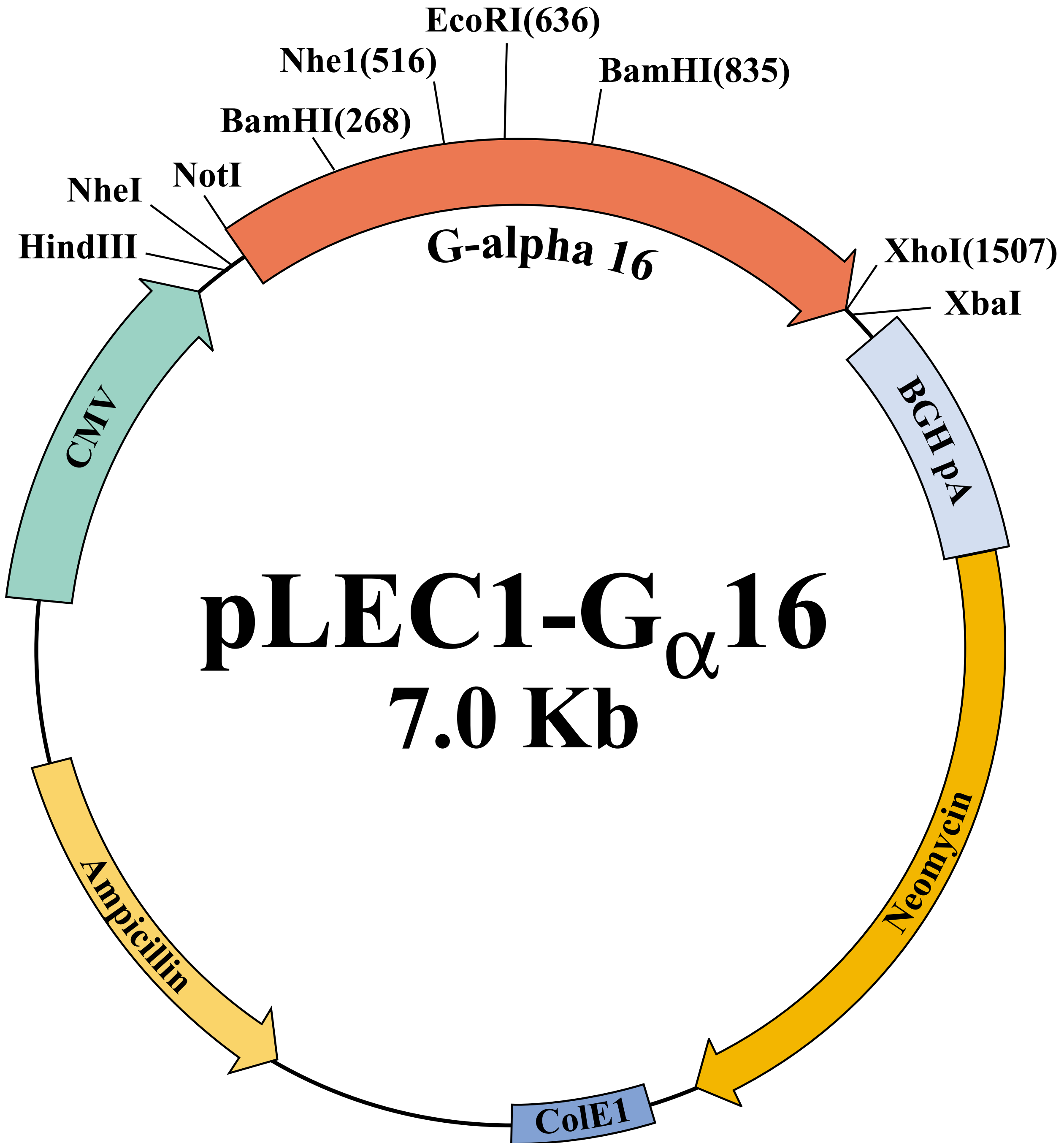
SV40 pA
CMV
EBV Ori
Hygromycin
pCEP-Gα16
11.9 Kb
EBNA-1
Amp/ColE1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BamHI(835)
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NotI
NheI
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XhoI(1507)
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BGH pA
CMV
pLEC1-Gα16
7.0 Kb
Neomycin
Ampicillin
ColE1