



**Promega**

## Technical Bulletin

---

# pCMVTNT™ Vector

INSTRUCTIONS FOR USE OF PRODUCT L5620.



[www.promega.com](http://www.promega.com)

PRINTED IN USA.  
Revised 5/09

Part# TB305

# pCMVTNT™ Vector

All technical literature is available on the Internet at: [www.promega.com/tbs](http://www.promega.com/tbs)  
 Please visit the web site to verify that you are using the most current version of this  
 Technical Bulletin. Please contact Promega Technical Services if you have questions on use  
 of this system. E-mail: [techserv@promega.com](mailto:techserv@promega.com).

1. Description.....	1
2. Product Components and Storage Conditions .....	1
3. Features of the pCMVTNT™ Vector.....	2
4. pCMVTNT™ Vector Multiple Cloning Site and Circle Map .....	3
5. pCMVTNT™ Vector Restriction Sites and Sequence Accession Number .....	5
6. References .....	7

## 1. Description

The pCMVTNT™ Vector<sup>(a,b)</sup> is designed for the convenient expression of cloned genes using in vivo or in vitro expression systems. Both the SP6 and the T7 polymerase promoters lie in tandem adjacent to the multiple cloning site, allowing for highly efficient synthesis of RNA in vitro from either promoter. Protein can be expressed in vitro from a gene cloned into the pCMVTNT™ Vector using an SP6- or T7-based, coupled in vitro transcription/translation system. The pCMVTNT™ Vector contains a 5' β-globin leader sequence reported to enhance expression of certain genes in vitro (1,2). For in vivo expression, the vector contains a cytomegalovirus (CMV) enhancer/promoter region that can allow strong constitutive expression in many cell types (3). A β-globin/IgG chimeric intron and a late SV40 polyadenylation site are located downstream of the enhancer/promoter region (4,5).

## 2. Product Components and Storage Conditions

Product	Size	Cat.#
pCMVTNT™ Vector	20µg	L5620

**Storage Conditions:** Store at -70°C to -20°C.

### 3. Features of the pCMVTnT™ Vector

#### Enhancer/Promoter Regions

The CMV enhancer/promoter region present in the pCMVTnT™ Vector allows strong, constitutive expression in many cell types. The promiscuous nature of the CMV enhancer/promoter has been demonstrated in transgenic mice, where expression of the chloramphenicol acetyltransferase (CAT) gene under the regulation of the CMV enhancer/promoter was observed in 24 of 28 tissues examined (3).

#### Chimeric Intron

Downstream of the enhancer/promoter region is a chimeric intron composed of the 5'-donor site from the first intron of the human  $\beta$ -globin gene and the branch and 3'-acceptor site from the intron that is between the leader and the body of an immunoglobulin gene heavy chain variable region (6). The sequences of the donor and acceptor sites, along with the branchpoint site, have been changed to match the consensus sequences for splicing (7). Transfection studies have demonstrated that the presence of an intron flanking the cDNA insert frequently increases the level of gene expression (8-11).

#### Tandem SP6 and T7 Promoters

Both SP6 and T7 promoters are located downstream of the intron (i.e., immediately upstream of the multiple cloning region). The presence of both of these promoters allows the convenient use of either an SP6- or T7-based in vitro coupled transcription/translation system.

#### Multiple Cloning Region

The multiple cloning region is immediately downstream from the T7 and SP6 promoters and the  $\beta$ -globin leader sequence. The sites in the multiple cloning region are compatible with subcloning cDNAs that have been prepared with the Universal RiboClone® cDNA Synthesis System (Cat.# C4360).

#### SV40 Late Polyadenylation Signal

Polyadenylation signals cause the termination of transcription by RNA polymerase II and signal the addition of approximately 200-250 adenosine residues to the 3'-end of the RNA transcript (12). Polyadenylation enhances RNA stability and translation (13,14). The late SV40 polyadenylation signal is extremely efficient and increases the steady-state level of RNA approximately fivefold more than the early SV40 polyadenylation signal (15).

