Promega

Technical Bulletin

pCI and pSI Mammalian Expression Vectors

INSTRUCTIONS FOR USE OF PRODUCTS E1721 AND E1731.

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pCI and pSI Mammalian Expression Vectors

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1. Description

The pCI[a] and pSI Mammalian Expression Vectors are designed to promote constitutive expression of cloned DNA inserts in mammalian cells. The major difference between the two vectors is the enhancer/promoter region controlling the expression of the inserted gene. The pSI Expression Vector contains the simian virus 40 (SV40) enhancer and early promoter region, whereas the pCI Expression Vector contains the human cytomegalovirus (CMV) major immediate-early gene enhancer/promoter region.

The pSI and pCI Vectors can be used for both transient and stable expression of genes. For stable expression, the pSI or pCI Vectors 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
pSI Mammalian Expression Vector	20µg	E1721

pCI and pSI Mammalian Expression Vectors are supplied frozen in TE buffer (pH 8.0).

Storage Conditions: Store the vectors at –20°C.

3. pCI Mammalian Expression Vector Multiple Cloning Sequence and Map

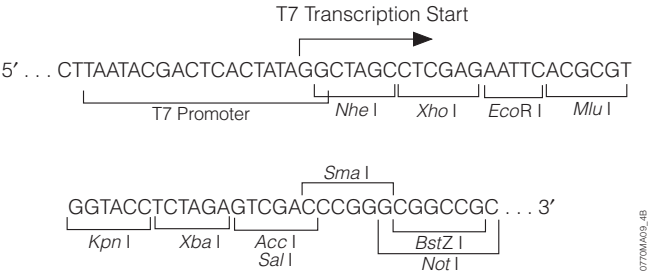
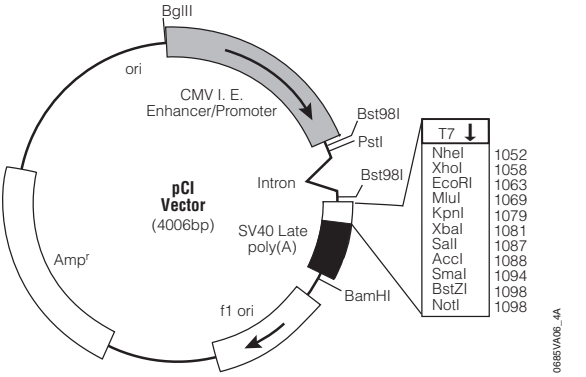


Figure 1. Multiple cloning region sequence and T7 promoter of the pCI Mammalian Expression Vector. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase. The strands shown are the same as the ssDNA strands produced by the pCI Vector.



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
*Col*El-derived origin of replication	3936

Use the T7 EEV Promoter Primer (Cat.# Q6700) to sequence ssDNA produced by the pCI and pSI Vectors. **Do Not** use the T7 Promoter Primer (Cat.# Q5021) to sequence the pCI or pSI Vectors. There is a sequence difference between the primer and the promoter sequences.

4. pSI Mammalian Expression Vector Multiple Cloning Sequence and Map

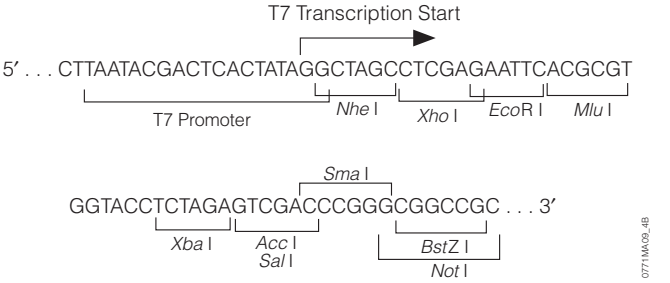
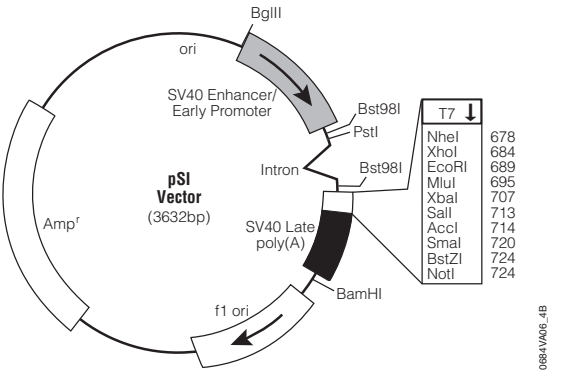


