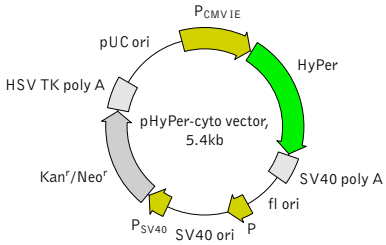
pHyPer-cyto vector

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-cyto vector	**FP941**	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 fluorescent hydrogen peroxide sensor HyPer in mammalian cells under the control of CMV promoter; source of HyPer coding sequence

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
HyPer
Kozak consensus translation initiation site: 596-606
Start codon (ATG): 603-605; Stop codon: 2037-2039
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2238-2243 & 2267-2272
mRNA 3' ends: 2276 & 2288
f1 single-strand DNA origin: 2335-2790
Eukaryotic promoter for expression of Kan^r gene
-35 region: 2852-2857; -10 region: 2875-2880
Transcription start point: 2887
SV40 origin of replication: 3131-3266
SV40 early promoter
Enhancer (72-bp tandem repeats): 2964-3035 & 3036-3107
21-bp repeats: 3111-3131, 3132-3152 & 3154-3174
Early promoter element: 3187-3193
Major transcription start points: 3183, 3221, 3227 & 3232
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3315-3317; Stop codon: 4107-4109
G->A mutation to remove Pst I site: 3497
C->A (Arg to Ser) mutation to remove BssH II site: 3843
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4345-4350 & 4358-4363
pUC plasmid replication origin: 4694-5337

Vector description

pHyPer-cyto is a mammalian expression vector encoding a fluorescent sensor HyPer. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the HyPer coding sequence [Kozak 1987].

The vector can be also used as a source of HyPer coding sequence. Flanking restriction sites are convenient for excision of HyPer sequence and its further insertion into other expression vectors of choice. Alternatively, HyPer coding sequence can be amplified by PCR.

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-cyto vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive HyPer expression in eukaryotic cells. 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.

Kozak, M. (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." Nucleic Acids Res, 15 (20): 8125–8148 / pmid: 3313277