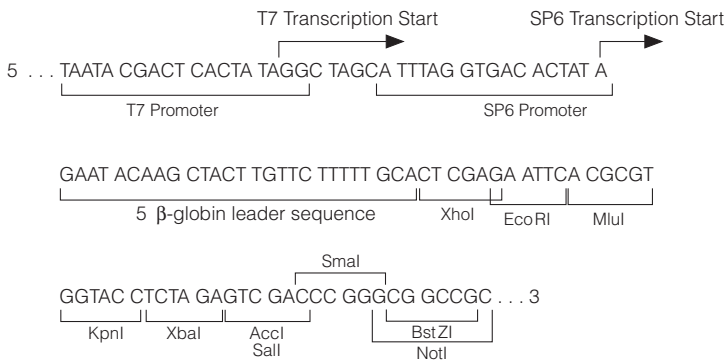
## 5' Leader Sequence of $\beta$ -Globin

Several factors have been identified that lead to efficient translation in vitro and in vivo. Among these are a cap site, an untranslated region, and a consensus sequence surrounding the AUG start site. As an approach to generating highly efficient mRNA for translation of foreign genes, hybrid RNAs have been synthesized in which the cognate leader is replaced with one derived from a highly efficient viral or eukaryotic mRNA (1). The 5' UTR of  $\beta$ -globin has been reported to increase the translation of several genes for more rapid initiation of translation (1,2).

## f1 Origin of Replication

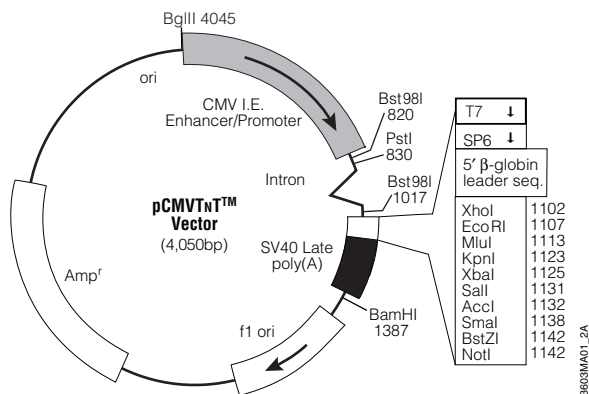
For generation of single-stranded DNA (ssDNA) from the f1 origin, bacteria transformed with the pCMVTnT™ Vector carrying the DNA insert of interest are infected with an appropriate helper phage. The plasmid then enters the f1 replication mode, and the resulting ssDNA is exported from the cell as an encapsidated virus particle. The ssDNA molecule exported has the sequence of the strand shown for the multiple cloning region (Figure 1).

## 4. pCMVTnT™ Vector Multiple Cloning Site and Circle Map



**Figure 1. pCMVTnT™ Vector multiple cloning site.** The sequence shown corresponds to RNA synthesized by the T7 or SP6 RNA polymerases. The strand shown is the same as the ssDNA strand produced by this vector.

#### 4. pCMVTnT™ Vector Multiple Cloning Site and Circle Map (continued)



**Figure 2. pCMVTnT™ Vector circle map and sequence reference points.**

Cytomegalovirus immediate-early enhancer/promoter region	1-795
Chimeric intron	857-989
T7 RNA polymerase promoter	1034-1052
SP6 RNA polymerase promoter	1058-1074
5' β-globin leader sequence	1075-1101
Multiple cloning region	1102-1148
SV40 late polyadenylation signal	1155-1376
Phage f1 region	1466-1921
β-Lactamase (Amp <sup>r</sup> ) coding region	2358-3218

**!** **Note:** Use the T7 EEV Promoter Primer (Cat.# Q6700) to sequence the pCMVTnT™ Vector. **Do not** use the T7 Promoter Primer (Cat.# Q5021) to sequence this vector as there is a sequence difference between the T7 Promoter Primer and the T7 promoter sequence in the pCMVTnT™ Vector.

