



Technical Bulletin

pGEM[®]-*luc* Vector

INSTRUCTIONS FOR USE OF PRODUCT E1541.



www.promega.com

PRINTED IN USA.
Revised 4/07



Part# TB104

pGEM[®]-*luc* 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

I. Description	1
II. Product Components	2
III. pGEM [®] - <i>luc</i> Vector Multiple Cloning Region and Circle Map	2
IV. pGEM [®] - <i>luc</i> Vector Restriction Sites	4
V. Related Products	6
VI. References	6

I. Description

The pGEM[®]-*luc* Vector^(a) is a cassette vector designed to be a source of the *luc* gene encoding firefly luciferase, which is found in the pGL2 Vectors. The plasmid is not intended for the expression of luciferase in eukaryotic cells.

The pGEM[®]-*luc* Vector was constructed by positioning the luciferase gene (*luc*) (1–3) in the center of the multiple cloning region of the pGEM[®]-11Zf(–) Vector, providing a number of unique restriction sites at both ends of the gene (Figure 1). Sites that are surrounded by parentheses are not unique, as additional sites for each also exists in the luciferase gene. To make use of these nonunique sites, a partial restriction enzyme digest should be performed. Note also that using HindIII or NsiI to clone the luciferase gene will include upstream ATG codons, which may reduce the efficiency of expression in eukaryotes.

Luciferase is a 61kDa monomeric protein that does not require post-translational modifications for enzymatic activity. Thus, it can function as a genetic reporter immediately upon translation (2,3). Luciferase synthesized by in vitro translation can be labeled with ³⁵S, as the protein contains 4 cysteine and 14 methionine residues. To ensure full enzymatic activity of luciferase, no more than 5 codons can be deleted from either the 5'- or 3'-end of the coding region.

For some experiments, antisense RNA to luciferase mRNA may be useful as a nucleic acid probe. Such antisense RNA can be generated from the T7 RNA polymerase promoter in pGEM[®]-*luc* Vector.

The sequences of Promega vectors are available online at: **www.promega.com/vectors/** and are also available from the GenBank[®] database (the GenBank[®]/EMBL Accession Number for the pGEM[®]-*luc* Vector is X65316).

II. Product Components

Product	Size	Cat.#
pGEM®-luc DNA	20µg	E1541

The pGEM®-luc Vector is supplied with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain the vector and are not competent cells.

Storage Conditions: Store the pGEM®-luc Vector at -20°C and the glycerol stock of JM109 cells at -70°C.

III. pGEM®-luc Vector Multiple Cloning Region and Circle Map

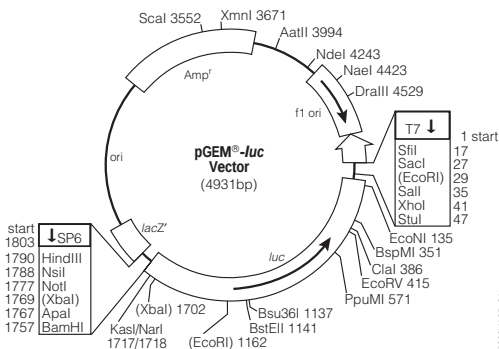


Figure 1. pGEM®-luc Vector circle map. Sites shown in parentheses are not unique.

pGEM®-luc Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10-50; 1757-1795
luciferase cDNA sequences	57-1756
luciferase coding region	105-1754
SP6 RNA polymerase promoter (-17 to +3)	1801-1820
SP6 RNA polymerase transcription initiation site	1803
lac operon sequences	1828-2057; 4754-4912
binding site of pUC/M13 reverse sequencing primer	1838-1854
lacZ start codon	1842
lac operator	1853-1880
β-lactamase coding region	3002-3859
phage f1 region	4296-4749
binding site of pUC/M13 forward sequencing primer	4872-4888
T7 RNA polymerase promoter (-17 to +3)	4915-3

Note: lacZ start codon is disrupted and therefore inactive.

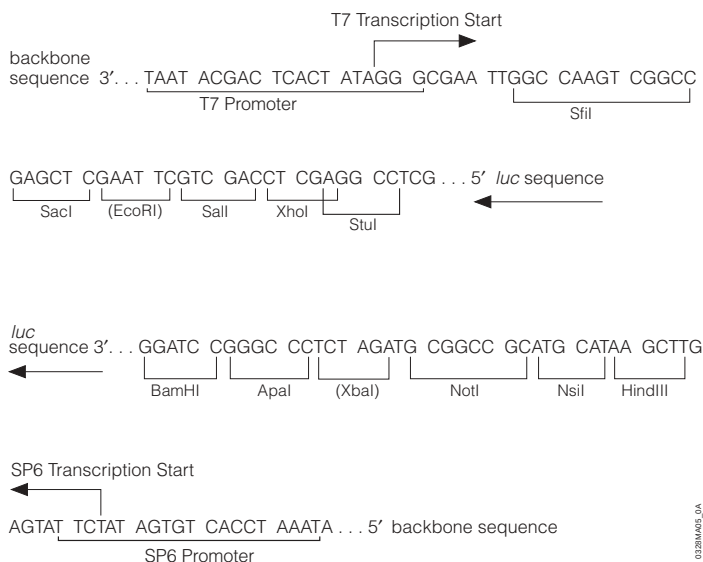


Figure 2. pGEM®-*luc* Vector promoter and adjacent unique restriction enzyme sites. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The bold arrow indicates the orientation of the *luc* gene open reading frame.

