TECHNICAL BULLETIN

pGEM®-*luc* Vector

Instructions for Use of Product
E1541

Revised 6/17
TB104

Promega

pGEM®-*luc* Vector

All technical literature is available at: www.promega.com/protocols/
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1. Description

The pGEM®-*luc* Vector 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 ^{35}S, 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: **ww.promega.com/vectors/** and are also available from the GenBank® database (the GenBank®/EMBL Accession Number for the pGEM®-*luc* Vector is X65316).

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2. 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 –30°C to –10°C and the glycerol stock of JM109 cells below –65°C.

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

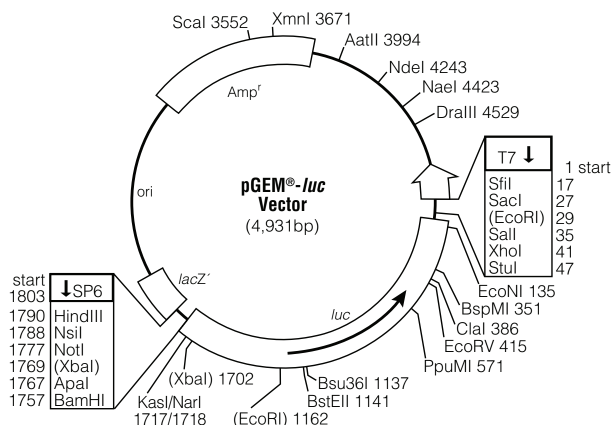

Figure 1. pGEM®-*luc* Vector circle map. Sites shown in parentheses are not unique.

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
*lac*Z start codon	1842
lac operator	1853–1880
β-lactamase coding region	3002–3859
T7 RNA polymerase promoter (–17 to +3)	4915–3

Note: *lac*Z start codon is disrupted and therefore inactive.

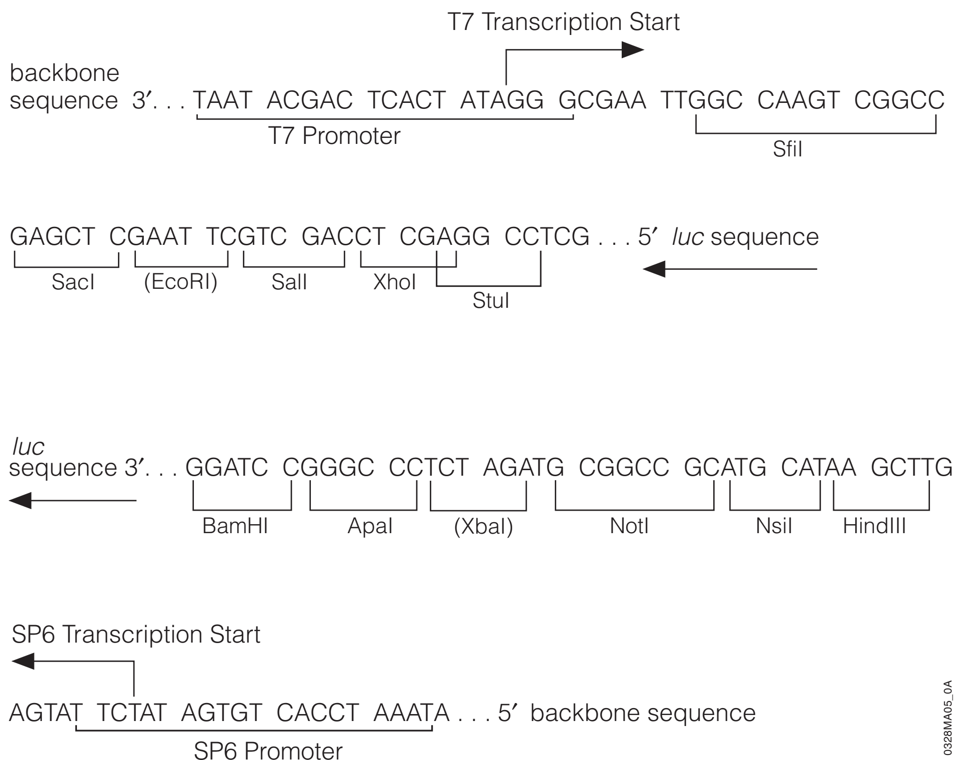

Figure 2. pGEM®-*luc* Vector promoter and adjacent unique restriction enzyme sites. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The bold arrow indicates the orientation of the *luc* gene open reading frame.

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

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

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

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

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

AciI	Bst71I	**FokI**	MboI	PleI
AluI	BstOI	**HaeIII**	**MboII**	**RsaI**
Alw26I	BstUI	HgaI	MnlI	**Sau3AI**
AspH	**CfoI**	**HhaI**	MseI	Sau96I
Bbv	DdeI	**HinfI**	**MspI**	ScrFI
BsaOI	**DpnI**	**HpaII**	**MspA1I**	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.

5. 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® Luciferse Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
Bright-Glo™ Luciferse Assay System	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650

6. 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.

7. Summary of Changes

The following change was made to the 6/17 revision of this document:

The f1 origin of replication was removed from the reported vector sequence.

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