Technical Bulletin

pCI-neo Mammalian Expression Vector

INSTRUCTIONS FOR USE OF PRODUCT E1841.

www.promega.com

PRINTED IN USA.
Revised 9/09

Part# TB215

pCI-neo Mammalian Expression Vector

All technical literature is available on the Internet at: 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

1. Description

The pCI-neo Mammalian Expression Vector[a] carries the human cytomegalovirus (CMV) immediate-early enhancer/promoter region to promote constitutive expression of cloned DNA inserts in mammalian cells. The pCI-neo Vector contains the neomycin phosphotransferase gene, a selectable marker for mammalian cells. This vector can be used for transient expression or for stable expression by selecting transfected cells with the antibiotic G-418.

The sequences of Promega vectors are available online at: **www.promega.com/vectors/** and are also available from the GenBank® database (GenBank®/EMBL Accession Number for the pCI-neo Mammalian Expression Vector is U47120).

2. Product Components and Storage Conditions

Product	Size	Cat.#
pCI-neo Mammalian Expression Vector	20µg	E1841

The pCI-neo Vector is supplied frozen at a concentration of 1mg/ml in TE buffer (pH 8.0).

Storage Conditions: Store the pCI-neo Mammalian Expression Vector at –20°C.

3. Vector Components

3.A. Enhancer/Promoter Regions

The CMV immediate-early enhancer/promoter region present in the pCI-neo Vector allows for strong, constitutive expression in a variety of cell types. The promiscuous nature of the CMV enhancer/promoter has been demonstrated in transgenic mice, where expression of the chloramphenicol acetyltransferase (CAT) gene regulated by the CMV enhancer/promoter was observed in 24 of the 28 tissues examined (1). The pCI-neo Vector also contains the SV40 enhancer and early promoter region upstream of the neomycin phosphotransferase gene. The SV40 early promoter contains the SV40 origin of replication, which will induce transient, episomal replication of the pCI-neo Vector in cells expressing the SV40 large T antigen such as COS-1 or COS-7 cells (2).

3.B. Chimeric Intron

Downstream of the CMV 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 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). The intron is located upstream of the cDNA insert in order to prevent utilization of possible cryptic 5´-donor splice sites within the cDNA sequence (5).

Transfection studies have demonstrated that an intron flanking the cDNA insert frequently increases the level of gene expression (6–9). The increase in expression level due to the intron depends on the particular cDNA insert. For example, in transient transfections of 293 cells, we have found that the presence of this chimeric intron results in an approximate 20-fold increase in expression of the CAT gene (10). In contrast, the chimeric intron increases the gene expression level from the luciferase cDNA by only threefold (10). In transgenic experiments, an intron is necessary to promote a high level of expression for virtually all cDNA inserts (11–13).

3.C. T7 and T3 RNA Polymerase Promoters

In the pCI-neo Vector, T7 and T3 RNA polymerase promoters flank the multiple cloning region. These promoters can be used to synthesize RNA from the sense and the antisense strand of the cloned DNA insert. **Note:** Due to a sequence

difference between the T7 Promoter Primer (Cat.# Q5021) and the T7 RNA polymerase promoter in the pCI-neo Mammalian Expression Vector, the T7 Promoter Primer cannot be used for sequencing these expression vectors. The sequence difference is downstream of the transcriptional start site and is located at the 3´-end of the T7 Promoter Primer. The T7 EEV (Eukaryotic Expression Vector) Promoter Primer (Cat.# Q6700) has been designed specifically to prime sequencing reactions from the mammalian expression vectors, pCI-neo (Cat.# E1841), pSI (Cat.# E1721), pCI (Cat.# E1731) and pALTER®-MAX (Cat.# Q5761).

Note: Use the T7 EEV Promoter Primer (Cat.# Q6700) to sequence ds- or ssDNA produced by the pCI-neo Vector. **Do not** use the T7 Promoter Primer (Cat.# Q5021) to sequence the pCI-neo Vector. The primer and the promoter sequences differ.

