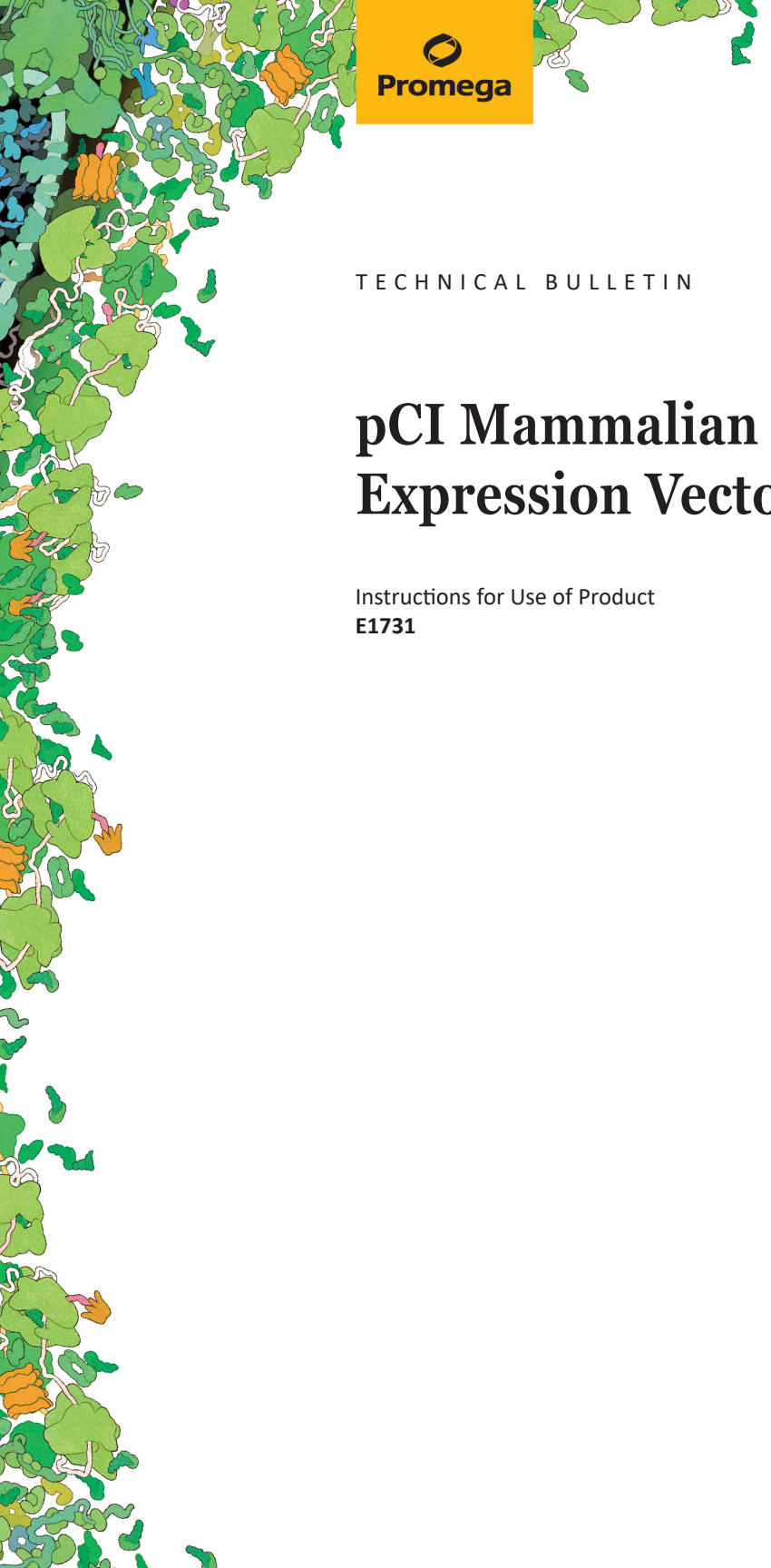


TECHNICAL BULLETIN

pCI Mammalian Expression Vector

Instructions for Use of Product
E1731

Revised 8/21
TB206

pCI Mammalian Expression Vector

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The pCI Mammalian Expression Vector is designed to promote constitutive expression of cloned DNA inserts in mammalian cells. The pCI Expression Vector contains the human cytomegalovirus (CMV) major immediate-early gene enhancer/promoter region to express the inserted gene.

