



Restriction Map and Multiple Cloning Site (MCS) of pZsYellow1-N1 Vector. Unique restriction sites are shown in bold. The *Not I* site follows the ZsYellow1 stop codon.

Description

pZsYellow1-N1 encodes a human codon-optimized variant of wild-type *Zoanthus* sp. yellow fluorescent protein, ZsYellow1 (1). The ZsYellow1 coding sequence contains a series of silent base-pair changes, which correspond to human codon-usage preferences, for optimal expression in mammalian cells (2). Additionally, an upstream sequence—located just 5' to the ZsYellow1 start codon—has been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells. A single amino acid substitution (Met-128 to Val) has been made to enhance the emission characteristics of ZsYellow1 (excitation maximum = 529 nm; emission maximum = 539 nm).

The multiple cloning site (MCS) in pZsYellow1-N1 is positioned between the immediate-early promoter of CMV ($P_{CMV IE}$) and the ZsYellow1 coding sequence. Thus, genes cloned into the MCS will be expressed as fusions to the N-terminus of ZsYellow1 if they are in the same reading frame as ZsYellow1 and there are no intervening stop codons. The SV40 polyadenylation signals (SV40 polyA) downstream of the ZsYellow1 gene direct proper processing of the 3' end of ZsYellow1 mRNA.

The vector backbone contains an SV40 origin (SV40 ori) for replication in mammalian cells that express the SV40 T antigen, a pUC origin of replication (pUC ori) for propagation in *E. coli*, and an f1 origin (f1 ori) for single-stranded DNA production. In addition, a neomycin-resistance cassette—consisting of the SV40 early promoter (P_{SV40e}), the neomycin/kanamycin resistance gene of Tn5 (Neo^r/Kan^r), and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK poly A) gene—allows stably transfected eukaryotic cells to be selected using G418 (4). A bacterial promoter (*P*) upstream of this cassette drives expression of the Neo^r/Kan^r gene in *E. coli* host cells, which can be selected with kanamycin.

(080612; published 06 August 2012)



Clontech

United States/Canada
800.662.2566

Asia Pacific
+1.650.919.7300

Europe
+33.(0)1.3904.6880

Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
ATakara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Use

Fusions to the N terminus of ZsYellow1 retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pZsYellow1-N1 so that it is in frame with the ZsYellow1 coding sequence, with no intervening, in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant pZsYellow1-N1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (available from Clontech; Cat. Nos. 631307 & 631308). We recommend selecting mammalian cell cultures in 500–1,300 µg/ml G418, depending on the cell line. Be sure to establish a kill curve for each cell line and each lot of G418 to determine the optimal selection concentration. Unmodified (i.e., non-recombinant) pZsYellow1-N1 can also be used simply to express ZsYellow1 in a cell line of interest (e.g., as a transfection marker).

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
Enhancer region: 59–465; TATA box: 554–560
Transcription start point: 583
C→G mutation to remove *Sac* I site: 569
- Multiple Cloning Site (MCS): 591–665
- *Zoanthus* sp. yellow fluorescent protein (ZsYellow1) coding sequence
Kozak consensus translation initiation site: 672–682
Start codon (ATG): 679–681; stop codon: 1372–1374
Met-128 to Val mutation (A→G): 1063
- SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1528–1533 & 1557–1562; mRNA 3' ends: 1566 & 1578
- f1 single-strand DNA origin: 1625–2080 (Packages the noncoding strand of ZsYellow1.)
- Bacterial promoter for expression of Kan^r gene:
–35 region: 2142–2147; –10 region: 2165–2170
Transcription start point: 2177
- SV40 origin of replication: 2421–2556
- SV40 early promoter
Enhancer (72-bp tandem repeats): 2254–2325 & 2326–2397
21-bp repeats: 2401–2421, 2422–2442 & 2444–2464
Early promoter element: 2477–2483
Major transcription start points: 2473, 2511, 2517 & 2522
- Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences: Start codon (ATG): 2605–2607; stop codon: 3397–3399
G→A mutation to remove *Pst* I site: 2787
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3133
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3635–3640 & 3648–3653
- pUC plasmid replication origin: 3984–4627

Propagation in *E. coli*

- Suitable host strains: DH5 α , HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Gorman, C. (1985). In *DNA Cloning: A Practical Approach, Vol. II*. Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

Notice to Purchaser

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with the licensing requirements described on the product's web page at <http://www.clontech.com>. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

Clontech and the Clontech logo are trademarks of Clontech Laboratories, Inc. All other marks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions. Clontech is a Takara Bio Company. ©2012 Clontech Laboratories, Inc.

This document has been reviewed and approved by the Clontech Quality Assurance Department.