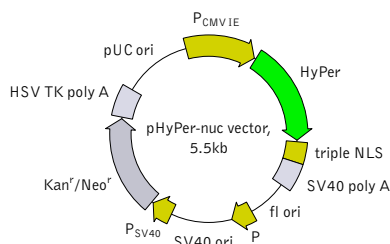


pHyPer-nuc vector

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>

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

| 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 | |

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 Vecso 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 Vecso (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

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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.

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