Certificate of Analysis

pEF1α-mCherry-C1 Vector

Table of Contents

Product Information 1
Description 2
Location of Features 3
Additional Information 3
Quality Control Data 4

Catalog No.	Amount	Lot Number
631972	10 µg	Specified on product label.

Product Information

pEF1α-mCherry-C1 is a mammalian expression vector that constitutively expresses a protein of interest fused to the C-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-C1 Vector (20 µl/tube)

Storage Conditions

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

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

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

Concentration

- 500 ng/µl

Shipping Conditions

- Dry ice (–70°C)

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

United States/Canada 800.662.2566
Asia Pacific +1.650.919.7300
Europe +33.(0)1.3904.6880
Japan +81.(0)77.543.6116

(PA124330)

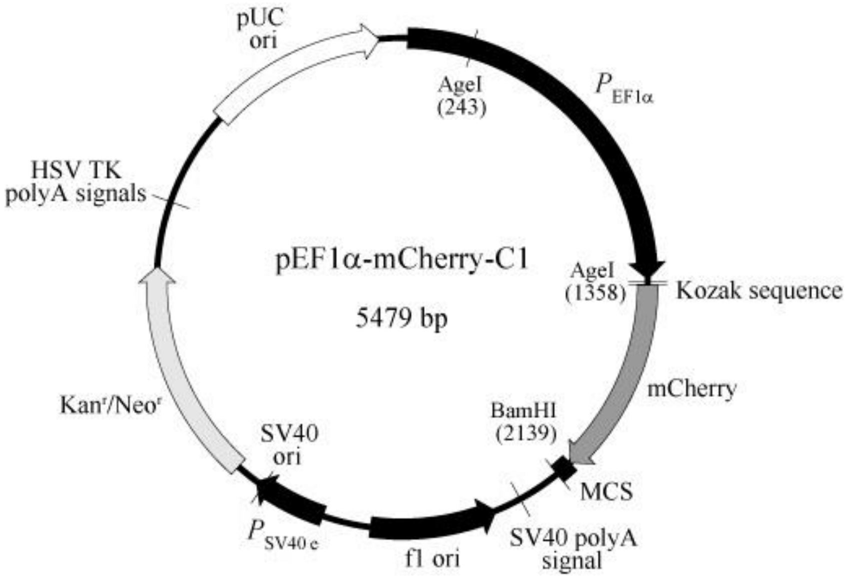

Figure 1. pEF1α-mCherry-C1 vector map.

End of mCherry (CTG TAC AAG); BspEI (TCC GGA); HindIII (CAA GCT); EcoRI (TCG AAT)

2069 CTG TAC AAG TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA

AccI / SalI (GTC GAC); KpnI / Acc65I (GGT ACC); XmaI / SmaI (GGG CCC); BamHI (GGA TCC); Stop (TGA); Stop (TAA); Stop (CTG A...)

2117 GTC GAC GGT ACC GCG GGC CCG GGA TCC ACC GGA TCT AGA TAA CTG AT

Figure 2. pEF1α-mCherry-C1 multiple cloning site (MCS).

Description

pEF1α-mCherry-C1 is designed to constitutively express a protein of interest fused to the C-terminus of mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma* sp. red fluorescent protein, DsRed (1). 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-C1 is positioned downstream of the mCherry coding sequence. Expression of the fusion protein is driven by the EF1α promoter ($P_{EF1α}$), which remains constitutively active even after stable integration of the vector into the host cell genome (2). A Kozak consensus sequence located immediately upstream of the mCherry gene enhances the translational efficiency of the fusion in eukaryotic systems (3), and SV40 polyadenylation signals downstream of the mCherry gene direct proper processing of the 3' end of the 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 (4). 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
- Kozak consensus sequence: 1363–1373
- mCherry: 1370–2077
- MCS (multiple cloning site): 2078–2143
- 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

Genes cloned into the MCS must be in-frame with the mCherry coding sequence, and do not require start or stop codons. The pEF1α-mCherry-C1 vector can be transfected into mammalian cells using any standard transfection method. pEF1α-mCherry-C1 can be used as a cotransfection marker, as the unmodified vector will express mCherry in mammalian cells. If required, stable transfectants can be selected using G418 (4).

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

1. Shaner, N. C. *et al*. (2004) *Nat. Biotechnol*. **22**(12):1567−72.
2. Wang, R. *et al.* (2008) *Stem Cells Dev.* **17**(2):279−289.
3. Kozak, M. (1987) *Nucleic Acids Res*. **15**(20):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.

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(s)	Fragment(s)
BamHI	5.5 kb
AgeI	1.1 & 4.4 kb

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

Notice to Purchaser

pEF1alpha-mCherry-C1 Vector

CATALOG NO.

631972

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 listed below and 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.

STATEMENT 44

The DsRed-Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,005,511; 7,157,566; 7,393,923 and 7,250,298.

STATEMENT 72

Living Colors Fluorescent Protein Products: Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at. Any and all uses of this product will be subject to the terms and conditions of the Non-Commercial Use License Agreement (the “Non-Commercial License”), a copy of which can be found below. As a condition of sale of this product to you, and prior to using this product, you must agree to the terms and conditions of the Non-Commercial License. Under the Non-Commercial License, Clontech grants Not-For-Profit Entities a non-exclusive, non-transferable, non-sublicensable and limited license to use this product for internal, non-commercial scientific research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the product may be made without express written permission from Clontech. Any other use of this product requires a different license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com. For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.

TRADEMARKS:

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

4/3/2014

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.543.6116

Notice to Purchaser

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

4/3/2014

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.543.6116