## 5. pCMVTNT™ Vector Restriction Sites and Sequence Accession Number

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3'-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pCMVTNT™ Vector sequence is available on the Internet at: [www.promega.com/vectors](http://www.promega.com/vectors) and in the GenBank® database (Accession Number AF477200).

**Table 1. Restriction Enzymes That Cut the pCMVTNT™ Vector 1-5 Times.**

<b>Enzyme</b>	<b># of Sites</b>	<b>Location</b>	<b>Enzyme</b>	<b># of Sites</b>	<b>Location</b>
<i>Aat</i> II	5	278, 331, 414, 600, 2226	<i>Bst</i> O I	5	243, 436, 3874, 3887, 4008
<i>Acc</i> I	1	1132	<i>Bst</i> Z I	1	1142
<i>Acc</i> 65 I	1	1119	<i>Cfr</i> 10 I	2	1591, 3060
<i>Afl</i> II	2	820, 1017	<i>Cla</i> I	1	1380
<i>Afl</i> III	1	1113	<i>Dra</i> I	4	1346, 2567, 3259, 3278
<i>Alw</i> 44 I	3	1976, 2473, 3719	<i>Dra</i> II	1	2165
<i>Alw</i> N I	1	3624	<i>Dra</i> III	1	1699
<i>Asp</i> H I	5	721, 1980, 2477, 2562, 3723	<i>Drd</i> I	4	809, 1743, 2062, 3931
<i>Ava</i> I	2	1102, 1136	<i>Dsa</i> I	1	513
<i>Ava</i> II	2	2781, 3003	<i>Eae</i> I	4	8, 62, 1142, 2752
<i>Bal</i> I	2	10, 64	<i>Eag</i> I	1	1142
<i>Bam</i> H I	1	1387	<i>Ear</i> I	2	1404, 2346
<i>Ban</i> I	5	618, 943, 1119, 1655, 3192	<i>Ecl</i> HK I	1	3145
<i>Ban</i> II	2	721, 1625	<i>Eco</i> 52 I	1	1142
<i>Bbs</i> I	1	928	<i>Eco</i> ICR I	1	719
<i>Bgl</i> II	1	4045	<i>Eco</i> R I	1	1107
<i>Bsa</i> I	2	882, 3079	<i>Fok</i> I	5	950, 2063, 2706, 2993, 3174
<i>Bsa</i> O I	5	1145, 1426, 2627, 2776, 3699	<i>Fsp</i> I	2	1445, 2922
<i>Bsa</i> A I	2	493, 1696	<i>Hae</i> II	3	1541, 1549, 3793
<i>Bsa</i> B I	1	1386	<i>Hinc</i> II	3	669, 1133, 1285
<i>Bsa</i> J I	3	513, 1136, 3873	<i>Hind</i> II	3	669, 1133, 1285
<i>Bsa</i> M I	2	1206, 1299	<i>Hind</i> III	1	748
<i>Bsm</i> I	2	1206, 1299	<i>Hpa</i> I	1	1285
<i>Bsp</i> H I	3	2200, 2305, 3313	<i>Kpn</i> I	1	1123
<i>Bsp</i> M I	1	844	<i>Mlu</i> I	1	1113
<i>Bsr</i> G I	1	96	<i>Msp</i> A1 I	4	2043, 2509, 3450, 3695
<i>Bss</i> S I	3	2169, 2476, 3860	<i>Nae</i> I	1	1593
<i>Bst</i> 98 I	2	820, 1017			

