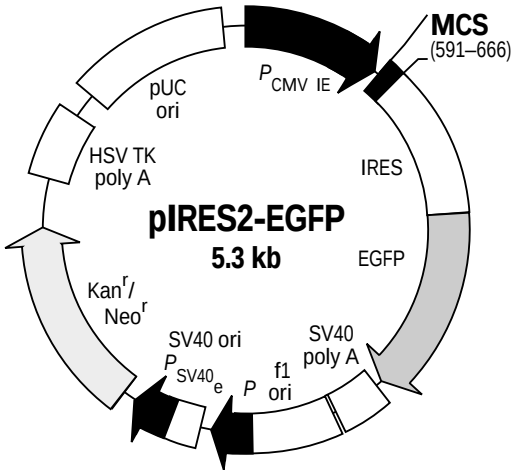

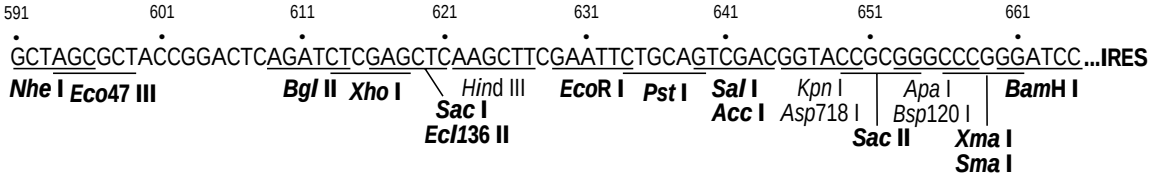

Restriction Map and Multiple Cloning Site (MCS) of pIRES2-EGFP Vector. Unique restriction sites are in bold. Note that the *Eco*47 III site has not been confirmed in the final construct.

Description:

pIRES2-EGFP contains the internal ribosome entry site (IRES; 1, 2) of the encephalomyocarditis virus (ECMV) between the MCS and the enhanced green fluorescent protein (EGFP) coding region. This permits both the gene of interest (cloned into the MCS) and the EGFP gene to be translated from a single bicistronic mRNA. pIRES2-EGFP is designed for the efficient selection (by flow cytometry or other methods) of transiently transfected mammalian cells expressing EGFP and the protein of interest. This vector can also be used to express EGFP alone or to obtain stably transfected cell lines without time-consuming drug and clonal selection.

EGFP is a red-shifted variant of wild-type GFP (3–5) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) EGFP encodes the GFPmut1 variant (6) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (7). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (8) to further increase the translation efficiency in eukaryotic cells. The MCS in pIRES2-EGFP is between the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the IRES sequence. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the bicistronic mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pIRES2-EGFP backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. pIRES2-EGFP replaces (but is not derived from) the pIRES-EGFP Vector previously sold by BD Biosciences Clontech. pIRES2-EGFP is functionally similarly to pIRES-EGFP; however, pIRES2-EGFP gives brighter EGFP fluorescence than the older vector. Note that the *Xba* I site at position

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1987 is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Use:
Genes inserted into the MCS should include the initiating ATG codon. pIRES2-EGFP and its derivatives can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (9).

Location of features:
- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560; Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- MCS: 591–665
- IRES sequence: 666–1250
- Enhanced green fluorescent protein (EGFP) gene
  Kozak consensus translation initiation site: 1247–1257
  Start codon (ATG): 1254–1256; Stop codon: 1971–1973
  Insertion of Val at position 2: 1257–1259
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 1446–1451
  His-231 to Leu mutation (A→T): 1948
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2127–2132 & 2156–2161; mRNA 3' ends: 2165 & 2177
- f1 single-strand DNA origin: 2224–2679 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2741–2746; –10 region: 2764–2769
  Transcription start point: 2776
- SV40 origin of replication: 3020–3155
- SV40 early promoter/enhancer
  72-bp tandem repeats: 2853–2996; 21-bp repeats (3): 3000–3063
  Early promoter element: 3076–3082
- Kanamycin/neomycin resistance gene: 3204–3998
  G→A mutation to remove *Pst* I site: 3386; C→A (Arg to Ser) mutation to remove *Bss*H II site: 3732
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals: 4234–4252
- pUC plasmid replication origin: 4583–5226

Propagation in *E. coli*
- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

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3. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
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7. Jang, S. K., *et al.* (1988) *J. Virol.* **62**:2636–2643.
8. Huang, M. T. F. & Gorman, C. M. (1990) *Nucleic Acids Res.* **18**(4):937–947.
9. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

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