TECHNICAL BULLETIN

pTNT™ Vector

Instructions for Use of Product
L5610

Revised 8/14
TB304

Promega

pTNT™ Vector

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1. Description

The pTNT™ Vector is designed for the convenient expression of cloned genes using in vitro expression systems. Both the SP6 and the T7 polymerase promoters lie in tandem adjacent to the multiple cloning site, allowing for the highly efficient synthesis of RNA in vitro from either promoter. Protein can be expressed in vitro from a gene cloned into the pTNT™ Vector using an SP6- or T7-based, in vitro coupled transcription/translation system. The pTNT™ Vector contains a 5´ β-globin leader sequence and a synthetic poly$(A)_{30}$ tail, which have both been reported to enhance expression of certain genes (1–3). The vector also contains a T7 terminator site (4).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
pTNT™ Vector	20μg	L5610

Storage Conditions: Store at –70°C to –20°C.

3. Features of the pTNT™ Vector

Tandem SP6 and T7 Promoters

Both the SP6 and T7 promoters are located 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. 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).

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).

Poly(A) Tail

A synthetic poly(A) tail of 30 residues has been added downstream of the multiple cloning site. The presence of a poly(A) tail at the 3´ end of an RNA transcript has been referenced for enhanced translation of RNAs containing a consensus Kozak sequence or for transcripts containing only an initial start codon (3,5).

f1 Origin of Replication

For generation of single-stranded DNA (ssDNA) from the f1 origin, bacteria transformed with the pTNT™ 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. pTNT™ Vector Multiple Cloning Site and Circle Map

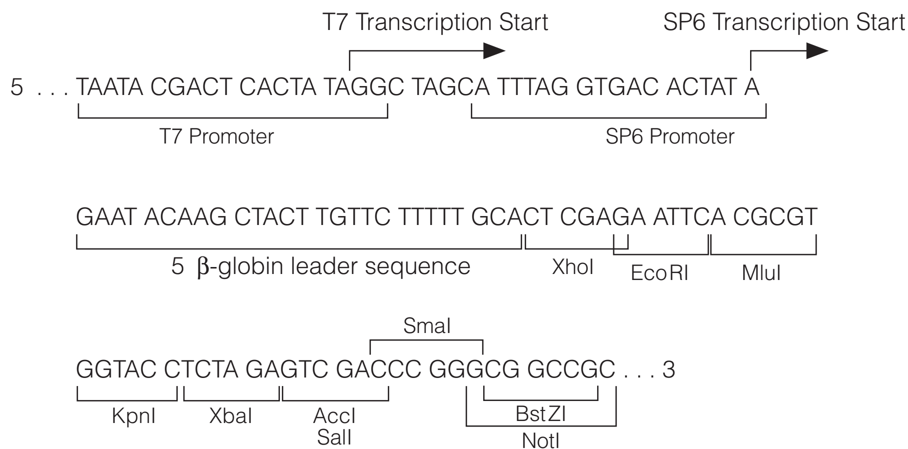

Figure 1. pTNT™ Vector multiple cloning site. The sequence shown corresponds to RNA synthesized by the T7 or SP6 RNA polymerases. The strand shown is the same as the ssDNA strand produced by this vector.

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

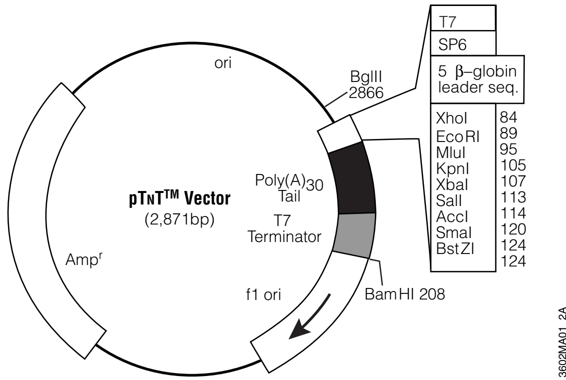

Figure 2. pTNT™ Vector circle map and sequence reference points.

T7 RNA polymerase promoter	16–34
SP6 RNA polymerase promoter	40–56
5´ β-globin leader sequence	57–83
Multiple cloning region	84–130
Synthetic poly(A)$_{30}$ region	131–160
T7 transcription terminator sequence	161–208
Phage f1 region	287–742
β-Lactamase (Amp^{r}) coding region	1179–2039

Note: Use the T7 EEV Promoter Primer (Cat.# Q6700) to sequence the pTNT™ 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 pTNT™ Vector.

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5. pTNT™ 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 pTNT™ Vector sequence is available at: **www.promega.com/vectors/** and in the GenBank® database (GenBank®/EMBL Accession Number **AF479322**).