The pCI Vector can be used for both transient and stable expression of genes. For stable expression, the pCI Vector must be co-transfected with an expression vector containing a selectable gene for mammalian cells.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
pCI Mammalian Expression Vector	**20µg**	**E1731**

pCI Mammalian Expression Vector is supplied frozen in TE buffer (pH 8.0).

Storage Conditions: Store the vector at –10°C to –30°C.

3. pCI Mammalian Expression Vector Multiple Cloning Sequence and Map

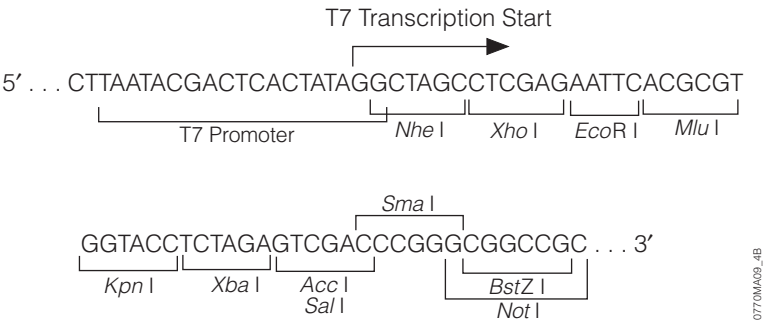
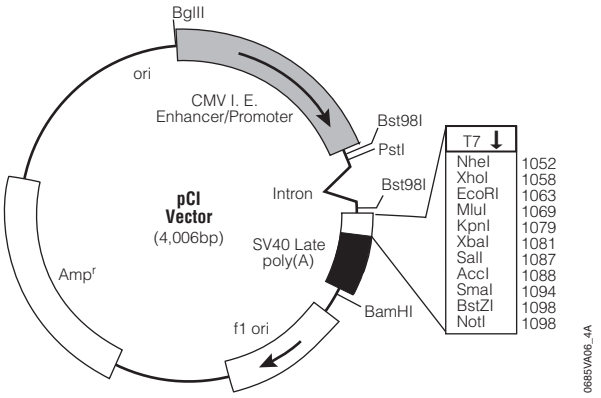


Figure 1. Multiple cloning region sequence and T7 promoter of the pCI Mammalian Expression Vector. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase. The strands shown are the same as the ssDNA strands produced by the pCI Vector.



pCI Mammalian Expression Vector sequence reference points:

Cytomegalovirus immediate-early enhancer/promoter region	1–742
Chimeric intron	857–989
T7-EEV sequencing primer binding site	1020–1041
T7 RNA Polymerase Promoter (–17 to +2)	1034–1052
T7 promoter transcription start site	1051
Multiple cloning region	1052–1104
SV40 late polyadenylation signal	1111–1332
Phage f1 region	1422–1877
β-lactamase (Amp^r) coding region	2314–3174
ColEl-derived origin of replication	3936

4. Vector Components

4.A. Enhancer/Promoter Regions

The CMV enhancer/promoter region present in the pCI Vector allow 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 the 28 tissues examined (1).

4.B. 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 (2). The sequences of the donor and acceptor sites, along with the branchpoint site, have been changed to match the consensus sequences for splicing (3).

4.B. Chimeric Intron (continued)

Transfection studies have demonstrated that the presence of an intron flanking the cDNA insert frequently increases the level of gene expression (4–7). The increase in expression level due to the presence of the intron depends on the particular cDNA insert.

The intron is located 5´ to the cDNA insert in order to prevent utilization of possible cryptic 5´-donor splice sites within the cDNA sequence (8). In transgenic experiments, the presence of an intron is necessary to promote a high level of expression for virtually all cDNA inserts (9–11).