3.D. Multiple Cloning Region and Convenient Restriction Enzyme Sites

The multiple cloning region in the pCI-neo Vector is immediately downstream from the T7 promoter. The unique restriction sites available in the multiple cloning region of the pCI-neo Vector are identical to those found in the pCI and pSI Mammalian Expression Vectors (Cat.# E1731 and E1721, respectively), except that the pCI-neo Vector does not contain a unique KpnI or BstZI site within this region.

Large hairpin structures in the 5´-end of untranslated mRNAs have been shown to reduce the level of in vitro and in vivo translation in higher eukaryotes (14–18). RNA transcribed from the multiple cloning region of the pCI-neo Vector is not predicted to contain hairpin structures that would interfere with translation (17). Because there are no ATG sequences in either the multiple cloning region or between the transcriptional start site and the multiple cloning region, an ATG for the initiation of translation must be present in the inserted DNA.

Note: The insert must contain an ATG for translation initiation.

Unique restriction sites flank the CMV enhancer (BglII and SgfI) and the CMV promoter (SgfI and I-PpoI), allowing easy replacement of these regulatory regions with other regulatory regions of interest.

3.E. SV40 Late Polyadenylation Signal

Polyadenylation signals terminate transcription by RNA polymerase II and cause the addition of approximately 200–250 adenosine residues to the 3´-end of the RNA transcript (19). Polyadenylation has been shown to enhance RNA stability and translation (20,21). To facilitate efficient processing of cloned DNA inserts not containing polyadenylation signals, the SV40 late polyadenylation signal has been positioned downstream from the multiple cloning region. The SV40 late polyadenylation signal is extremely efficient and has been shown to increase the steady-state level of RNA approximately fivefold more than the SV40 early polyadenylation signal (22).

3.F. Neomycin Phosphotransferase Selectable Marker

The neomycin phosphotransferase gene from Tn5 is present in the pCI-neo Vector and is under the regulation of the SV40 enhancer and early promoter region. A synthetic polyadenylation signal based on the highly efficient polyadenylation signal of the rabbit β-globin gene (23) is located downstream of the neomycin phosphotransferase gene. To increase the translational efficiency of the neomycin phosphotransferase gene, the upstream, out-of-frame ATG sequences present in the wildtype neomycin phosphotransferase gene have been eliminated. Additionally, the sequence upstream of the initiator ATG has been changed to match a sequence shown to improve the context for initiating translation (24).

Expression of the neomycin phosphotransferase gene in mammalian cells confers resistance to the antibiotic G-418 (Cat.# V7981) (25). G-418 is an aminoglycoside antibiotic produced by streptomycetes that induces cytotoxicity by blocking translation. Neomycin phosphotransferase inactivates G-418 through phosphorylation, thereby blocking its toxic effects (26).

3.G. f1 Origin of Replication and Plasmid Replicon

The backbone for the pCI-neo Vector was derived from the pGEM®-3Zf(+) Vector. As a result, this vector is a high-copy plasmid and contains the origin of replication of the filamentous phage f1. To generate single-stranded DNA (ssDNA) from the f1 origin, bacteria transformed with the pCI-neo 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).

The "poison" sequence present in pBR322, which has been shown to inhibit replication of vectors containing SV40 origins in COS cells, has been deleted in the pCI-neo Vector (27). 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.

4. Transfection of Mammalian Cells with the pCI-neo Vector

The pCI-neo Vector can be used for both transient and stable expression of genes. Transfection of DNA into mammalian cells may be mediated by cationic lipids (28,29), calcium phosphate (30,31), DEAE-dextran (32–34), polybrene-DMSO (35,36) or electroporation (37,38). Calcium phosphate and transfection systems based on the cationic lipids such as Transfectam® Reagent, Tfx™ Reagents and TransFast™ Transfection Reagent are available from Promega. For more information, please request the *Transfectam® Reagent Technical Bulletin* (#TB116), the *Tfx™ Reagents Technical Bulletin* (#TB216), the *TransFast™ Transfection Reagent Technical Bulletin* (#TB260) or the *ProFection® Mammalian Transfection Systems Technical Manual* (#TM012).

