Cat. # 3240
3241
3242

For Research Use

TaKaRa

pBApo-CMV Neo DNA
pBApo-CMV Pur DNA
pBApo-CMV DNA

Product Manual

v201612Da

Table of Contents

I. Description

pBApo-CMV is a simple gene expression vector for mammalian cells. This vector carries a promoter from cytomegalovirus (CMV IE promoter) and a polyA signal site from herpes simplex virus thymidine kinase gene. The vectors are useful for the construction of expression plasmids by inserting the ORF of a target gene at the multicloning site. This vector can also be used to express microRNA precursors and other transcripts in addition to ordinary genes.
In addition to a basic vector, the pBApo-CMV series includes vectors carrying a neomycin-resistance gene or a puromycin resistance gene as a selection marker in mammalian cells.

II. Product Information

pBApo-CMV DNA (Cat. #3242)	20 μg
pBApo-CMV Neo DNA (Cat. #3240)	20 μg
pBApo-CMV Pur DNA (Cat. #3241)	20 μg

Concentration : 0.5 μg/μl
Form : 10 mM Tris-HCl, pH8.0, 1 mM EDTA

III. Storage

-20℃
2 years from date of receipt under proper storage conditions.

IV. Vector Map and Cloning Sites

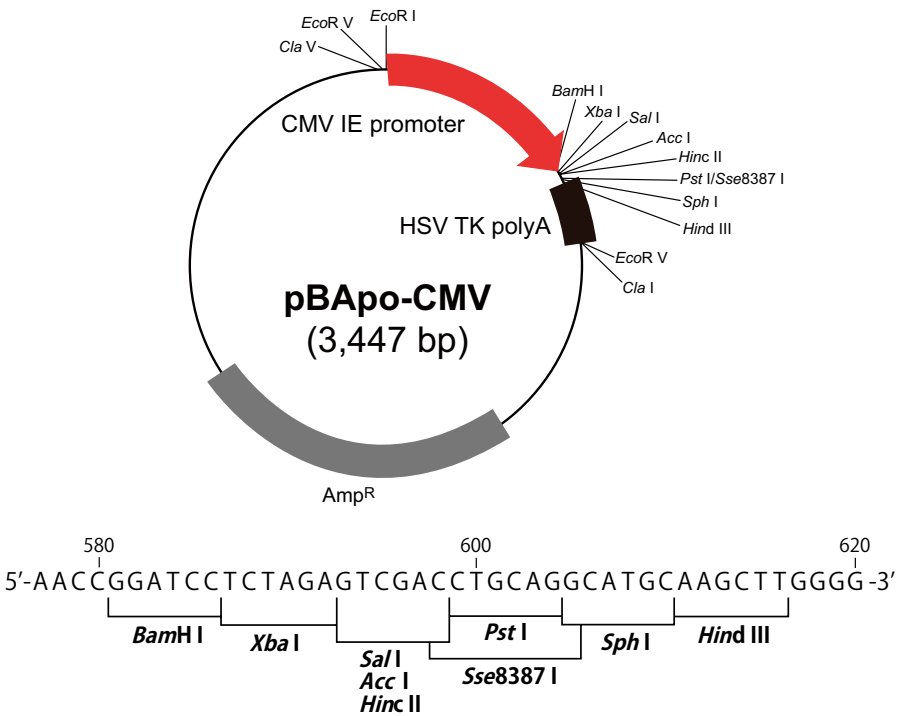

Figure 1. Vector map and cloning sites of pBApo-CMV

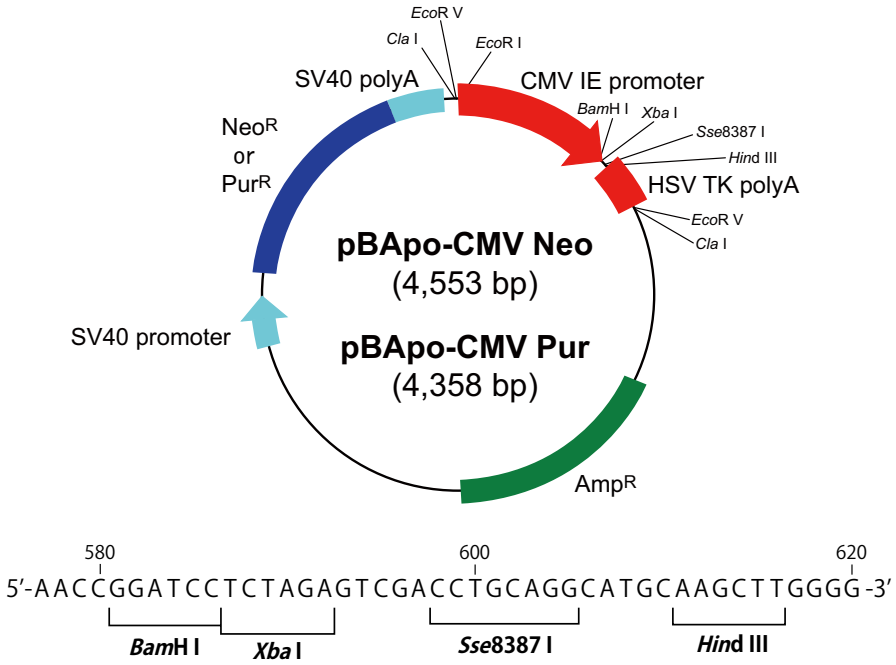

Figure 2. Vector map and cloning sites of pBApo-CMV Neo and pBApo-CMV Pur

V. Protocol

1. Gene insertion
   Insert the target gene's ORF into the cloning site of one of the pBApo-CMV plasmid vectors. The ampicillin resistance gene carried by the vector allows for selection of transformed *E. coli*.
2. Transfection into cultured cells
   Transfect plasmids into cultured cells using a transfection reagent such as Xfect™ (Cat. #631317 etc.) under the conditions specified in its protocol.
3. Selection of transfected cells
   pBApo-CMV Neo DNA has a neomycin resistance gene and pBApo-CMV Pur DNA has a puromycin resistance gene to allow for drug selection of transfected cells.