4.C. T7 Promoter

A T7 promoter is located downstream of the intron (i.e., immediately upstream of the multiple cloning region). This promoter can be used to synthesize RNA transcripts in vitro using T7 RNA Polymerase (Cat.# P2075).

4.D. Multiple Cloning Region

The multiple cloning region is immediately downstream from the T7 promoter. The sites in the multiple cloning region are compatible with subcloning cDNAs that have been prepared with the Universal RiboClone® cDNA Synthesis Systems (Cat.# C4360).

Note: There are no ATG sequences in either the multiple cloning region or between the transcription start site and the multiple cloning region. Thus, an ATG for the initiation of translation must be present in the inserted DNA.

4.E. 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 has been shown to enhance RNA stability and translation (13,14). The late SV40 polyadenylation signal is extremely efficient and has been shown to increase the steady-state level of RNA approximately 5-fold more than the early SV40 polyadenylation signal (15).

4.F. f1 Origin of Replication and Plasmid Replicon

The backbone for the pCI Vector was derived from the pGEM®-3Zf(+) Vector. As a result, these vectors are high-copy plasmids and contain the origin of replication of the filamentous phage f1. For generation of single-stranded DNA (ssDNA) from the f1 origin, bacteria transformed with the pCI 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). For further information, please contact your local Promega Branch Office or Distributor. In the U.S., contact Technical Services at 1-800-356-9526.

The “poison” sequence present in pBR322 that has been shown to inhibit replication of SV40 origin-containing vectors in COS cells has been deleted in the pCI Vector (16). This results in more efficient expression of the cloned cDNAs in COS cells and other cells that have been transformed with the SV40 large T antigen.

5. Related Products

Product	Size	Cat. #
Flexi® Cloning System Entry/Transfer	5 entry, 20 transfer reactions	C8640
Flexi® Cloning System Transfer	100 transfer reactions	C8820
Carboxy Flexi® System, Transfer	50 transfer reactions	C9320
pCI-neo Mammalian Expression Vector	20µg	E1841
pAdVAntage™ Vector	20µg	E1711
ProFection® Mammalian Transfection System—Calcium Phosphate	40 transfections	E1200
TransFast™ Transfection Reagent	1.2mg	E2431

Product	Size	Cat.#
Universal RiboClone® cDNA Synthesis System	1 system	C4360
T7 RNA Polymerase*	1,000 units	P2075

*For Laboratory Use.

6. References

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2. 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.
3. 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.
4. 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.
5. Buchman, A.R. and Berg, P. (1988) Comparison of intron-dependent and intronindependent gene expression. *Mol. Cell. Biol.* **8**, 4395–405.
6. Evans, M.J. and Scarpulla, R.C. (1989) Introns in the 3′-untranslated region can inhibit chimeric CAT and β-galactosidase gene expression. *Gene* **84**, 135–42.
7. Huang, M.T. and Gorman, C.M. (1990) Intervening sequences increase efficiency of RNA 3′ processing and accumulation of cytoplasmic RNA. *Nucleic Acids Res.* **18**, 937–47.
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10. Choi, T. *et al.* (1991) A generic intron increases gene expression in transgenic mice. *Mol. Cell. Biol.* **11**, 3070–4.

6. References (continued)

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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.
16. Lusky, M. and Botchan, M. (1981) Inhibition of SV40 replication in simian cells by specific pBR322 DNA sequences. *Nature* **293**, 79–81.

7. pCI Mammalian Expression Vector Restriction Enzyme Sites

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. Vector sequences are available in the GenBank® database (GenBank®/EMBL Accession Number U47119) and online at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pCI Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	5	278, 331, 414, 600, 2182
AccI	1	1088
Acc65I	1	1075
AflII	2	820, 1017
AflIII	1	1069
Alw44I	3	1932, 2429, 3675
AlwNI	1	3580
AspHI	5	721, 1936, 2433, 2518, 3679
AvaI	2	1058, 1092
AvaII	2	2737, 2959
BalI	2	10, 64
BamHI	1	1343
BanI	5	618, 943, 1075, 1611, 3148
BanII	2	721, 1581
BbsI	1	928
BglII	1	4001
BsaI	2	882, 3035
BsaOI	5	1101, 1382, 2583, 2732, 3655
BsaAI	2	493, 1652