Figure 2. Multiple cloning region sequence and T7 promoter of the pSI Mammalian Expression Vector. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase. The strands shown are the same as the ssDNA strands produced by the pSI Vector.



pSI Mammalian Expression Vector sequence reference points:

SV40 enhancer and early promoter region	1–419
Chimeric intron	483–615
T7 EEV sequencing primer binding site	646–667
T7 RNA Polymerase Promoter (–17 to +2)	660–678
T7 promoter transcription start site	677
Multiple cloning region	678–730
SV40 late polyadenylation signal	737–958
Phage f1 region	1048–1503
β-lactamase (Amp^r) coding region	1940–2800

Use T7 EEV Promoter Primer (Cat.# Q6700) to sequence ssDNA produced by the pCI and pSI Vectors. **Do Not** use the T7 Promoter Primer (Cat.# Q5021) to sequence the pCI or pSI Vectors. There is a sequence difference between the primer and the promoter sequences.

5. Vector Components

5.A. Enhancer/Promoter Regions

The SV40 enhancer/promoter region present in the pSI Vector and 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). The pSI Vector contains the SV40 origin of replication, which allows transient, episomal replication in cells expressing the SV40 large T antigen such as COS-1 or COS-7 cells (2).

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

Transfection studies have demonstrated that the presence of an intron flanking the cDNA insert frequently increases the level of gene expression (5–8). The increase in expression level due to the presence of the intron depends on the particular cDNA insert. For example, in transient transfections of 293s cells, we have found that the presence of the chimeric intron in the pSI Vector results in a 21-fold increase in expression of the CAT gene. In contrast, the chimeric intron increases the gene expression level from the luciferase cDNA by only 3-fold.

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 (9). In transgenic experiments, the presence of an intron is necessary to promote a high level of expression for virtually all cDNA inserts (10–12).

5.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).

5.D. Multiple Cloning Region

The multiple cloning region is immediately downstream from the T7 promoter. The unique restriction sites available in the multiple cloning region of the pCI and pSI Vectors are identical, **except that the pSI Vector does not contain a unique *Kpn* I site within this region**. 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.

5.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 (13). Polyadenylation has been shown to enhance RNA stability and translation (14,15). 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 (16).

5.F. f1 Origin of Replication and Plasmid Replicon

The backbones for the pCI and pSI Vectors were 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 pSI or pCI Vectors 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 (Figures 1 and 2). 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 and pSI Vectors (17). 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.

6. 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
T7 EEV Promoter Primer	2µg	Q6700
ProFection® Mammalian Transfection System—Calcium Phosphate	40 transfections	E1200
TransFast™ Transfection Reagent	1.2mg	E2431
Tfx™-50 Transfection Reagent	2.1mg	E1811
Tfx™-20 Transfection Reagent	4.8mg	E2391

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

*For Laboratory Use.

7. References

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7. References (continued)

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8. Appendix

8.A. 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
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
EcoI	1	1063
FokI	5	950, 2019, 2662, 2949, 3130
FspI	2	1401, 2878
HaeII	3	1497, 1505, 3749
HincII	3	669, 1089, 1241
HindII	3	669, 1089, 1241
HindIII	1	748
HpaI	1	1241
KpnI	1	1079
MluI	1	1069

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

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

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

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

AciI	Bst71I	**HinfI**	MnlI	Sau96I
AcyI	BstUI	**HpaII**	MseI	ScrFI
AluI	**CfoI**	HphI	**MspI**	SfaNI
Alw26I	**DdeI**	**Hsp92I**	**NciI**	**TaqI**
BbvI	**DpnI**	**Hsp92II**	**NdeII**	**Tru9I**
BglI	DpnII	MaeI	NlaIII	**XhoII**
BsaHI	Fnu4HI	MaeII	NlaIV	
Bsp1286 I	**HaeIII**	MaeIII	PleI	
BsrI	HgaI	MboI	**RsaI**	
BsrSI	**HhaI**	**MboII**	**Sau3A I**	

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

8.B. pSI 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 U47121) and online at: **www.promega.com/vectors/**