Note: DEAE-dextran has not proven efficient for the generation of stable transfectants.

For stable expression, the transfected cells must be selected with the antibiotic G-418. Following the transfection, seed the cells at a low cell density and apply the G-418 antibiotic to the medium at a concentration between 100µg/ml and 1mg/ml. The concentration of G-418 required to select and maintain drug resistance depends on the cell type and growth rate. In general, mammalian cells require a concentration of 400–600µg/ml of G-418 for selection and 200–400µg/ml of G-418 for maintenance of stable transformants (25). A stock solution of 100mg/ml of G-418 can be made up in 40mM HEPES (pH 7.3), PBS or water. (Store the stock solution of G-418 at –20°C.) For effective selection, the cells should be subconfluent since confluent, nongrowing cells are very resistant to the effects of G-418. Change the medium every 3 days until drug-resistant clones appear (2–5 weeks, depending on the cell type). Death of cells not expressing neomycin phosphotransferase may require 3–9 days following addition of G-418.

Note: Prepare a control plate for all selection experiments by treating untransfected cells with G-418 in medium under the experimental conditions. This control plate will indicate whether the conditions of antibiotic selection were sufficiently stringent to eliminate cells not expressing neomycin phosphotransferase.

5. pCI-neo Vector Multiple Cloning Region and Circle Map

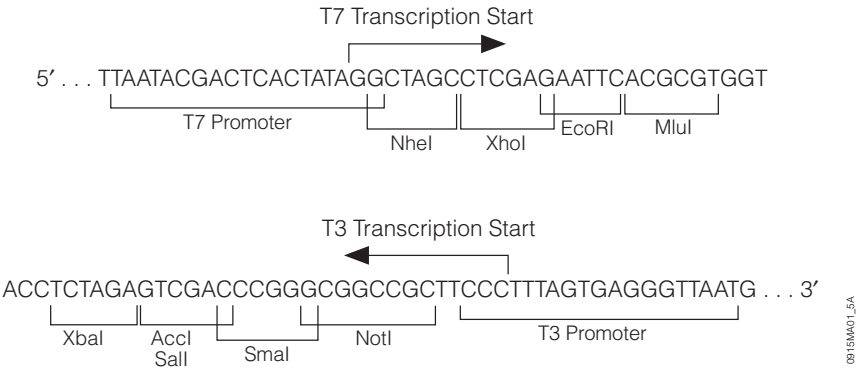
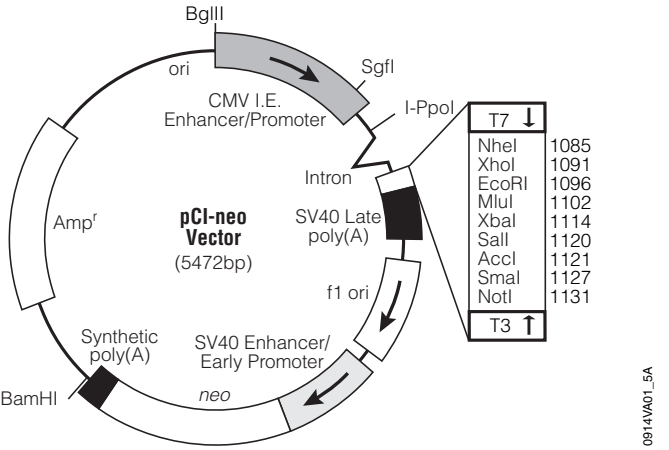


Figure 1. T7 and T3 RNA polymerase promoters and multiple cloning region sequence of the pCI-neo Vector. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by T3 RNA polymerase. The strand shown is the same as the ssDNA produced by the vector.

Note: Use the T7 EEV Promoter Primer (Cat.# Q6700) to sequence ds- or ssDNA produced by the pCI-neo Vector. **Do not** use the T7 Promoter Primer (Cat.# Q5021) to sequence the pCI-neo Vector. The primer and the promoter sequences differ.

