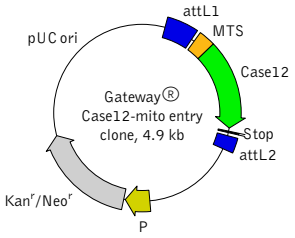
Gateway® Case12-mito entry clone

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.



For vector sequence, please visit our Web site at http://www.evrogen.com/products/vectors.shtml

Product	Cat.#	Size
Gateway® Case12-mito entry clone	**FP995**	20 μg

Vector type	Gateway® entry clone
Reporter	Case12
Reporter codon usage	mammalian
Promoter for Case12	NO
Host cells	prokaryotic
Selection	kanamycin
Replication	pUC ori
Use	Transfer of Case12-mito coding sequence into Gateway® destination vectors

Location of features

attL1 site: 14-113
Case12-mito: 120-1475
attL2 site: 1494-1593
Kanamycin resistance gene: 2811-3605
pUC origin of replication: 4190-4833

Vector description

Gateway® Case12-mito entry clone is a vector containing coding sequence of mitochondria-targeted fluorescent sensor Case12. Mitochondrial targeting sequence (MTS) is fused to the Case12 N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase [Rizzuto et al. 1989; Rizzuto et al. 1995]. Case12-mito coding sequence is flanked by attL1 and attL2 sites allowing easy site-specific recombination. The Invitrogen Gateway® Technology provides a rapid and highly efficient way to transfer the Case12-mito gene into a number of Gateway® destination vectors for expression in different experimental systems.

The vector backbone contains pUC origin of replication and kanamycin resistance gene (Kan^r) for propagation and selection in *E. coli*.

LR site-specific recombination

Please refer to Invitrogen Gateway® Technology description for detailed instructions regarding LR site-specific recombination reaction. In general, to transfer Case12-mito gene into the destination vector you will need:

- Purified plasmid DNA of Gateway® Case12-mito
- A destination vector of choice
- Invitrogen LR Clonase ™ II enzyme mix (Invitrogen Cat.# 11791-020)
- Proteinase K solution (supplied with the LR Clonase™ II enzyme mix)
- TE-Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- Appropriate chemically competent *E. coli* host and growth media for expression
- Appropriate selective plates.

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.

References

Rizzuto, R. et al. (1989) "A gene specifying subunit VIII of human cytochrome c oxidase is localized to chromosome 11 and is expressed in both muscle and non-muscle tissues." J Biol Chem, 264 (18): 10595–10600 / pmid: 2543673

Rizzuto, R. et al. (1995) "Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells." Curr Biol, 5 (6): 635–642 / pmid: 7552174

Gateway® Technology. Ver. E. 13 May 2010, 25-0522. http://tools.invitrogen.com/content/sfs/manuals/gatewayman.pdf (visited on 17. 02. 2012)