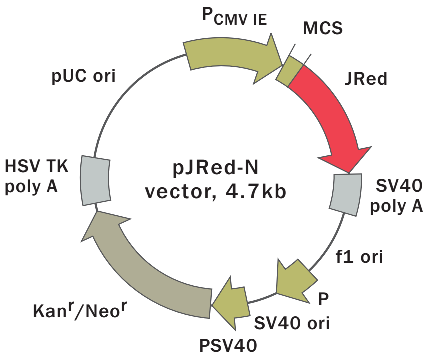
Mammalian expression vector pJRed-N



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

Product	Cat.#	Size
pJRed-N	**FP702**	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)

NheI — *BglII* — *SacI* — *EcoRI* — *SalI* — *SacII* — *SmaI/XmaI* — *AgeI* — JRed →

GCTA.GCG.CTA.CCG.GAC.TCA.GAT.CTC.GAG.CTC.AAG.CTT.CGA.ATT.CTG.CAG.TCG.ACG.GTA.CCG.CGG.GCC.CGG.GAT.CCA.CCG.GTC.GCC.ACC.ATG.G..

AfeI — *XhoI* — *HindIII* — *PstI* — *KpnI* — *ApaI* — *BamHI* — *NcoI**

* — not unique sites.

Use

- Generation of fusions to the JRed N-terminus

- Expression of JRed or its fusions in mammalian cells

Vector description

pJRed-N vector is an eukaryotic (mammalian) expression vector encoding true-red fluorescent protein JRed. The vector allows to generate fusions to the JRed N-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 JRed sequence (Kozak, 1987). Multiple cloning site (MCS) is located between $P_{CMV\ IE}$ and JRed coding sequence.

The vector backbone comprises 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 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 cloned into MCS of the vector. It will be expressed as a fusion to the JRed N-terminus when inserted in the same reading frame as JRed and no intervening stop codons are present. The inserted sequence should contain an initiating ATG codon. TurboFP635-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

Notes: The plasmid DNA was isolated from dam$^+$-methylated *E.coli*. Therefore some restriction sites are bloked 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 to use TagFP635 or TagRFP 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). Unmodified pJRed-N will express JRed, when transfected into eukaryotic (mammalian) cells.

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
MCS: 591-671
JRed gene
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681; Stop codon: 1405-1407
Polyadenylation signals: 1561-1566 & 1590-1595
mRNA 3' ends: 1599 & 1611
f1 single-strand DNA origin: 1658-2113
(packages the noncoding strand of JRed)
Bacterial promoter expression of Kan^r gene:
-35 region: 2175-2180; -10 region: 2198-2203
Transcription start point: 2210
SV40 origin of replication: 2454-2589
SV40 early promoter
Enhancer (72-bp tandem repeats): 2287-2358 & 2359-2430
21-bp repeats: 2434-2454, 2455-2475, & 2477-2497
Early promoter element: 2510-2516
Major transcription start points: 2506, 2544, 2550 & 2555
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2638-2640; stop codon: 3430-3432
G->A mutation to remove PstI site: 2820
C->A (Arg to Ser) mutation to remove BssHII site: 3166
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3668-3673 & 3681-3686
pUC plasmid replication origin: 4017-4660

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.