Figure 2. pCI-neo Vector circle map and sequence reference points. Neo = neomycin phosphotransferase; CMV I.E. = cytomegalovirus immediate-early.



pCI-neo Sequence Reference Points:

CMV immediate-early enhancer/promoter region	1–750
Chimeric intron	890–1022
T7-EEV sequencing primer binding region	1053–1074
T7 RNA polymerase promoter (–17 to +2)	1067–1085
Multiple cloning region	1085–1137
T3 RNA polymerase promoter (–17 to +3)	1140–1158
SV40 late polyadenylation signal	1167–1388
Phage f1 region	1483–1938
SV40 enhancer and early promoter	2000–2418
SV40 minimum origin of replication	2316–2381
Coding region of neomycin phosphotransferase	2463–3257
Synthetic polyadenylation signal	3321–3369
β-lactamase (Amp^r) coding region	3780–4640

6. pCI-neo Vector Restriction 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. The vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number U47120) and on the Internet at: **www.promega.com/vectors/**

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

Enzyme	# of Sites	Location
AatII	5	278, 331, 414, 600, 3648
AccI	1	1121
Acc65I	2	1108 , 2047
AflII	4	828, 847, 1050, 2445
AflIII	1	1102
Alw44I	3	3398, 3895, 5141
AlwI	1	5046
AvaI	2	1091, 1125
AvaII	3	3107, 4203, 4425
AvrII	1	2397
BalI	3	10, 64, 2673
BamHI	1	3385
BanII	3	729, 1642, 2956
BbeI	1	2594
BbsI	1	961
BbuI	3	2145, 2217, 2996
BglII	1	5467
BsaI	2	915, 4501
BsaAI	3	493, 1713, 2895
BsaBI	2	1399, 3384
BsaMI	2	1218, 1311
BsmI	2	1218, 1311
BspHI	3	3622, 3727, 4735
BspMI	4	877, 2478, 2859, 3309
BsrGI	1	96
BssHII	1	2988
BssSI	4	3183, 3591, 3898, 5282
Bst98I	4	828, 847, 1050, 2445
BstXI	1	3312
BstZI	2	1131, 2497
Cfr10I	4	1608, 2910, 3091, 4482
ClaI	2	1403, 3372
CspI	1	3107
Csp45I	1	3273
DraI	4	1358, 3989, 4681, 4700
DraII	1	3587
DraIII	1	1716
DsaI	4	513, 2008, 2304, 3023
EagI	2	1131, 2497
EarI	4	1421, 2935, 3145, 3768
EclHKI	1	4567
Eco52I	2	1131, 2497
EcoICRI	1	727
EcoRI	1	1096
EheI	1	2592
FspI	4	1462, 2001, 2693, 4344
HaeII	4	1558, 1566, 2594, 5215
HincII	3	677, 1122, 1297
HindII	3	677, 1122, 1297
HindIII	2	756, 2413
HpaI	1	1297
I-PpoI	1	851
KasI	1	2590
KpnI	2	1112, 2051
MluI	1	1102
NaeI	2	1610, 3093
NarI	1	2591
NcoI	4	513, 2008, 2304, 3023
NdeI	1	387
NgoMIV	2	1608, 3091
NheI	1	1085
NotI	1	1131
NsiI	2	2147, 2219
NspI	4	2145, 2217, 2996, 3542
PaeR7I	1	1091
Ppu10I	2	2143, 2215
PspAI	1	1125
PstI	2	838, 2644
PvuI	3	664, 1443, 4198
PvuII	2	2073, 2697
RsrII	1	3107
SacI	1	729
SalI	1	1120

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

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

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
ScaI	2	1063, 4086	**StyI**	5	513, 2008, 2304, 2397, 3023
SfiI	1	2350			
SgfI	1	664	TfiI	4	2419, 3076, 3210, 3369
SinI	3	3107, 4203, 4425			
SmaI	1	1127	**Tth111I**	1	2709
SnaBI	1	493	**VspI**	2	160, 4392
SpeI	1	152	**XbaI**	1	1114
SphI	3	2145, 2217, 2996	**XhoI**	1	1091
SspI	4	5, 52, 1921, 3762	**XmaI**	1	1125
StuI	1	2396	**XmnI**	1	3967

