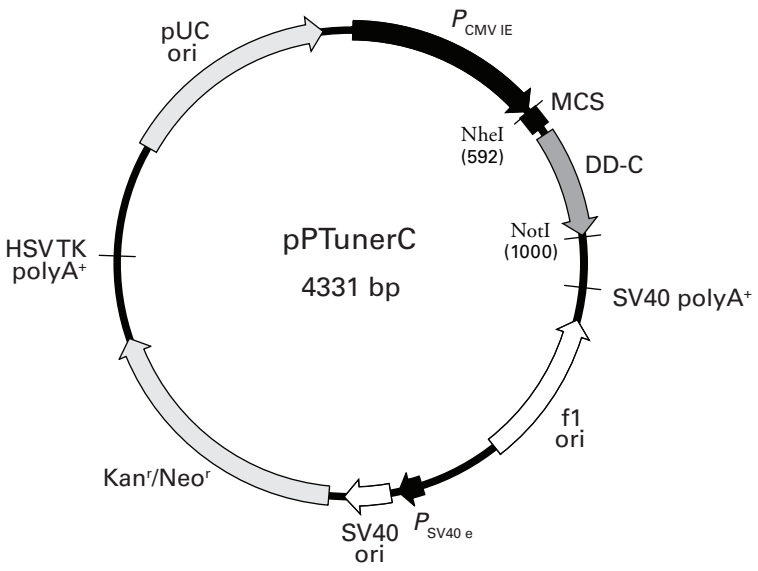
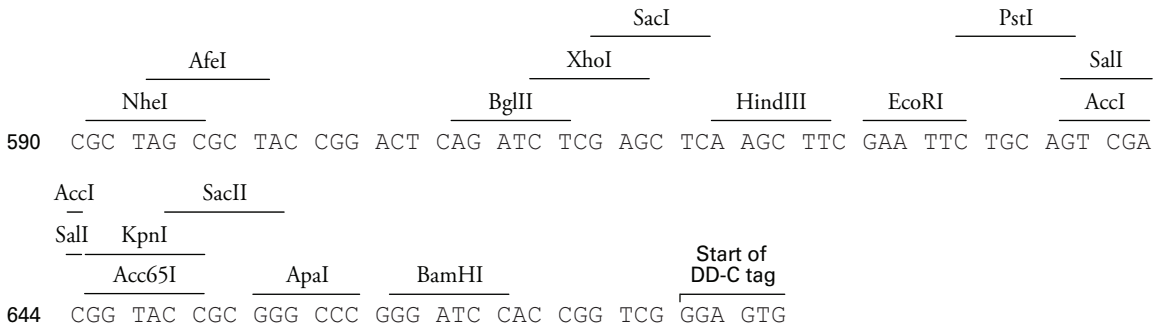
Vector Information

pPTunerC Vector Information PT4437-5

Catalog No. 631072





pPTunerC Vector Map and Multiple Cloning Site (MCS).

Description

pPTunerC is an expression vector that allows you to precisely regulate the amount of your protein of interest in mammalian cells. The vector encodes a 12 kDa, FKBP-based destabilization domain (DD-C) that has been optimized for use as a C-terminal tag (1). This domain, located just downstream of the multiple cloning site (MCS), causes the rapid degradation of any protein to which it is fused. Once expressed, the amount of DD-C-tagged protein present in the cell can be rapidly increased by the addition of Shield1 stabilizing ligand to the medium. Shield1 is a membrane permeant molecule that binds to the DD-C tag, 'shielding' the fusion protein from proteasomal degradation.

Expression of the fusion protein is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located just upstream of the multiple cloning site (MCS). pPTunerC contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen. The vector also contains a neomycin/kanamycin resistance cassette (Kan^r/Neo^r) that allows G418 selection of stably transfected eukaryotic cells (2). In addition, a bacterial promoter located upstream of this cassette allows kanamycin resistance in *E. coli*. The vector also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR113805; published 17 January 2011)

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Use

pPTunerC is available in the ProteoTuner™ Shield System C (Cat. No. 631072), and is designed to express your C-terminally DD-C-tagged protein of interest in mammalian cells. To create your DD-C-tagged protein of interest, your gene of interest must contain no in-frame stop codons, and must be cloned into the MCS in the same reading frame as the DD-C tag sequence.

When cells expressing a DD-C-tagged protein of interest are grown in medium containing Shield1, the ligand binds to the DD-C tag and protects the fusion protein from degradation. As a result, the protein quickly accumulates inside the cells in amounts that are directly proportional to the concentration of Shield1 in the medium. If the cells are subsequently grown in medium lacking Shield1, the DD-C tag is no longer stabilized, and the fusion protein is rapidly degraded. Because the effects of Shield1 are concentration-dependent and reversible, it is possible to fine-tune the amount of fusion protein present in the cells simply by adjusting the concentration of Shield1 in the medium (3).

Location of Features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- MCS (multiple cloning site): 591–665
- DD-C (destabilization domain, optimized for use as a C-terminal tag): 674–994
- SV40 polyA$^+$ (SV40 polyA signal): 1150–1184
- f1 origin of replication (allows the production of single-stranded DNA): 1247–1702 (complementary)
- P_{Kan^r} (Bacterial promoter for Kan^r gene expression): 1764–1792
- $P_{SV40\ e}$ (SV40 early promoter and enhancer): 1876–2019
- SV40 origin of replication: 2043–2181
- Kan^r/Neo^r (Tn5 kanamycin/neomycin resistance gene): 2227–3021
- HSV TK polyA$^+$ (herpes simples virus thymidine kinase polyA signal): 3257–3275
- pUC origin of replication: 3606–4249

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to G418.

Propagation in *E. coli*

- Suitable host strains: Stellar™ Competent Cells. 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) in *E. coli* hosts.
- *E. coli* replication origin: pUC

References

1. Chu, B.W. *et al.* (2008) *Bioorg. Med. Chem. Lett.* **18**(22):5941–5944.
2. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.
3. Banaszynski, L. *et al.* (2006) *Cell* **126**(5):995–1004.

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.