IV. pGEM®-luc 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. Vector sequences are available in the GenBank® database (GenBank®/EMBL Accession Number X65316) and on the Internet at: www.promega.com/vectors/

Table 1. Restriction Enzymes That Cut the pGEM®-luc Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AatII	1	3994	BstEII	1	1141
AccI	1	36	BstZI	2	18, 1777
AccIII	2	538, 1054	Bsu36I	1	1137
Acyl	5	325, 1718, 1744, 3609, 3991	Cfr10I	4	321,1480, 3152, 4421
AflIII	2	1256, 2179	Clal	1	386
Alw44 I	3	2493, 3739, 4236	Csp45I	2	794, 1582
AlwNI	1	2595	DraI	3	2938, 2957, 3649
Apal	1	1767	DraII	3	571, 1764, 4048
Aval	2	41, 693	DraIII	1	4529
Avall	3	571, 3210, 3432	DrdI	3	2287, 4156, 4573
BalI	1	11	EagI	2	18, 1777
BamHI	1	1757	EclHKI	1	3072
BanI	4	1717, 1923, 3020, 4485	Eco52I	2	18, 1777
BanII	4	27, 733, 1767, 4455	Eco81I	1	1137
BbeI	1	1721	EcoICRI	1	25
BbsI	3	345, 461, 1739	EcoNI	1	135
BbuI	2	1094, 1786	EcoRI	2	29, 1162
BglI	3	17, 3192, 4764	EcoRV	1	415
BsaI	1	3133	EheI	1	1719
BsaAI	2	1599, 4526	FspI	2	3294, 4771
BsaHI	5	325, 1718, 1744, 3609, 3991	HaeII	5	1721, 2057, 2427, 4371, 4379
Bsp120I	1	1763	HincII	2	37, 449
BspHI	3	2899, 3907, 4012	HindII	2	37, 449
BspMI	1	351	HindIII	1	1790
BsrGI	1	1259	Hsp92I	5	325, 1718, 1744, 3609, 3991
BssSI	3	235, 3736, 4043	KasI	1	1717

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

Table 1. Restriction Enzymes That Cut the pGEM®-luc Vector Between 1 and 5 Times. (continued)

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NaeI	1	4423	SacI	1	27
NarI	1	1718	SalI	1	35
NdeI	1	4243	ScaI	1	3552
NgoMIV	1	4421	SfiI	1	17
NotI	1	1777	SgrAI	1	321
NsiI	1	1788	SinI	3	571, 3210, 3432
NspI	4	1094, 1786, 2183, 4100	SphI	2	1094, 1786
PacI	1	431	SplI	1	1595
PaeR7I	1	41	SspI	2	3876, 4734
Ppu10I	1	1784	StuI	1	47
PpuMI	1	571	VspI	3	1950, 2009, 3244
Psp5II	1	571	XbaI	2	1702, 1769
PvuI	2	3442, 4792	XcmI	1	1019
PvuII	2	2003, 4821	XhoI	1	41
			XmnI	1	3671

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-luc Vector.

Acc B7I	Bpu1102I	Eco47III	PflMI	SmaI
Acc65I	BsaBI	Eco72I	PinAI	SnaBI
AflII	BsaMI	FseI	PmeI	SpeI
AgeI	BsmI	HpaI	PmlI	SrfI
AscI	BssHII	I-PpoI	PshAI	Sse8387I
AvrII	Bst1107I	KpnI	PspAI	StyI
BbrPI	Bst98I	MluI	PstI	SwaI
BclI	BstXI	NcoI	RsrII	Tth111I
BglII	CspI	NheI	SacII	XmaI
BlpI	DsaI	NruI	SgfI^(b)	

Table 3. Restriction Enzymes That Cut the pGEM®-luc Vector 6 or More Times.

AcI	Bst71I	FokI	MboI	PleI
AluI	BstOI	HaeIII	MboII	RsaI
Alw26I	BstUI	HgaI	MnII	Sau3AI
AspH	CfoI	HhaI	MseI	Sau96I
Bbv	DdeI	HinfI	MspI	ScrFI
BsaOI	DpnI	HpaII	MspAII	SfaNI
BsaJI	DpnII	HphI	NciI	TaqI
Bsp1286I	EaeI	Hsp92II	NdeII	TfiI
BsrI	EarI	MaeI	NlaIII	Tru9I
BsrSI	Fnu4HI	MaeIII	NlaIV	XhoII

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

V. Related Products

Product	Size	Cat.#
Luciferase Assay System	100 Assays	E1500
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030
Luciferase Assay System, 10-Pack	1,000 assays	E1501
Luciferase Assay System Freezer Pack	1,000 assays	E4530
Luciferase 1000 Assay System	1,000 assays	E4550
Luciferase Assay Reagent	1,000 assays	E1483
Steady-Glo® Luciferase Assay System	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
Dual-Glo® Luciferase Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
Bright-Glo™ Luciferase Assay System*	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650

*For Laboratory Use.

VI. References

1. Ow, D. *et al.* (1986) Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* **234**, 856–9.
2. de Wet, J.R. *et al.* (1987) Firefly luciferase gene: structure and expression in mammalian cells. *Mol. Cell Biol.* **7**, 725–37.
3. Wood, K.V. (1990) Firefly Luciferase: A new tool for molecular biologists. *Promega Notes* **28**, 1–3.

^(a)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

^(b)U.S. Pat. No. 5,391,487 has been issued to Promega Corporation for Restriction Endonuclease Sgf I.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

© 1988–2007 Promega Corporation. All Rights Reserved.

pGEM is a registered trademark of Promega Corporation.

DNAStar is a registered trademark of DNAStar, Inc. GenBank is a registered trademark of the U.S. Department of Health and Human Services.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Notes