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

Enzyme	# of Sites	Location
AatII	1	1047
AccI	1	114
Acc65I	1	101
AcyI	2	1044, 1426
AflIII	1	95
Alw26I	4	929, 971, 1124, 1900
Alw44I	3	797, 1294, 2540
AlwNI	1	2445
AspHI	4	801, 1298, 1383, 2544
AvaI	2	84, 118
AvaII	2	1602, 1824
BamHI	1	208
BanI	3	101, 476, 2013
BanII	1	446
BglI	2	276, 1848
BglII	1	2866
BsaI	1	1900
BsaOI	5	127, 247, 1448, 1597, 2520
BsaAI	1	517
BsaHI	2	1044, 1426
BsaJI	3	118, 172, 2694
Bsp1286I	5	446, 801, 1298, 1383, 2544
BspHI	3	1021, 1126, 2134
BssSI	3	990, 1297, 2681
BstOI	1	2695, 2708, 2829
BstZI	1	124
Cfr10I	2	412, 1881
DraI	3	1388, 2080, 2099
DraII	2	177, 986
DraIII	1	520
DrdI	3	564, 883, 2752
EaeI	2	124, 1573
EagI	1	124
EarI	2	225, 116
EclHKI	1	1966
Eco52I	1	124
EcoRI	1	89
FokI	4	884, 1527, 1814, 1995
FspI	2	266, 1743
HaeII	3	362, 370, 2614
HgaI	5	295, 876, 1434, 2164, 2742
HincII	1	115
HindII	1	115
Hsp92I	2	1044, 1426
KpnI	1	105
MluI	1	95
MspAI	4	864, 1330, 2271, 2516
NaeI	1	414
NdeI	1	792
NgoMIV	1	412
NheI	1	34
NotI	1	124
NspI	1	941
PaeR7I	1	84
PspAI	1	118
PvuI	2	247, 1597
RsaI	4	12, 103, 809, 1485
SalI	1	113
ScaI	2	12, 1485
SinI	2	1602, 1824
SmaI	1	120
SspI	2	725, 1161
StyI	1	172
VspI	1	1791
XbaI	1	107
XhoI	1	84
XmaI	1	118
XmnI	1	1366

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

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

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

AccB7I	BbuI	Bst1107I	Eco81I	**NcoI**	Psp5II	**SphI**
AccIII	**BclI**	**Bst98I**	**EcoICRI**	**NruI**	**PstI**	SplI
AflII	BlpI	**BstEII**	EcoNI	**NsiI**	**PvuII**	SrfI
AgeI	Bpu1102I	**BstXI**	**EcoRV**	PacI	RsrII	Sse8387I
ApaI	BsaBI	**Bsu36I**	EheI	PflMI	**SacI**	**StuI**
AscI	BsaMI	**ClaI**	FseI	PinAI	**SacII**	SwaI
AvrII	BsmI	**CspI**	**HindIII**	PmeI	**SfiI**	TfiI
BalI	Bsp120I	**Csp45I**	**HpaI**	PmlI	**SgfI**	Tth111I
BbeI	BspMI	DsaI	**I-PpoI**	Ppu10I	SgrAI	XcmI
BbrPI	BsrGI	**Eco47III**	KasI	PpuMI	**SnaBI**	
BbsI	**BssHII**	Eco72I	**NarI**	PshAI	**SpeI**	

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

AciI	**DdeI**	HphI	MseI	Sau96I
AluI	**DpnI**	**Hsp92I**	**MspI**	ScrFI
BbvI	DpnII	MaeI	NciI	SfaNI
BsrI	Fnu4HI	MaeII	**NdeII**	**TaqI**
BsrSI	**HaeIII**	MaeIII	NlaIII	**Tru9I**
Bst71I	**HhaI**	**MboI**	NlaIV	**XhoII**
BstUI	**HinfI**	**MboII**	PleI	
CfoI	**HpaII**	MnlI	**Sau3AI**	

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

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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. Wakiyama, M., Futami, T. and Miura, K. (1997) Poly(A) dependent translation in rabbit reticulocyte lysate. *Biochimie* **79**, 781–785.
4. Sengupta, D., Chakravarti, D. and Maitra, U. (1989) Relative efficiency of utilization of promoter and termination sites by bacteriophage T3 RNA polymerase. *J. Biol. Chem.* **264**, 14246–55.
5. Betz, N. (2000) Characterization of TNT® T7 Quick for PCR DNA. *Promega Notes* **77**, 19–22.

7. Summary of Changes

The following change was made in the 8/14 revision of this document:

Patent and licensing information was updated.

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