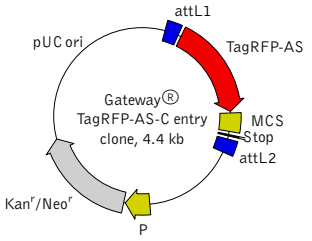
Gateway® TagRFP-AS-C entry clone

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.

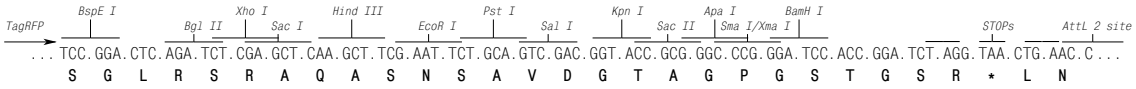


For vector sequence, please visit our Web site at http://www.evrogen.com/products/vectors.shtml

Product	Cat.#	Size
Gateway® TagRFP-AS-C entry clone	**FP148**	20 μg

Vector type	Gateway® entry clone
Reporter	TagRFP
Reporter codon usage	Arabidopsis and Saccharomyces
Promoter for TagRFP	NO
Host cells	prokaryotic
Selection	prokaryotic
Replication	pUC ori
Use	Generation of fusions to the C-terminus of TagRFP; transfer of the construct encoding TagRFP or its fusion into Gateway® destination vectors

Multiple cloning site (MCS)



Location of features

attL1 site: 14-113
Kozak translation initiation site: 129-139
TagRFP-AS: 136-846
MCS: 847-924
attL2 site: 932-1031
Kanamycin resistance gene: 2256-3050
pUC origin of replication: 3635-4278

Vector description

Gateway® TagRFP-AS-C entry clone is a vector containing red (orange) fluorescent protein TagRFP gene variant with codon usage optimized for high expression in Arabidopsis and Saccharomyces. TagRFP 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 TagRFP gene into a number of Gateway® destination vectors for expression in different experimental systems. Multiple cloning site (MCS) located at the 3'-end of TagRFP gene allows to generate fusions to the TagRFP C-terminus for expression, localization and cellular dynamics studies.

To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the TagRFP 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*.

Generation of TagRFP fusion proteins

A localization signal or a gene of interest can be cloned into MCS of the vector both before and after site-specific recombination with a destination vector. It will be expressed as a fusion to the TagRFP C-terminus when inserted in the same reading frame as TagRFP and no in-frame stop codons are present.

Alternatively, TagRFP gene can be fused to the 3'-end of a gene of interest by LR recombination of the Gateway® TagRFP-AS-C with a destination vector containing this gene in a correct reading frame. In this case, the protein of interest will be expressed as a fusion to the TagRFP N-terminus.

TagRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

Note: The plasmid DNA was isolated from dam$^+$-methylated *E.coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam$^-$ host and make fresh DNA.

LR site-specific recombination

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

- Purified plasmid DNA of Gateway® TagRFP-AS-C
- 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)