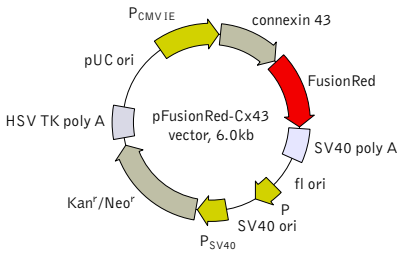
pFusionRed-Cx43 vector

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
pFusionRed-Cx43 vector	**FP417**	20 μg

Vector type	mammalian expression vector
Reporter	FusionRed
Reporter codon usage	mammalian
Promoter for FusionRed	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	red fluorescent labeling of connexin 43

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
Connexin 43-FusionRed fusion: 824-2689
Start codon: 824-826
Connexin 43: 824-1969
FusionRed: 1991-2689
Stop codon: 2687-2689
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2842-2847 & 2871-2876
mRNA 3' ends: 2880 & 2892
f1 single-strand DNA origin: 2939-3394
Bacterial promoter for expression of Kan^r gene
-35 region: 3456-3461; -10 region: 3479-3484
Transcription start point: 3491
SV40 origin of replication: 3735-3870
SV40 early promoter
Enhancer (72-bp tandem repeats): 3568-3639 & 3640-3711
21-bp repeats: 3715-3735, 3736-3756 & 3758-3778
Early promoter element: 3791-3797
Major transcription start points: 3787, 3825, 3831 & 3836
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3919-3921; Stop codon: 4711-4713
G->A mutation to remove Pst I site: 4101
C->A (Arg to Ser) mutation to remove BssH II site: 4447
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4949-4954 & 4962-4967
pUC plasmid replication origin: 5298-5941

Vector description

pFusionRed-Cx43 is a mammalian expression vector encoding FusionRed-Cx43 fusion protein. The vector can be used for fluorescent labeling of connexin 43 in living cells.

FusionRed codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Rat connexin 43 is fused to the FusionRed N-terminus.

pFusionRed-Cx43 vector can be used as a source of FusionRed-Cx43 hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

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.

The vector backbone contains immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli*, and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA.

SV40 early promoter (P_{SV40}) provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Expression in mammalian cells

pFusionRed-Cx43 vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the FusionRed-Cx43 fusion in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman 1985].

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

Gorman, C. (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143–190.

Haas, J. et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." Curr Biol, 6 (3): 315–324 / pmid: 8805248