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

AccB7I	BlpI	Eco72I	PflMI	**SacII**
AccIII	Bpu1102I	Eco81I	PinAI	SgrAI
AgeI	Bsp120I	EcoNI	PmeI	SplI
ApaI	Bst1107I	**EcoRV**	PmlI	SrfI
AscI	**BstEII**	FseI	PpuMI	Sse8387I
BbrPI	**Bsu36I**	**NruI**	PshAI	SwaI
BclI	**Eco47III**	PacI	Psp5II	XcmI

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

AciI	**Bsp1286I**	EaeI	MaeI	NlaIII
AcyI	BsrI	Fnu4HI	MaeII	NlaIV
AluI	**BsrSI**	**FokI**	MaeIII	PleI
Alw26I	Bst71I	**HaeIII**	**MboI**	**RsaI**
AspHI	**BstOI**	HgaI	**MboII**	**Sau3AI**
BanI	BstUI	**HhaI**	MnlI	Sau96I
BbvI	**CfoI**	**HinfI**	MseI	ScrFI
BglI	**DdeI**	**HpaII**	**MspI**	SfaNI
BsaHI	**DpnI**	HphI	**MspA1I**	**TaqI**
BsaJI	DpnII	**Hsp92I**	**NciI**	**Tru9I**
BsaOI	DrdI	**Hsp92II**	**NdeII**	**XhoII**

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

7. Related Products

Product	Size	Cat.#
pCI Mammalian Expression Vector	20µg	E1731
pSI Mammalian Expression Vector	20µg	E1721
pAdVAntage™ Vector	20µg	E1711
ProFection® Mammalian Transfection System—Calcium Phosphate	40 transfections	E1200
Transfectam® Reagent for the Transfection of Eukaryotic Cells	1mg	E1231
	0.5mg	E1232
TransFast™ Transfection Reagent	1.2mg	E2431
Tfx™-20 Reagent	4.8mg	E2391
Tfx™-50 Reagent	2.1mg	E1811
Antibiotic G-418 Sulfate	100mg	V7981
T7 EEV Promoter Primer	2µg	Q6700

8. References

1. Schmidt, E.V. *et al*. (1990) The cytomegalovirus enhancer: A pan-active control element in transgenic mice. *Mol. Cell. Biol.* **10**, 4406–11.
2. Gluzman, Y. (1981) SV40-transformed simian cells support the replication of early SV40 mutants. *Cell* **23**, 175–82.
3. Bothwell, A.L.M. *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.
4. 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. *Meth. Enzymol.* **183**, 252–78.
5. Huang, M.T.F. and Gorman, C.M. (1990) The simian virus 40 small-t intron, present in many common expression vectors, leads to aberrant splicing. *Mol. Cell. Biol.* **10**, 1805–10.
6. 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.
7. Buchman, A.R. and Berg, P. (1988) Comparison of intron-dependent and intron-independent gene expression. *Mol. Cell. Biol.* **8**, 4395–405.
8. 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.
9. Huang, M.T.F. and Gorman, C.M. (1990) Intervening sequences increase efficiency of RNA 3´ processing and accumulation of cytoplasmic RNA. *Nucl. Acids Res.* **18**, 937–47.

8. References (continued)

10. Brondyk, B. (1994) pCI and pSI Mammalian Expression Vectors. *Promega Notes* **49**, 7–11.

11. Brinster, R.L. *et al.* (1988) Introns increase transcriptional efficiency in transgenic mice. *Proc. Natl. Acad. Sci. USA* **85**, 836–40.

12. Choi, T. *et al.* (1991) A generic intron increases gene expression in transgenic mice. *Mol. Cell. Biol.* **11**, 3070–4.