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

Table 1. Restriction Enzymes That Cut the pCI Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location
BsaBI	1	1342
BsaJI	3	513, 1092, 3829
BsaMI	2	1162, 1255
BsmI	2	1162, 1255
BspHI	3	2156, 2261, 3269
Bsp I	1	844
BsrGI	1	96
BssSI	3	2125, 2432, 3816
Bst98I	2	820, 1017
BstOI	5	243, 436, 3830, 3843, 3964
BstZI	1	1098
Cfr10I	2	1547, 3016
ClaI	1	1336
DraI	4	1302, 2523, 3215, 3234
DraII	1	2121
DraIII	1	1655
DrdI	4	809, 1699, 2018, 3887
DsaI	1	513
EaeI	4	8, 62, 1098, 2708
EagI	1	1098
EarI	2	1360, 2302
EclHKI	1	3101
Eco52I	1	1098
EcoICRI	1	719
EcoRI	1	1063
FokI	5	950, 2019, 2662, 2949, 3130
FspI	2	1401, 2878
HaeII	3	1497, 1505, 3749
HincII	3	669, 1089, 1241

Enzyme	# of Sites	Location
HindII	3	669, 1089, 1241
HindIII	1	748
HpaI	1	1241
KpnI	1	1079
MluI	1	1069
MspA1	4	1999, 2465, 3406, 3651
NaeI	1	1549
NcoI	1	513
NdeI	2	387, 1927
NgoMIV	1	1547
NheI	1	1052
NotI	1	1098
NspI	1	2076
PaeR7I	1	1058
PspAI	1	1092
PstI	1	830
PvuI	2	1382, 2732
SacI	1	721
SalI	1	1087
ScaI	2	1030, 2620
SinI	2	2737, 2959
SmaI	1	1094
SnaBI	1	493
SpeI	1	152
SspI	4	5, 52, 1860, 2296
StyI	1	513
VspI	2	160, 2926
XbaI	1	1081
XhoI	1	1058
XmaI	1	1092
XmnI	1	2501

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

7. pCI Mammalian Expression Vector Restriction Enzyme Sites (continued)

Table 2. Restriction Enzymes That Do Not Cut the pCI Vector.

AccB7I	BbuI	BstXI	**EcoRV**	PacI	Psp5II	SplI
AccIII	**BclI**	Bsu36I	EheI	PflMI	PvuII	SrfI
AgeI	BlpI	CspI	FseI	PinAI	RsrII	Sse8387 I
ApaI	Bpu1102 I	Csp45I	**I-PpoI**	PmeI	**SacII**	StuI
AscI	Bsp120I	Eco47III	KasI	PmlI	SfiI	SwaI
AvrII	BssHII	Eco72I	NarI	Ppu10I	**SgfI**	TfiI
BbeI	Bst1107 I	Eco81I	NruI	PpuMI	SgrAI	Tth111 I
BbrPI	BstEII	EcoNI	NsiI	PshAI	SphI	XcmI

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

AciI	Bsp1286 I	**DpnI**	**HpaII**	MboI	NlaIII	SfaNI
AcyI	BsrI	DpnII	HphI	MboII	NlaIV	**TaqI**
AluI	BsrSI	Fnu4HI	Hsp92I	MnlI	PleI	Tru9I
Alw26I	Bst71I	**HaeIII**	Hsp92II	MseI	**RsaI**	XhoII
BbvI	BstUI	HgaI	MaeI	**MspI**	Sau3AI	
BglI	**CfoI**	**HhaI**	MaeII	NciI	Sau96I	
BsaHI	**DdeI**	**HinfI**	MaeIII	NdeII	ScrFI	

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

8. Summary of Changes

The following changes were made to the 8/21 revision of this document:

1. Removed discontinued Cat.# E1721 and associated information.
2. Updated the cover page.
3. Removed disclaimers.