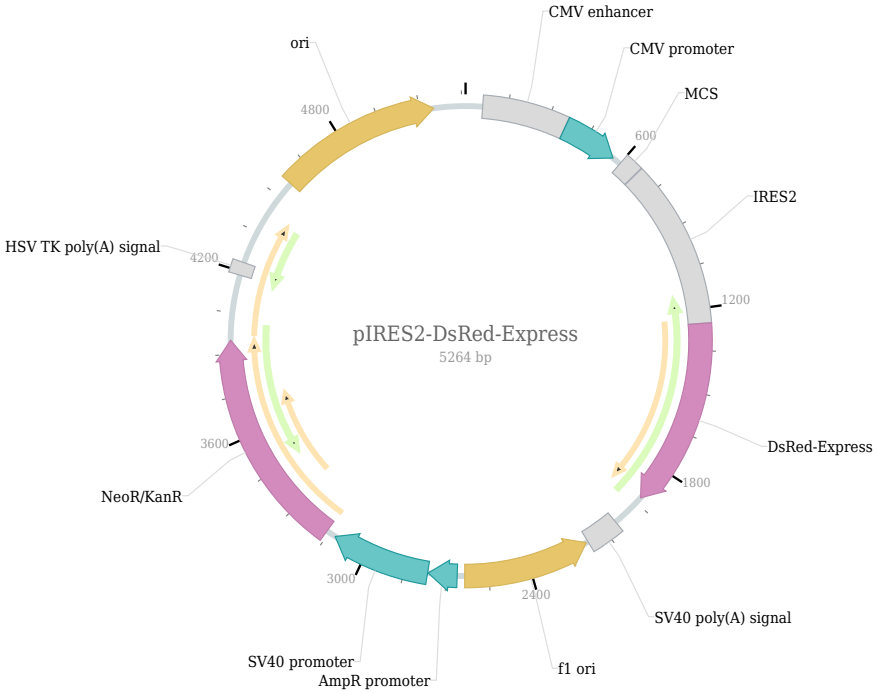
pIRES2-DsRed-Express

Vector ID	V007487
Antibiotic Resistance	Kanamycin
Length	5264 bp
Vector Type	Mammalian Expression Vectors

Product Information

Replication Origin	ori
Selection Marker	Neomycin
Copy Number	High copy number
Promoter	CMV
Cloning Method	Enzyme digestion and ligation
5' Primer	CMV-F: CGCAAATGGGCGGTAGGCGTG
3' Primer	IRES-R: CCTCACATTGCCAAAAGACG
Growth Strain(s)	DH10B
Comment	pIRES2-DsRed-Express contains the internal ribosome entry site (IRES; 1, 2) of the encephalomyocarditis virus (ECMV) between the MCS and a mutant of the Discosoma sp. red fluorescent protein DsRed-Express coding region (3–5). This permits both the gene of interest (cloned into the MCS) and the DsRed-Express gene to be translated from a single bicistronic mRNA. pIRES2-DsRed-Express is designed for the efficient selection by flow cytometry of transiently transfected mammalian cells expressing DsRed-Express and the protein of interest. This vector can also be used to express DsRed-Express alone or to obtain stably transfected cell lines without timeconsuming drug and clonal selection.DsRed-Express is a human codon-optimized (4) DsRed variant of Discosoma sp. red fluorescent protein (6). Its coding sequence contains nine amino acid substitutions which improve solubility of the protein and reduce the time from transfection to detection of red fluorescence (exitation and emission maxima = 557 nm and 579 nm, respectively). In addition, these substitutions reduce the level of residual green emission (5). Although DsRed-Express most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express displays a reduced tendency to aggregate (3, 5).The MCS in pIRES2-DsRed-Express is between the immediate early promoter of cytomegalovirus (P CMV IE) and the IRES sequence. SV40 polyadenylation signals downstream of the DsRed-Express 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-DsRed-Express backbone also provides a pUC origin of replication for propagation in E. coli and an f1 origin for single-stranded DNA production.

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.