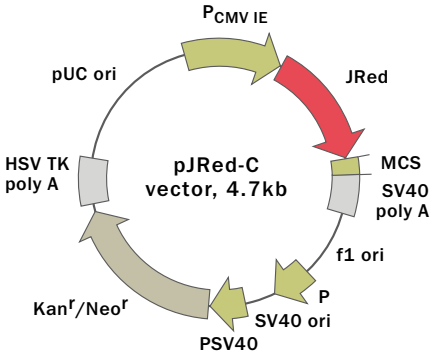
Mammalian expression vector pJRed-C



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pJRed-C	**FP701**	20 µg

Please contact your local distributor for exact prices and delivery information.

Vector type	mammalian expression vector
Reporter	JRed
Reporter codon usage	mammalian
Promoter for JRed	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)

JRed → BspE1 BglII SacI EcoRI SalI KpnI ApaI BamHI STOPs

TCC.GGA.CTC.AGA.TCT.CGA.GCT.CAA.GCT.TCG.AAT.TCT.GCA.GTC.GAC.GGT.ACC.GCG.GGC.CCG.GGA.TCC.ACC.GGA.TCT.AGA.TAA.CTG.ATC.A

XhoI HindIII PstI SacII SmaI/XmaI XbaI# BclI#

— sites are blocked by methylation.

Use

- Generation of fusions to the JRed C-terminus

- Expression of JRed or its fusions in mammalian cells

Vector description

pJRed-C vector is an eukaryotic (mammalian) expression vector encoding true red fluorescent protein, JRed. The vector allows to generate fusions to the JRed C-terminus and to express JRed fusions or JRed alone in mammalian cells.

JRed codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al*., 1996). To increase JRed translation, Kozak consensus translation initiation site is generated upstream of the JRed sequence (Kozak, 1987). Multiple cloning site (MCS) is located between JRed coding sequence and SV40 polyadenylation signal (SV40 polyA).

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 polyA direct proper processing of the 3' end of the reporter mRNA.

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

Generation of fusions

A localization signal (or a gene of interest) should be inserted into MCS of the vector. It will be expressed as a fusion to the JRed C-terminus when inserted in the same reading frame as JRed and no intervening stop codons are present. JRed-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express JRed, when transfected into eukaryotic (mammalian) cells.

Notes: 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.

Despite its dimeric structure, JRed is still suitable for generation of fusions with proteins of interest, however we recommend that you use TagRFP or TagFP635 for these purposes.

Expression in mammalian cells

The vector can be transfected into mammalian cells by any known transfection method. 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.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465; TATA box: 554-560
Transcription start point: 583
JRed gene
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615; Stop codons: 1417-1419
Last amino acid in JRed: 1336-1338
MCS: 1339-1427
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1559-1564 & 1558-1593
mRNA 3' ends: 1597 & 1609
f1 single-strand DNA origin: 1656-2111
(packages the noncoding strand of JRed)
Bacterial promoter for expression of Kan^r gene
-35 region: 2173-2178; -10 region: 2196-2201
Transcription start point: 2208
SV40 origin of replication: 2452-2587
SV40 early promoter
Enhancer (72-bp tandem repeats): 2285-2356 & 2357-2428
21-bp repeats: 2432-2452, 2453-2473, & 2475-2495
Early promoter element: 2508-2514
Major transcription start points: 2504, 2542, 2548 & 2553
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2636-2638; stop codon: 3428-3430
G->A mutation to remove PstI site: 2818
C->A (Arg to Ser) mutation to remove BssHII site: 3164
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3666-3671 & 3679-3684
pUC plasmid replication origin: 4015-4658

References

Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. *et al*. (1996) Curr. Biol. 6: 315-324.

Kozak M. (1987) Nucleic Acids Res. 15:8125-8148.