General Information

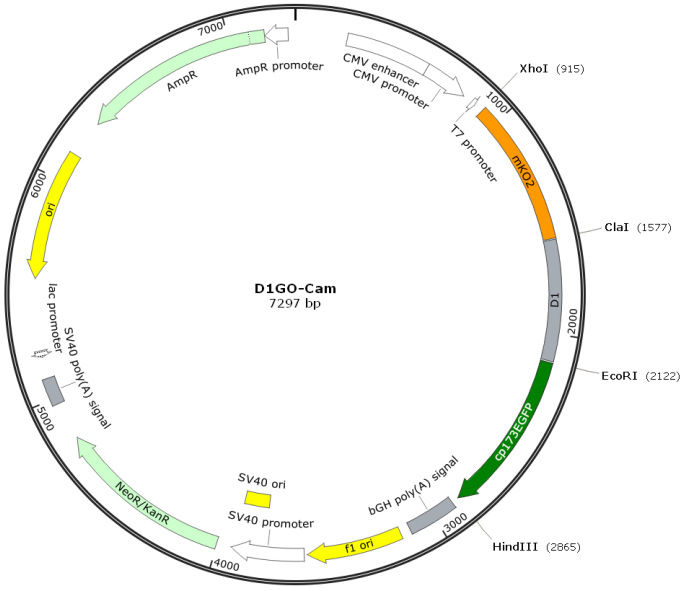
This product is non-toxic, non-contagious and is not intended for human use. The plasmid DNA in the package is synthetic and only for research and development purposes. It does not present any danger for humans, animals or the environment. The product is only for in vitro studies and is not for commercial use.

D1GO-Cam vector

This vector has not been completely sequenced. All provided information regarding the vector composition was compiled using the information from published literature, other sources together with partial sequences obtained by NGFI.

Vector description

D1GO-Cam is a genetically encoded FRET-based probe for imaging cytosolic Ca^{2+} of intact mammalian cells. In order to express D1GO-Cam in cells of interest, 20 µg of purified endotoxin-free plasmid DNA coding for D1GO-Cam is provided. The plasmid coding for D1GO-Cam represents a mammalian expression vector with a strong viral promotor. For plasmid amplification in E.coli ampicillin should be used. 1 – 1.5 µg DNA is required for cell transfection in a single well of a stand ard 6-well dish following standard transfection procedures. Usually cells express high amounts of D1GO-Cam 24 – 48 hours after cell transfection. Standard optical filters for GFP/OFP or alternatively GFP/RFP FRET imaging should be used. The vector can be also used as a source of D1GO-Cam coding sequence. Flanking restriction sites are convenient for excision of D1GO-Cam sequence and its further insertion into other expression vectors of choice.



Expression in mammalian cells

D1GO-Cam vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive D1GO-Cam expression in eukaryotic cells.

Propagation in E. coli

Suitable host strains for propagation in E. coli include DH5alpha, HB101, XL1-Blue, and other general purpose strains. The vector confers resistance to ampicillin (100 µg/ml) to E. coli hosts.

Note: The higher excitation wavelength of red-shifted cameleons (~ 480 nm) allows a combination with UV-excitable chemical indicators such as fura-2 for simultaneous real-time imaging of Ca^{2+} dynamic within the cytosol in a ratiometric manner.

References

Waldeck-Weiermair M. (2012) “Spatiotemporal Correlations between Cytosolic and Mitochondrial Ca2+ Signals Using a Novel Red-Shifted Mitochondrial Targeted Cameleon” (http://journals.plos.org/plosone/article/asset?id=10.1371%2Fjournal.pone.0045917.PDF)

Neue Stiftingtalstrasse 6/6, 8010 Graz, Austria
+43 316 385 71960
sales@ngfi.eu
www.ngfi.eu