

## Product Contents

### pF5K CMV-neo Flexi<sup>®</sup> Vector:

| Part No. | Size (units) |
|----------|--------------|
| C941A    | 20µg         |

**Description:** The pF5K CMV-neo Flexi<sup>®</sup> Vector<sup>(a,b,c)</sup> is designed for use with the Flexi<sup>®</sup> System, Entry/Transfer (Cat.# C8640) and the Flexi<sup>®</sup> System, Transfer (Cat.# C8820). The pF5K CMV-neo Flexi<sup>®</sup> Vector contains a CMV immediate early enhancer/promoter region plus a chimeric intron for mammalian expression and a T7 promoter for in vitro expression of the protein-coding region. The vector also contains the barnase gene for positive selection of the insert, and unique SgfI and PmeI sites that allow easy insertion and transfer of the insert of interest. The pF5K CMV-neo Flexi<sup>®</sup> Vector also contains a neomycin resistance gene with dual bacterial and SV40 promoters for kanamycin resistance and selection of the plasmid in *E. coli* and for long-term selection capabilities in mammalian cells. Transfection of a pF5K CMV-neo Flexi<sup>®</sup> Vector containing a cloned protein-coding region into mammalian cells provides resistance to the antibiotic G-418 (Cat.# V7981, V8091), allowing long-term selection of transfectants. **Do not use the pF5K CMV-neo Flexi<sup>®</sup> Vector without an insert as a negative control** because the barnase gene decreases the viability of the transfected cells. Cloned protein-coding regions can be transferred from the pF5K CMV-neo Flexi<sup>®</sup> Vector to other Flexi<sup>®</sup> Vectors with different expression options using the Flexi<sup>®</sup> Entry/Transfer Systems (Cat.# C8640 and C8820). For more information, see the *Flexi<sup>®</sup> Vector Systems Technical Manual #TM254*.

### Usage Information

**Concentration:** 100ng/µl.

**GenBank<sup>®</sup> Accession Number:** DQ487156.

**Storage Buffer:** The pF5K CMV-neo Flexi<sup>®</sup> Vector is supplied in 10mM Tris-HCl (pH 8.0), 1mM EDTA.

**Storage Conditions:** See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

**Usage Notes:** Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

## Quality Control Assays

**Nuclease Assay:** Following incubation of 1µg of pF5K CMV-neo Flexi<sup>®</sup> Vector in Restriction Enzyme Buffer B at 37°C for 16 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

**Physical Purity:**  $A_{260}/A_{280} \geq 1.80$ .

**Restriction Digestion:** The presence of unique restriction sites for PmeI and SgfI is confirmed by showing that the vector is linearized and yields the expected fragment sizes after digesting 1µg of vector for 2 hours with 10 units of PmeI, SgfI and Bgl II.

<sup>(a)</sup>Patent Pending.

<sup>(b)</sup>For research use only. Persons wishing to use this product or its derivatives in other fields of use, including without limitation, commercial sale, diagnostics or therapeutics, should contact Promega Corporation for licensing information.

<sup>(c)</sup>The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

Part# 9PIC941

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**Promega**

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## pF5K CMV-neo Flexi® Vector Features and Circle Map

The following features are present in the vector based on nucleotide sequence.

|  |           |
|--|-----------|
| CMV immediate early enhancer/promoter                | 1–742     |
| chimeric intron                                      | 857–989   |
| T7 RNA polymerase promoter (–17 to +3)               | 1033–1052 |
| Sgf I site   | 1056–1063 |
| barnase coding region                                | 1087–1422 |
| Pme I site   | 1424–1431 |
| SV40 late poly(A) signal                             | 1583–1804 |
| SV40 enhancer and early promoter                     | 1903–2320 |
| EM7 bacterial promoter                               | 2337–2394 |
| neomycin phosphotransferase coding region            | 2409–3203 |
| synthetic polyadenylation signal                     | 3267–3313 |
| ColE1-derived plasmid replication of origin          | 3551–3587 |
| cer site (site for <i>E. coli</i> XerCD recombinase) | 4258–4543 |

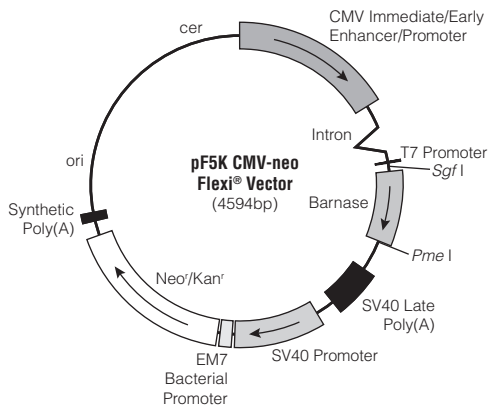


Figure 1. pF5K CMV-neo Flexi® Vector circle map.

## Related Products

| Product   | Size                              | Cat. # |
|---|-----------------------------------|--------|
| Flexi® System, Entry/Transfer                           | 5 entry and 20 transfer reactions | C8640  |
| Flexi® System, Transfer                                 | 100 transfer reactions            | C8820  |
| Carboxy Flexi® System, Transfer                         | 50 transfer reactions             | C9320  |
| 10X Flexi® Enzyme Blend (Sgf I & Pme I)                 | 25µl                              | R1851  |
|   | 100µl                             | R1852  |
| Carboxy Flexi Enzyme Blend (Sgf I & EcoI/CRI)           | 50µl                              | R1901  |
| HaloTag® Flexi® Vectors–CMV Dilution Series Sample Pack | 9 × 2µg                           | G3780  |
| Single Step (KRX) Competent Cells                       | 5 × 200µl                         | L3001  |

There are Flexi® Vectors available for many different applications.  
 Visit: [www.promega.com/applications/cloning](http://www.promega.com/applications/cloning) to find out more.

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