Table 4. Restriction Enzymes That Cut the pSI Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AatII	1	1808	BspHI	3	1782, 1887, 2895
AccI	1	714	BspMI	1	470
Acc65I	2	48, 701	BssSI	3	1751, 2058, 3442
AcyI	2	1805, 2187	**Bst98I**	2	446, 643
AflII	2	446, 643	**BstZI**	1	724
AflIII	1	695	Cfr10I	2	1173, 2642
Alw44I	3	1558, 2055, 3301	**ClaI**	1	962
AlwNI	1	3206	**DraI**	4	928, 2149, 2841, 2860
AspHI	4	1562, 2059, 2144, 3305	DraII	1	1747
AvaI	2	684, 718	DraIII	1	1281
AvaII	2	2363, 2585	DrdI	4	435, 1325, 1644, 3513
AvrII	1	398			
BamHI	1	969	DsaI	2	9, 305
BanI	5	48, 569,701, 1237, 2774	EaeI	2	724, 2334
			EagI	1	724
BanII	1	1207	Ear I	2	986, 1928
BbsI	1	554	**EclHKI**	1	2727
BbuI	2	146, 218	**Eco52I**	1	724
BglI	3	351, 1037, 2609	**EcoRI**	1	689
BglII	1	3627	FspI	2	1027, 2504
BsaI	2	508, 2661	**HaeII**	3	1123, 1131, 3375
BsaOI	5	727, 1008, 2209, 2358, 3281	HgaI	5	1056, 1637, 2195, 2925, 3503
BsaAI	1	1278	**HincII**	2	715, 867
BsaBI	1	968	HindII	2	715, 867
BsaHI	2	1805, 2187	**HindIII**	1	414
BsaMI	2	788, 881	**HpaI**	1	867
BsmI	2	788, 881	**Hsp92I**	2	1805, 2187
Bsp1286I	5	1207, 1562, 2059, 2144, 3305	**KpnI**	2	52, 705
			MluI	1	695

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

Table 4. Restriction Enzymes That Cut the pSI Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
MspA1I	5	74, 625, 2091, 3032, 3277	**RsaI**	5	50, 656, 703, 1570, 2246
NaeI	1	1175	**SalI**	1	713
NcoI	2	9, 305	**ScaI**	2	656, 2246
NdeI	1	1553	**SfiI**	1	351
NgoMIV	1	1173	SinI	2	2363, 2585
NheI	1	678	**SmaI**	1	720
NotI	1	724	**SphI**	2	146, 218
NsiI	2	148, 220	**SspI**	2	1486, 1922
NspI	3	146, 218, 1702	**StuI**	1	397
PaeR7I	1	684	**StyI**	3	9, 305, 398
Ppu10I	2	144, 216,	TfiI	1	420
PspAI	1	718	**VspI**	1	2552
PstI	1	456	**XbaI**	1	707
PvuI	2	1008, 2358	**XhoI**	1	684
PvuII	1	74	**XmaI**	1	718
			XmnI	1	2127

Table 5. Restriction Enzymes That Do Not Cut the pSI Vector.

Acc B7I	Bsp120I	Eco81I	PflMI	SgrAI
AccIII	BsrGI	**EcoICRI**	PinAI	**SnaBI**
AgeI	**BssHII**	EcoNI	PmeI	**SpeI**
ApaI	Bst1107 I	**EcoRV**	PmlI	SplI
AscI	**BstEII**	EheI	PpuMI	SrfI
BalI	**BstXI**	FseI	PshAI	Sse8387I
BbeI	**Bsu36I**	**I-PpoI**	Psp5II	SwaI
BbrPI	**CspI**	KasI	RsrII	**Tth111I**
BclI	**Csp45I**	**NarI**	**SacI**	XcmI
BlpI	**Eco47III**	**NruI**	**SacII**	
Bpu1102 I	Eco72I	PacI	**SgfI**	

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

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

AciI	**CfoI**	**HpaII**	MseI	ScrFI
AluI	**DdeI**	HphI	**MspI**	SfaNI
Alw26I	**DpnI**	**Hsp92II**	**NciI**	**TaqI**
BbvI	DpnII	MaeI	**NdeII**	**Tru9I**
BsaJI	Fnu4HI	MaeII	NlaIII	**XhoII**
BsrI	FokI	MaeIII	NlaIV	
Bsr SI	**HaeIII**	MboI	PleI	
Bst71I	**HhaI**	**MboII**	**Sau3AI**	
BstOI	**HinfI**	Mnl I	Sau96I	

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

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