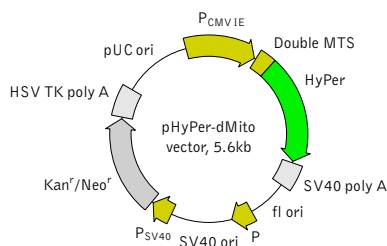


## pHyPer-dMito vector

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>

### Location of features

P<sub>CMV IE</sub>: 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<sup>r</sup> 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

### References

Gorman (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 et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248

Rizzuto 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 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

### Notice to Purchaser:

The HyPer-related materials (also referred to as "Products") are intended for research use only. Some elements of these materials may be covered by third party patents issued and applicable in certain countries. No license under these patents is conveyed expressly or by implication to the recipient of the materials. Users of these materials may be required to obtain a patent license depending upon the particular application and country in which the materials are received or used.

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839, and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

**MATERIAL SAFETY DATA SHEET INFORMATION:** To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

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 <sub>CMV IE</sub>	
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	

### 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<sup>+</sup>-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<sup>-</sup> host and make fresh DNA.

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) 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<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> 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.