



Next Generation Fluorescence Imaging

Smart Sensor Solutions

General Information

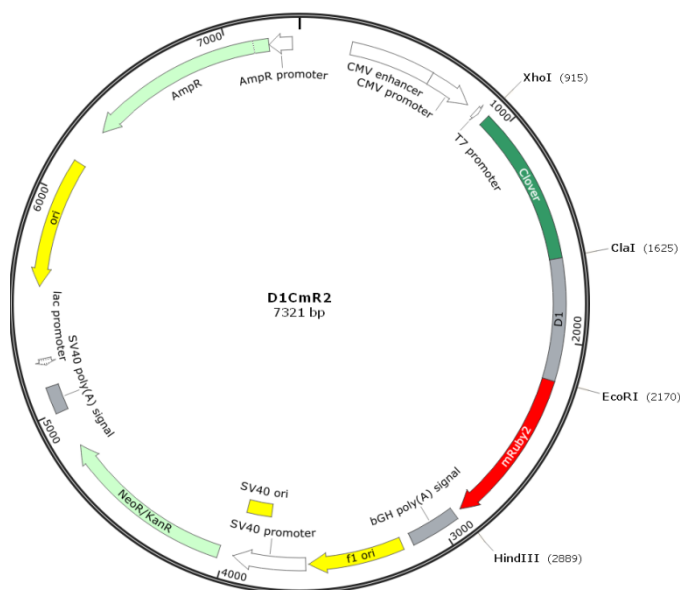
This product is non-toxic, non-contagious and is not intended for human use. The plasmid DNA in the package is synthetic and only for research and development purposes. It does not present any danger for humans, animals or the environment. The product is only for in vitro studies and is not for commercial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

D1CmR2 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

D1CmR2 is a genetically encoded FRET-based probe for imaging cytosolic Ca^{2+} with decreased Ca^{2+} sensitivity of intact mammalian cells. In order to express D1CmR2 in cells of interest, 20 μg of purified endotoxin-free plasmid DNA coding for D1CmR2 is provided. The plasmid coding for D1CmR2 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 standard 6-well dish following standard transfection procedures. Usually cells express high amounts of D1CmR2 24 – 48 hours after cell transfection. Standard optical filters for GFP/RFP or alternatively GFP/OPF FRET imaging should be used. The vector can be also used as a source of D1CmR2 coding sequence. Flanking restriction sites are convenient for excision of D1CmR2 sequence and its further insertion into other expression vectors of choice.



Expression in mammalian cells

D1CmR2 vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive D1CmR2 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 $\mu\text{g}/\text{ml}$) to E. coli hosts.

Note: The higher excitation wavelength of red-shifted cameleons ($\sim 480 \text{ nm}$) 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. (2015) "Generation of Red-Shifted Cameleons for Imaging Ca^{2+} Dynamics of the Endoplasmic Reticulum" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4507692/pdf/sensors-15-13052.pdf>)

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