13. Palmiter, R.D. *et al.* (1991) Heterologous introns can enhance expression of transgenes in mice. *Proc. Natl. Acad. Sci. USA* **88**, 478–82.

14. Fu, L. *et al.* (1991) Translational potentiation of messenger RNA with secondary structure in Xenopus. *Science* **251**, 807–10.

15. Kim, S.J. *et al.* (1992) Post-transcriptional regulation of the human transforming growth factor-beta 1 gene. *J. Biol. Chem.* **267**, 13702–707.

16. Kozak, M. (1986) Influences of mRNA secondary structure on initiation by eukaryotic ribosomes. *Proc. Natl. Acad. Sci. USA* **83**, 2850–4.

17. Kozak, M. (1989) Circumstances and mechanisms of inhibition of translation by secondary structure in eucaryotic mRNAs. *Mol. Cell. Biol.* **9**, 5134–42.

18. Rao, C.D. *et al.* (1988) The 5´ untranslated sequence of the c-sis/platelet-derived growth factor 2 transcript is a potent translational inhibitor. *Mol. Cell. Biol.* **8**, 284–92.

19. Proudfoot, N.J. (1991) Poly(A) signals. *Cell* **64**, 671–4.

20. Bernstein, P. and Ross, J. (1989) Poly(A), poly(A) binding protein and the regulation of mRNA stability. *Trends Biochem. Sci.* **14**, 373–7.

21. Jackson, R.J. and Standart, N. (1990) Do the poly(A) tail and 3´ untranslated region control mRNA translation? *Cell* **62**, 15–24.

22. 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.

23. Levitt, N. *et al.* (1989) Definition of an efficient synthetic poly(A) site. *Genes Dev.* **3**, 1019–25.

24. Kozak, M. (1989) The scanning model for translation: An update. *J. Cell Biol.* **108**, 229–41.

25. Southern, P.J. and Berg, P. (1982) Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Molec. Appl. Genet.* **1**, 327–41.

26. Davies, J. and Jimenez, A. (1980) A new selective agent for eukaryotic cloning vectors. *Am. J. Trop. Med. Hyg.* **29**, 1089–92.

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

28. Behr, J.P. *et al.* (1989) Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. *Proc. Natl. Acad. Sci. USA* **86**, 6982–6.

8. References (continued)

29. Loeffler, J.P. *et al.* (1990) Lipopolyamine-mediated transfection allows gene expression studies in primary neuronal cells. *J. Neurochem.* **54**, 1812.

30. Graham, F.L. and van der Eb, A.J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456–67.

31. Wigler, M. *et al.* (1977) Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. *Cell* **11**, 223–32.

32. McCutchan, J.H. and Pagano, J.S. (1968) Enchancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethylaminoethyl-dextran. *J. Natl. Cancer Inst.* **41**, 351–7.

33. Al-Moslih, M.I. and Dubes, G.R. (1973) The kinetics of DEAE-dextran-induced cell sensitization to transfection. *J. Gen. Virol.* **18**, 189–93.

34. Luthman, H. and Magnusson, G. (1983) High efficiency polyoma DNA transfection of chloroquine treated cells. *Nucl. Acids Res.* **11**, 1295–308.

35. Kawai, S. and Nishizawa, M. (1984) New procedure for DNA transfection with polycation and dimethyl sulfoxide. *Mol. Cell. Biol.* **4**, 1172–4.

36. Aubin, R.J., Weinfeld, M. and Paterson, M.C. (1988) Factors influencing efficiency and reproducibility of polybrene-assisted gene transfer. *Som. Cell Mol. Genet.* **14**, 155–67.

37. Andreason, G.L. and Evans, G.A. (1988) Introduction and expression of DNA molecules in eukaryotic cells by electroporation. *BioTechniques* **6**, 650–60.

38. Neumann, E. *et al.* (1982) Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J.* **1**, 841.

[a]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.

Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA
Toll Free in USA 800-356-9526 · Phone 608-274-4330 · Fax 608-277-2516 · **www.promega.com**
Part# TB215 Printed in USA.