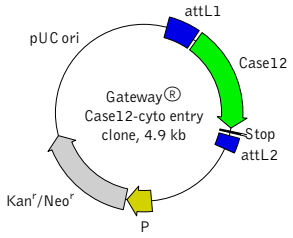
Gateway® Case12-cyto entry clone

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-cyto entry clone	**FP994**	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 coding sequence into Gateway® destination vectors

Location of features

attL1 site: 14-113
MCS: 117-194
Kozak translation initiation site: 195-205
Case12: 202-1449
attL2 site: 1468-1567
Kanamycin resistance gene: 2785-3579
pUC origin of replication: 4164-4807

Vector description

Gateway® Case12-cyto entry clone is a vector containing coding sequence of cytosolic fluorescent sensor Case12. Case12 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 gene into a number of Gateway® destination vectors for expression in different experimental systems.

To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the Case12 coding sequence [Kozak 1987].

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 gene into the destination vector you will need:

- Purified plasmid DNA of Gateway® Case12-cyto
- 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

Kozak, M. (1987) “An analysis of 5’-noncoding sequences from 699 vertebrate messenger RNAs.” Nucleic Acids Res, 15 (20): 8125–8148 / pmid: 3313277

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