

BD PharMingen Technical Data Sheet

pRK-5 MAMMALIAN EXPRESSION VECTOR

PRODUCT INFORMATION

Catalog Number:

556104 (Was: 40028P)

Contents:

20 µg in 20 µl vector

DESCRIPTION

The pRK-5 vector contains a powerful cytomegalovirus promoter and is designed for high level expression of cloned genes in cultured mammalian cells.^{1,2} pCMV promoters have been used to express different cDNAs and genes, including receptors, transcription factors, G-proteins and viral proteins. They have been used to transfect simian COS, mouse L, CHO, 293, HeLa, NIH 3T3 and many other cell lines. The pRK-5 vector works extremely well with simian SV40 transformed COS cells or adenovirus-transformed 293 cells (embryonic human kidney cells).

The pRK-5 vector offers the following features:

1. Powerful promoter/enhancer domain from the major immediate-early region of the human cytomegalovirus.
2. A multiple cloning region (MCS) including the following restriction sites listed from 5' to 3': XbaI, PstI, NotI, EcoRII, and HindIII.
3. SV40 polyadenylation signals for RNA processing in mammalian cells.
4. SV40 origin for episomal plasmid amplification in COS cells.
5. Bacteriophage f1 origin of replication for production of single-stranded plasmid DNA.
6. Ampicillin-resistant (Amp^R) gene for amplification in *E. coli* bacterial strains.

PREPARATION AND STORAGE

The plasmid DNA has been purified by silicon bead matrix and dissolved in TE buffer (10 mM Tris-HCl, pH 7.5; 1 mM EDTA). It should be kept at -20°C for long-term storage.

HANDLING

1. Insert your gene of interest into a suitable restriction site in the pRK-5 vector. Transform and amplify the plasmid DNA in *E. coli* strains (DH5α, HB101 or any other suitable strain) under ampicillin selection and purify using standard protocols.
2. For Stable transfection and expression of recombinant protein, mammalian cells can be transfected with pRK-5 plasmid using DEAE-Dextran, CaPO₄, lipofectin, or electroporation protocols.²

REFERENCES

1. Eaton, D., P.E. Hass, P. Hollingshead, K. Wion, J. Mather, R.M. Lawn, G.A. Vehear, and C. Gorman. 1986. Construction and characterization of an active factor VIII variant lacking the central one-third of the molecule. *Biochemistry*. 25:8343-8347.
2. Kuphal, D., and P.M. Conn. 1995. Transient transfection of GGH3-1' cells [GH3 cells stably transfected with the gonadotropin-releasing hormone (GnRH) receptor complementary deoxyribonucleic acid] with the carboxyl-terminal of β-adrenergic receptor kinase 1 blocks prolactin release: evidence for a role of the G protein βγ-subunit complex in GnRH signal transduction. *Endocrinology*. 136:3031-3036.

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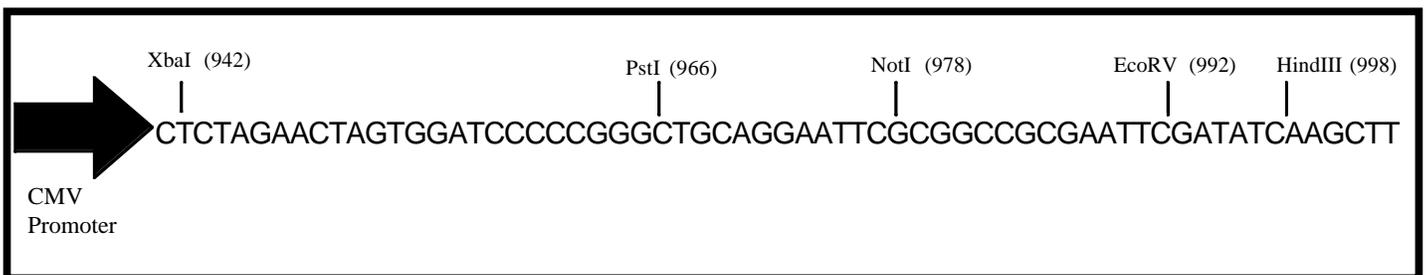
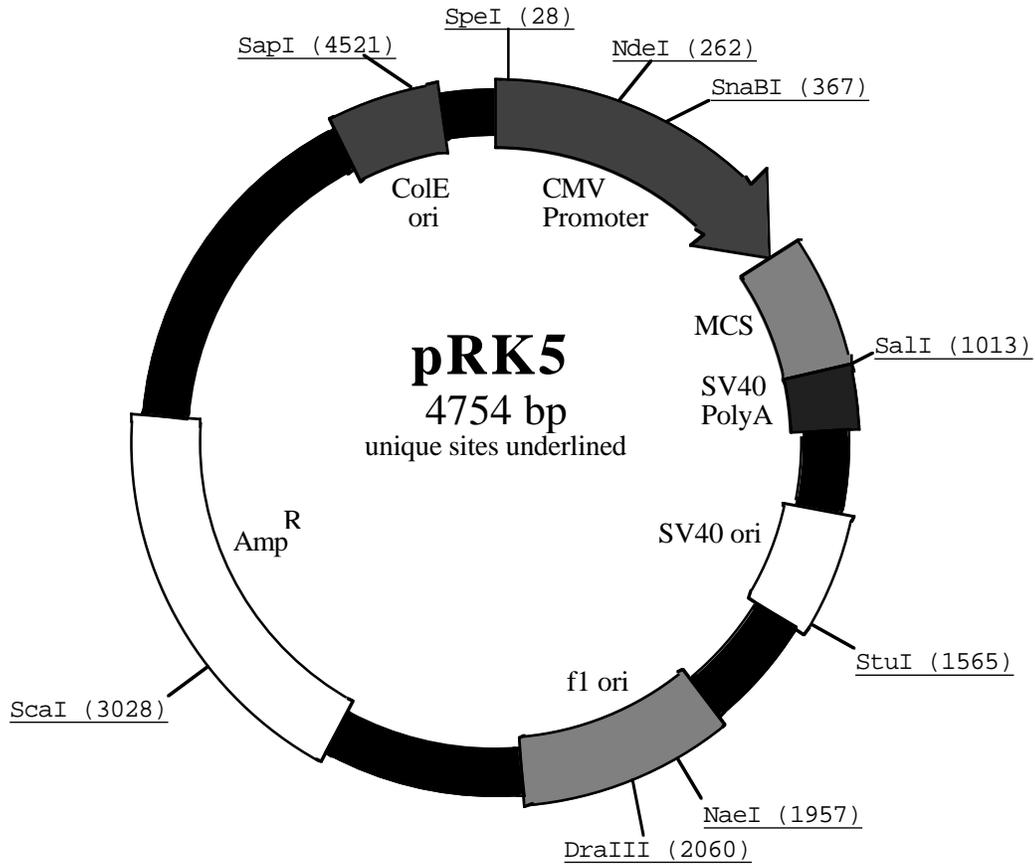
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