TECHNICAL BULLETIN

pCMVTnT™ Vector

Instructions for Use of Product
L5620

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TB305

pCMVTnT™ Vector

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1. Description

The pCMVTnT™ Vector 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 β-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 β-globin leader sequence. The sites in the multiple cloning region are compatible with subcloning cDNA 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 β-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 β-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

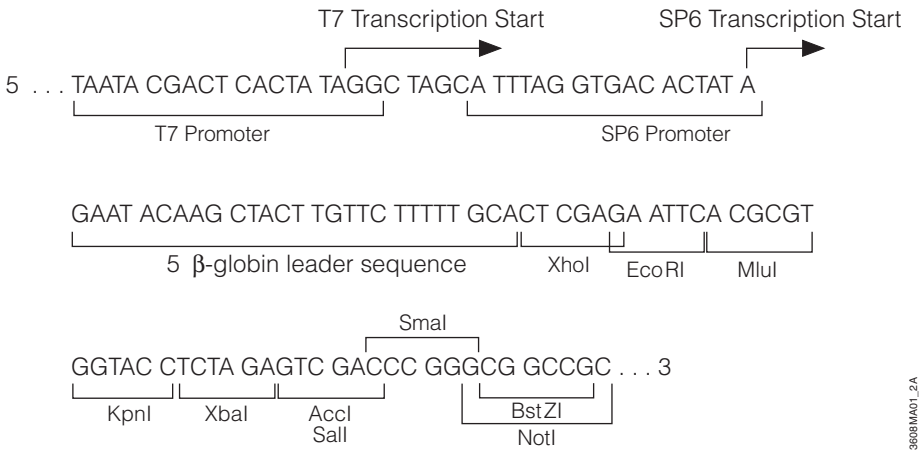

Figure 1. pCMVTNT™ Vector multiple cloning site. The sequence shown corresponds to RNA synthesized by the T7 or SP6 RNA polymerases. The strand shown is the same as the ssDNA strand produced by this vector.

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

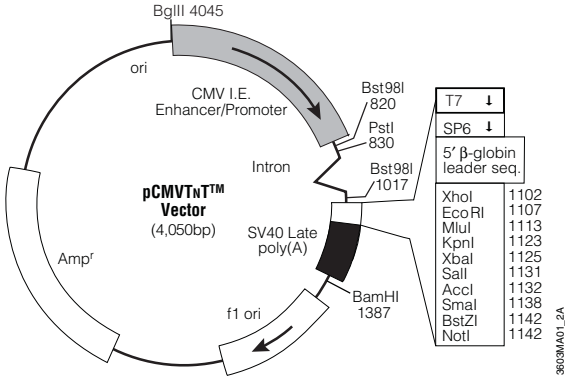

Figure 2. pCMVTNT™ Vector circle map and sequence reference points.

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 (Ampr) 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 at: **www.promega.com/vectors/** and in the GenBank® database (GenBank®/EMBL Accession Number **AF477200**).

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

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AatII	5	278, 331, 414, 600, 2226	EarI	2	1404, 2346
AccI	1	1132	**EclHKI**	1	3145
Acc65I	1	1119	Eco52I	1	1142
AflII	2	820, 1017	**EcoICRI**	1	719
AflIII	1	1113	**EcoRI**	1	1107
Alw44I	3	1976, 2473, 3719	**FokI**	5	950, 2063, 2706, 2993, 3174
AlwNI	1	3624	FspI	2	1445, 2922
AspHI	5	721, 1980, 2477, 2562, 3723	**HaeII**	3	1541, 1549, 3793
AvaI	2	1102, 1136	**HincII**	3	669, 1133, 1285
AvaII	2	2781, 3003	HindII	3	669, 1133, 1285
BalI	2	10, 64	**HindIII**	1	748
BamHI	1	1387	HpaI	1	1285
BanI	5	618, 943, 1119, 1655, 3192	**KpnI**	1	1123
BanII	2	721, 1625	**MluI**	1	1113
BbsI	1	928	**MspAI**	4	2043, 2509, 3450, 3695
			NaeI	1	1593
BglI	1	4045	**NcoI**	1	513
BsaI	2	882, 3079	**NdeI**	2	387, 1971
BsaOI	5	1145, 1426, 2627, 2776, 3699	NgoMIV	1	1591
BsaAI	2	493, 1696	**NheI**	1	1052
BsaBI	1	1386	**NotI**	1	1142
BsaJI	3	513, 1136, 3873	NspI	1	2120
BsaMI	2	1206, 1299	PaeR7I	1	1102
BsmI	2	1206, 1299	PspAI	1	1136
BspHI	3	2200, 2305, 3313	**PstI**	1	830
BspMI	1	844	**PvuI**	2	1426, 2776
BsrGI	1	96	**SacI**	1	721
BssSI	3	2169, 2476, 3860	**SalI**	1	1131
Bst98I	2	820, 1017	**ScaI**	2	1030, 2664
BstOI	5	243, 436, 3874, 3887, 4008	**SinI**	2	2781, 3003
BstZI	1	1142	**SmaI**	1	1138
Cfr10I	2	1591, 3060	**SnaBI**	1	493
ClaI	1	1380	**SpeI**	1	152
DraI	4	1346, 2567, 3259, 3278	**SspI**	4	5, 52, 1904, 2340
DraII	1	2165	**StyI**	1	513
DraIII	1	1699	**VspI**	2	160, 2970
DrdI	4	809, 1743, 2062, 3931	**XbaI**	1	1125
DsaI	1	513	**XhoI**	1	1102
EaeI	4	8, 62, 1142, 2752	**XmaI**	1	1136
EagI	1	1142	**XmnI**	1	2545

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

5. pCMVTNT™ Vector Restriction Sites and Sequence Accession Number (continued)

Table 2. Restriction Enzymes that Do Not Cut the pCMVTNT™ Vector.

AccB7I	BbuI	**BstXI**	**EcoRV**	PacI	Psp5II	SplI
AccIII	**BclI**	Bsu36I	EheI	PflMI	**PvuII**	SrfI
AgeI	BlpI	**CspI**	FseI	PinAI	RsrII	Sse8387I
ApaI	Bpu1102I	**Csp45I**	**I-PpoI**	PmeI	**SacII**	**StuI**
AscI	Bsp120I	**Eco47III**	KasI	PmlI	**SfiI**	SwaI
AvrII	**BssHII**	Eco72I	**NarI**	Ppu10I	**SgfI**	TfiI
BbeI	Bst1107I	Eco81I	**NruI**	PpuMI	SgrAI	Tth111I
BbrPI	**BstEII**	EcoNI	**NsiI**	PshAI	**SphI**	XcmI

Table 3. Restriction Enzymes that Cut the pCMVTNT™ Vector 6 or More Times.

AciI	BsrI	Fnu4HI	Hsp92 II	**MspI**	Sau96I
AcyI	**BsrSI**	**HaeIII**	MaeI	NciI	ScrFI
AluI	Bst71I	HgaI	MaeII	**NdeII**	SfaNI
Alw26I	BstUI	**HhaI**	MaeIII	NlaIII	**TaqI**
BbvI	**CfoI**	**HinfI**	**MboI**	NlaIV	**Tru9I**
BglI	**DdeI**	**HpaII**	**MboII**	PleI	**XhoII**
BsaHI	**DpnI**	HphI	MnlI	**Rsa I**	
Bsp1286I	DpnII	**Hsp92I**	MseI	**Sau3AI**	

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

6. References

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7. Summary of Changes

The following change was made in the 9/14 revision of this document:

Patent and licensing information was updated.