**Note:** The enzymes listed in boldface type are available from Promega.

Table 1. Restriction Enzymes That Cut the pCMVNT™ Vector 1-5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
<i>Nco</i> I	1	513	<i>Sca</i> I	2	1030, 2664
<i>Nde</i> I	2	387, 1971	<i>Sin</i> I	2	2781, 3003
<i>Ngo</i> M IV	1	1591	<i>Sma</i> I	1	1138
<i>Nhe</i> I	1	1052	<i>Sna</i> B I	1	493
<i>Not</i> I	1	1142	<i>Spe</i> I	1	152
<i>Nsp</i> I	1	2120	<i>Ssp</i> I	4	5, 52, 1904, 2340
<i>Pae</i> R7 I	1	1102	<i>Sty</i> I	1	513
<i>Psp</i> A I	1	1136	<i>Vsp</i> I	2	160, 2970
<i>Pst</i> I	1	830	<i>Xba</i> I	1	1125
<i>Pvu</i> I	2	1426, 2776	<i>Xho</i> I	1	1102
<i>Sac</i> I	1	721	<i>Xma</i> I	1	1136
<i>Sal</i> I	1	1131	<i>Xmn</i> I	1	2545

Table 2. Restriction Enzymes That Do Not Cut the pCMVNT™ Vector.

<i>Acc</i> B7 I	<i>Blp</i> I	<i>Eco</i> 47 III	<i>Nru</i> I	<i>Psp</i> 5 II	<i>Sse</i> 8387 I
<i>Acc</i> III	<i>Bpu</i> 1102 I	<i>Eco</i> 72 I	<i>Nsi</i> I	<i>Pvu</i> II	<i>Stu</i> I
<i>Age</i> I	<i>Bsp</i> 120 I	<i>Eco</i> 81 I	<i>Pac</i> I	<i>Rsr</i> II	<i>Swa</i> I
<i>Apa</i> I	<i>Bss</i> H II	<i>Eco</i> N I	<i>Pfl</i> M I	<i>Sac</i> II	<i>Tfi</i> I
<i>Asc</i> I	<i>Bst</i> 1107 I	<i>Eco</i> R V	<i>Pin</i> A I	<i>Sfi</i> I	<i>Tth</i> 111 I
<i>Avr</i> II	<i>Bst</i> E II	<i>Ehe</i> I	<i>Pme</i> I	<i>Sgf</i> I	<i>Xcm</i> I
<i>Bbe</i> I	<i>Bst</i> X I	<i>Fse</i> I	<i>Pml</i> I	<i>Sgr</i> A I	
<i>Bbr</i> P I	<i>Bsu</i> 36 I	<i>I-Ppo</i> I	<i>Ppu</i> 10 I	<i>Sph</i> I	
<i>Bbu</i> I	<i>Csp</i> I	<i>Kas</i> I	<i>Ppu</i> M I	<i>Spl</i> I	
<i>Bcl</i> I	<i>Csp</i> 45 I	<i>Nar</i> I	<i>Psh</i> A I	<i>Srf</i> I	

Table 3. Restriction Enzymes That Cut the pCMVNT™ Vector 6 or More Times.

<i>Aci</i> I	<i>Bsr</i> I	<i>Fnu</i> 4H I	<i>Hsp</i> 92 II	<i>Msp</i> I	<i>Sau</i> 96 I
<i>Acy</i> I	<b><i>Bsr</i>S I</b>	<b><i>Hae</i> III</b>	<i>Mae</i> I	<b><i>Nci</i> I</b>	<i>Scr</i> F I
<i>Alu</i> I	<i>Bst</i> 71 I	<i>Hga</i> I	<i>Mae</i> II	<b><i>Nde</i> II</b>	<i>Sfa</i> N I
<b><i>Alw</i>26 I</b>	<i>Bst</i> U I	<b><i>Hha</i> I</b>	<i>Mae</i> III	<i>Nla</i> III	<b><i>Taq</i> I</b>
<i>Bbv</i> I	<b><i>Cfo</i> I</b>	<b><i>Hinf</i> I</b>	<b><i>Mbo</i> I</b>	<i>Nla</i> IV	<b><i>Tru</i>9 I</b>
<b><i>Bgl</i> I</b>	<b><i>Dde</i> I</b>	<b><i>Hpa</i> II</b>	<b><i>Mbo</i> II</b>	<i>Ple</i> I	<b><i>Xho</i> II</b>
<i>Bsa</i> H I	<b><i>Dpn</i> I</b>	<i>Hph</i> I	<i>Mnl</i> I	<b><i>Rsa</i> I</b>	
<b><i>Bsp</i>1286 I</b>	<i>Dpn</i> II	<b><i>Hsp</i>92 I</b>	<i>Mse</i> I	<b><i>Sau</i>3A I</b>	

Note: The enzymes listed in boldface type are available from Promega.

