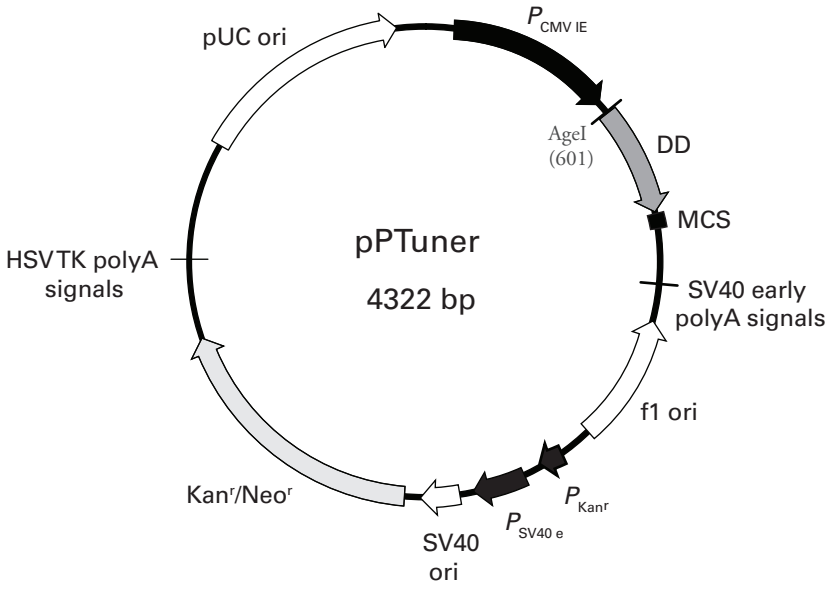
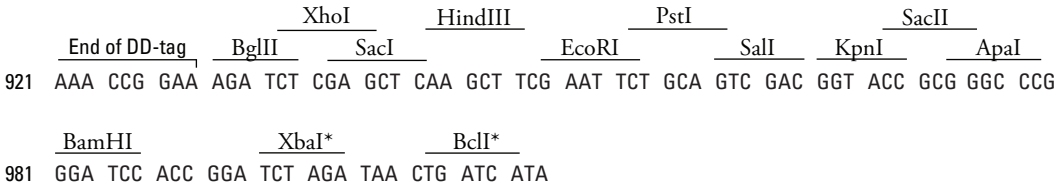
Vector Information

pPTuner Vector Information PT4046-5

Catalog No. 632172





pPTuner Vector Map and Multiple Cloning Site (MCS). *Note: The XbaI and BclI sites are methylated in the DNA provided by Clontech Laboratories, Inc. If you wish to digest the vector with these enzymes, you wil need to transform the vector into a dam$^-$ host and make fresh DNA.

Description

pPTuner 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 (L106P) destabilization domain (DD; 1) that is expressed as an N-terminal tag on your protein of interest; this domain causes the rapid degradation of any protein to which it is fused. Once expressed, the amount of DD-tagged protein present in the cell can be rapidly increased by the addition of Shield1 stabilizing ligand to the medium. Shield1 is a membrane permeable molecule that binds to the DD 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 DD tag sequence. pPTuner contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. The vector also contains a neomycin/kanamycin resistance cassette 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.

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Use

pPTuner is available in the ProteoTuner Shield System N (Cat. No. 632172), and is designed to express your DD-tagged protein of interest in mammalian cells. In order to create your DD-tagged protein of interest, your gene of interest must be cloned into the MCS in the same reading frame as the DD tag sequence, and it must contain a stop codon at the end of its coding sequence.

When cells expressing a DD-tagged protein of interest are grown in medium containing Shield1, the ligand binds to the DD 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 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 (1).

Location of Features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- DD (FKBP-L106P destabilization domain): 606–929
- MCS (multiple cloning site): 930–998
- SV40 early polyA signals: 1141–1175
- f1 origin of replication (allows the production of single-stranded DNA): 1238–1693 (complementary)
- P_{Kan^r} (Bacterial promoter for Kan^r gene expression): 1755–1783
- $P_{SV40\ e}$ (SV40 early promoter and enhancer): 1867–2096
- SV40 origin of replication: 2034–2172
- Kan^r/Neo^r (Tn5 kanamycin/neomycin resistance gene): 2218–3012
- HSV TK polyA signals (herpes simples virus thymidine kinase polyA signals): 3248–3266
- pUC origin of replication: 3597–4240

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
- Copy number: high

References

1. Banaszynski, L. *et al.* (2006) *Cell* **126**(5):995–1004.
2. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.