Certificate of Analysis

pEF1α-mCherry-N1 Vector

Catalog No.	Amount	Lot Number
631969	10 µg	Specified on product label.

Description

pEF1α-mCherry-N1 is a mammalian expression vector that constitutively expresses a protein of interest fused to the N-terminus of the red fluorescent protein mCherry, even after stable integration of the vector into the host cell genome. Stable, constitutive expression of the fusion protein is driven by the human elongation factor 1 alpha (EF1α) promoter, allowing the monitoring of a variety of cellular processes (such as differentiation in primary or stem cells) without the transgene silencing associated with CMV promoters. The unmodified vector can be used to express mCherry in mammalian cells.

Package Contents

- 1 tube of pEF1α-mCherry-N1 Vector (20 µl/tube)

Storage Conditions

- Store plasmid at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Expiration Date

- Specified on product label.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Concentration

- 500 ng/µl

Shipping Conditions

- Dry ice

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- pEF1alpha-mCherry-N1 Vector Information
- pEF1a-mCherry-N1 vector sequence in GenBank format

Takara Bio USA, Inc.
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada 800.662.2566
Asia Pacific +1.650.919.7300
Europe +33.(0)1.3904.6880
Japan +81.(0)77.565.6999

(032323)

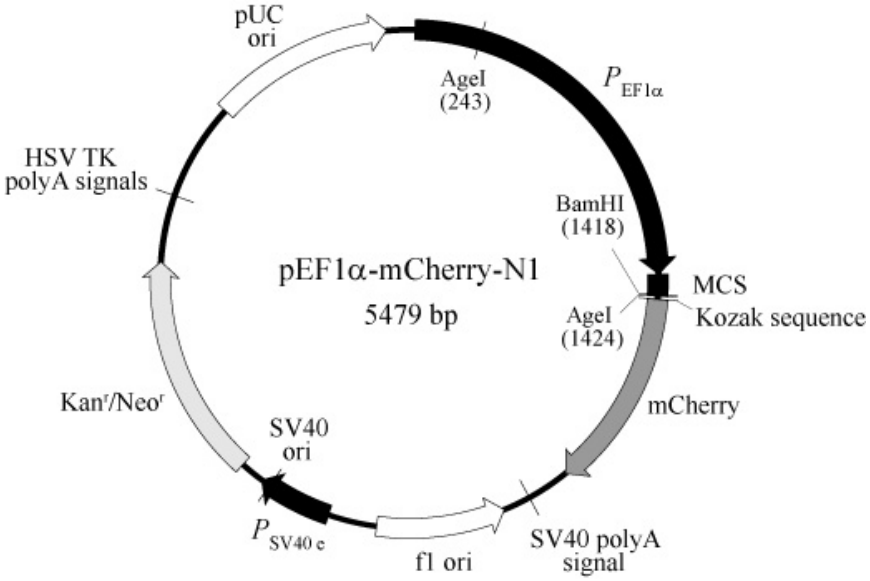

Figure 1. pEF1α-mCherry-N1 vector map.

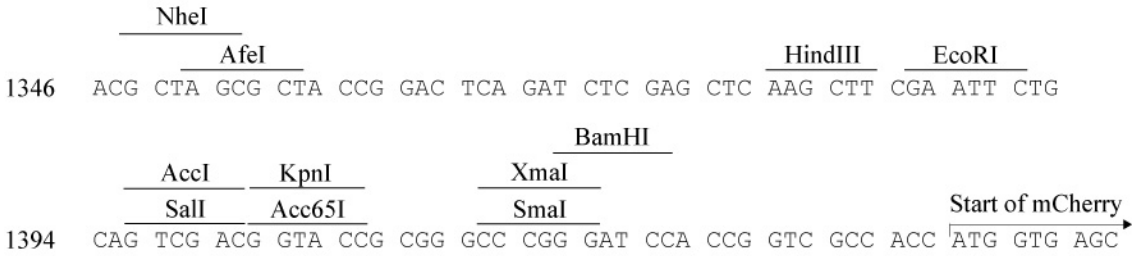

Figure 2. pEF1α-mCherry-N1 multiple cloning site (MCS).

Description

pEF1α-mCherry-N1 is designed to express a protein of interest fused to the N-terminus of mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma* sp. red fluorescent protein, DsRed (Shaner et al. 2004). The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively. Expression of fusion proteins that retain the fluorescence properties of the unmodified mCherry protein can be monitored by flow cytometry and localized by fluorescence microscopy.

The multiple cloning site (MCS) in pEF1α-mCherry-N1 is positioned between the EF1α promoter ($P_{EF1\alpha}$) and the mCherry coding sequence. Expression of the fusion protein is driven by the EF1α promoter, which remains constitutively active even after stable integration of the vector into the host cell genome (Wang et al. 2008). A Kozak consensus sequence, located immediately upstream of the mCherry gene, enhances the translational efficiency of mCherry in eukaryotic systems (Kozak 1987), and SV40 polyadenylation signals direct proper processing of the 3' end of the mCherry mRNA.

The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (Gorman 1985). This

cassette consists of the SV40 early promoter ($P_{SV40\ e}$), the Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette drives expression of the kanamycin resistance gene in *E. coli*.

Location of Features

- $P_{EF1\alpha}$ (human elongation factor 1 alpha promoter): 12–1346
- MCS (multiple cloning site): 1348–1422
- Kozak consensus sequence: 1429–1439
- mCherry: 1436–2143
- SV40 polyA signal: 2298–2332
- f1 origin of replication: 2395–2850 (complementary)
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 3024–3292
- SV40 origin of replication: 3191–3329
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 3375–4169
- HSV TK polyA signals: 4405–4423
- pUC origin of replication: 4754–5397

Additional Information

The gene of interest must be cloned into pEF1α-mCherry-N1 so that it is in-frame with the mCherry coding sequence. The gene must contain a start codon (ATG), and lack in-frame stop codons.

The pEF1α-mCherry-N1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (Gorman 1985). pEF1α-mCherry-N1 can also be used as a cotransfection marker, as the unmodified vector will express mCherry in mammalian cells.

For Western analysis, the mCherry protein can be detected with the Living Colors® mCherry Monoclonal Antibody (Cat. No. 632543), the DsRed Polyclonal Antibody (Cat. No. 632496) or the DsRed Monoclonal Antibody (Cat. Nos. 632392 & 632393).

Propagation in *E. coli*

- Suitable host strains: DH5α™, HB101 and other general-purpose strains. Single-stranded DNA production requires a host containing an F plasmid, such as the JM109 or XL1-Blue strains.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Excitation and Emission Maxima of mCherry

- Excitation: 587 nm
- Emission: 610 nm

References

Shaner, N. C. *et al*. (2004) *Nat. Biotechnol*. **22**(12):1567−72.

Wang, R. *et al.* (2008) *Stem Cells Dev.* **17**(2):279−289.

Kozak, M. (1987) *Nucleic Acids Res*. **15**(20):8125–8148.

Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*. Ed. D. M. Glover (IRL Press, Oxford, U.K.) pp. 143–190.

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzyme	Fragments
BamHI	5.5 kb
AgeI	1.2 & 4.3 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

pEF1alpha-mCherry-N1 Vector

CATALOG NO.

631969

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our 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 our prior written approval.

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

TRADEMARKS:

Takara Bio USA, Inc.
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

5/30/2025