## 6. References

1. Falcone, D. and Andrews, D.W. (1991) Both the 5' untranslated region and the sequences surrounding the start site contribute to efficient initiation of translation in vitro. *Mol. Cell. Biol.* **11**, 2656-64.
2. Annweiler, A., Hipskind, R.A. and Wirth, T. (1991) A strategy for efficient in vitro translation of cDNAs using the rabbit  $\beta$ -globin leader sequence. *Nucl. Acids Res.* **19**, 3750.
3. Schmidt, E.V. *et al.* (1990) The cytomegalovirus enhancer: A pan-active control element in transgenic mice. *Mol. Cell. Biol.* **10**, 4406-11.
4. Brinster, R.L. *et al.* (1988) Introns increase transcriptional efficiency in transgenic mice. *Proc. Natl. Acad. Sci. USA* **85**, 836-40.
5. Carswell, S. and Alwine, J.C. (1989) Efficiency of utilization of the simian virus 40 late polyadenylation site: Effects of upstream sequences. *Mol. Cell. Biol.* **9**, 4248-58.
6. Bothwell, A.L. *et al.* (1981) Heavy chain variable region contribution to the NPb family of antibodies: Somatic mutation evident in a gamma 2a variable region. *Cell* **24**, 625-37.
7. Senapathy, P., Shapiro, M.B. and Harris, N.L. (1990) Splice junctions, branch point sites, and exons: Sequence statistics, identification, and applications to genome project. *Methods Enzymol.* **183**, 252-78.
8. Gross, M.K., Kainz, M.S. and Merrill, G.F. (1987) Introns are inconsequential to efficient formation of cellular thymidine kinase mRNA in mouse L cells. *Mol. Cell. Biol.* **7**, 4576-81.
9. Buchman, A.R. and Berg, P. (1988) Comparison of intron-dependent and intron-independent gene expression. *Mol. Cell. Biol.* **8**, 4395-405.
10. Evans, M.J. and Scarpulla, R.C. (1989) Introns in the 3'-untranslated region can inhibit chimeric CAT and beta-galactosidase gene expression. *Gene* **84**, 135-42.
11. Huang, M.T. and Gorman, C.M. (1990) Intervening sequences increase efficiency of RNA 3' processing and accumulation of cytoplasmic RNA. *Nucl. Acids Res.* **18**, 937-47.
12. Proudfoot, N. (1991) Poly(A) signals. *Cell* **64**, 671-4.
13. Bernstein, P. and Ross, J. (1989) Poly(A), poly(A) binding protein and the regulation of mRNA stability. *Trends Biochem. Sci.* **14**, 373-7.
14. Jackson, R.J. and Standart, N. (1990) Do the poly(A) tail and 3' untranslated region control mRNA translation? *Cell* **62**, 15-24.
15. Carswell, S. and Alwine, J.C. (1989) Efficiency of utilization of the simian virus 40 late polyadenylation site: Effects of upstream sequences. *Mol. Cell. Biol.* **9**, 4248-58.

<sup>4a)</sup>Licensed for research and laboratory use only under U.S. Pat. No. 5,547,862.

<sup>6b)</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. Research Use includes contract research for which monetary or other consideration may be received. Other commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

© 2002, 2006, 2009 Promega Corporation. All Rights Reserved.

RiboClone is a registered trademark of Promega Corporation. pCMVINT is a trademark of Promega Corporation.

DNASTAR is a registered trademark of DNASTAR, Inc. GenBank is a registered trademark of the U.S. Department of Health and Human Services.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.