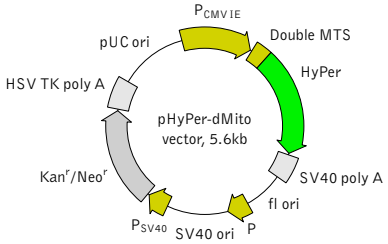
pHyPer-dMito vector

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
pHyPer-dMito vector	**FP942**	20 μg

Vector type	mammalian expression vector
Reporter	HyPer
Reporter codon usage	mammalian/E. coli
Promoter for HyPer	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Expression of mitochondria-targeted fluorescent hydrogen peroxide sensor HyPer in mammalian cells under the control of CMV promoter; source of mitochondria-targeted HyPer coding sequence

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
HyPer-dMito fusion
Start codon (ATG): 597-599
Mitochondrial localization signal 1 (MLS-1): 597-689
Mitochondrial localization signal 2 (MLS-2): 690-782
Start of HyPer coding sequence: 798-800
Stop codon: 2229-2231
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2385-2390 & 2414-2419
mRNA 3' ends: 2423 & 2435
f1 single-strand DNA origin: 2482-2937
Eukaryotic promoter for expression of Kan^r gene
-35 region: 2999-3004; -10 region: 3022-3027
Transcription start point: 3034
SV40 origin of replication: 3278-3413
SV40 early promoter
Enhancer (72-bp tandem repeats): 3111-3182 & 3183-3254
21-bp repeats: 3258-3278, 3279-3299 & 3301-3321
Early promoter element: 3334-3340
Major transcription start points: 3330, 3368, 3374 & 3379
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3462-3464; Stop codon: 4254-4256
G->A mutation to remove Pst I site: 3644
C->A (Arg to Ser) mutation to remove BssH II site: 3990
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4492-4497 & 4505-4510
pUC plasmid replication origin: 4841-5484

Vector description

pHyPer-dMito is a mammalian expression vector encoding mitochondria-targeted HyPer. HyPer codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Duplicated mitochondrial targeting sequence (MTS) is fused to the HyPer N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase [Rizzuto et al. 1989; Rizzuto et al. 1995].

pHyPer-dMito can be used as a source of dMTS-HyPer 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

pHyPer-dMito vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondria-targeted HyPer in many cell types. 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

Rizzuto, R. et al. (1989) "A gene specifying subunit VIII of human cytochrome c oxidase is localized to chromosome 11 and is expressed in both muscle and non-muscle tissues." J Biol Chem, 264 (18): 10595–10600 / pmid: 2543673

Rizzuto, R. et al. (1995) "Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells." Curr Biol, 5 (6): 635–642 / pmid: 7552174