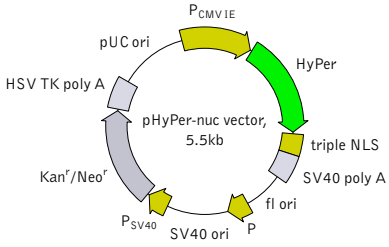
pHyPer-nuc vector

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-nuc vector	**FP944**	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 nuclear-targeted fluorescent hydrogen peroxide sensor HyPer in mammalian cells under the control of CMV promoter; source of nuclear-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-nuc fusion
Start codon (ATG): 603-605
HyPer coding sequence: 603-2036
Nuclear localization signals (NLS): 2058-2130
Stop codon: 2148-2150
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2290-2295 & 2319-2324
mRNA 3' ends: 2328 & 2340
f1 single-strand DNA origin: 2387-2842
Bacterial promoter for expression of Kan^r gene
-35 region: 2904-2909; -10 region: 2927-2932
Transcription start point: 2939
SV40 origin of replication: 3183-3318
SV40 early promoter
Enhancer (72-bp tandem repeats): 3016-3087 & 3088-3159
21-bp repeats: 3163-3183, 3184-3204 & 3206-3226
Early promoter element: 3239-3245
Major transcription start points: 3235, 3273, 3279 & 3284
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3367-3369; Stop codon: 4159-4161
G->A mutation to remove Pst I site: 3549
C->A (Arg to Ser) mutation to remove BssH II site: 3895
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4397-4402 & 4410-4415
pUC plasmid replication origin: 4746-5389

Vector description

pHyPer-nuc is a mammalian expression vector encoding nuclear-targeted HyPer. HyPer codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Three copies of the nuclear localization signal (NLS) fused to the HyPer C-terminus provide for efficient translocation of HyPer to the nuclei of mammalian cells [Fischer-Fantuzzi and Vesco 1988].

pHyPer-nuc vector can be used as a source of HyPer-NLS hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice. Alternatively, HyPer-NLS 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-nuc vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive HyPer expression 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

Fischer-Fantuzzi and Vesco (1988) "Cell-dependent efficiency of reiterated nuclear signals in a mutant simian virus 40 oncoprotein targeted to the nucleus." Mol Cell Biol, 8 (12): 5495–5503 / pmid: 2854199

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