   Drug selection should be started at least 24 hours after plasmid transfection. In case of a high cell density, reseed cells at appropriate dilution and replace the drug containing medium every 3 to 4 days. Generally, transfected cells can be obtained in 1 to 2 weeks. Since drug sensitivity varies from cell to cell, determine ahead of time the optimum concentration for the cell used. The concentration will generally be 500 - 1,000 μg/ml of G418 for Neo^R gene and 1 - 3 μg/ml of puromycin for Pur^R gene.

VI. Experimental Examples

Construction of a fluorescent protein expression vector (DsRed-Express)

- After digesting the pBApo-CMV Neo DNA with *Bam*H I and *Xba* I, a DNA fragment of approximately 4.6 kb was purified by agarose gel electrophoresis.
- The DNA Ligation Kit <Mighty Mix> (Cat. 6023) was used to ligate this fragment to a DsRed-Express gene that had been cut out from pDsRed-Express Vector (Cat. #632412).
- *E. coli* JM109 Competent Cells (Cat. #9052) were transformed with the ligation mix and plated on LB plates containing ampicillin.
- The colonies obtained were cultured in 2 to 5 ml of LB Amp liquid medium to prepare plasmids.
- One of the prepared plasmids was transfected into cultured 293 cells using *Trans*IT-293 Transfection Reagent (Mirus Bio).
- Cells were observed 2 days later using a fluorescence microscope to verify the expression of DsRed-Express. (Refer to Figure 3)

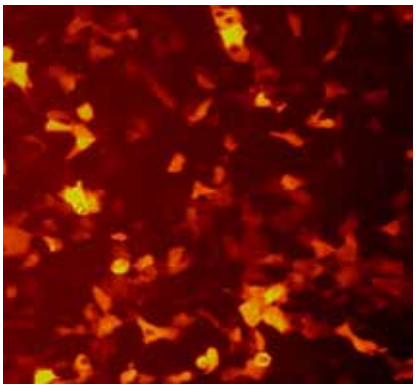

Figure 3: Fluorescence microscopy image. Day 2 after transfection of pBApo-CMV Neo / DsRed-Express.

VII. Related Products

pBApo-EF1 α Vector Series (Cat. #3243/3244)
DNA Ligation Kit < Mighty Mix > (Cat. #6023)
Xfect™ Transfection Reagent (Cat. #631317/631318)
pDsRed-Express Vector (Cat. #632412)
Cla I (Cat. #1034)
*Eco*R V (Cat. #1042)
Smi I (Cat. #1111)

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