TECHNICAL BULLETIN

pGEM®-4Z Vector

Instructions for Use of Product
P2161

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TB036

pGEM®-4Z 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 pGEM®-4Z Vector is intended for use as a standard cloning vector as well as for the highly efficient synthesis of RNA in vitro. The vector carries the *lac*Z α-peptide and multiple cloning region arrangement from pUC18 (1). In addition, the vector contains both the SP6 and T7 RNA polymerase promoters flanking the multiple cloning region. This arrangement gives rise to a functional α-peptide that is capable of complementing the product of the *lac*ZDM15 gene to produce functional β-galactosidase. Cells with the genotype, *lac*ZDM15, and also containing the pGEM®-4Z Vector will be blue in color when plated on indicator media containing IPTG and X-Gal. However, when the *lac*Z α-peptide is disrupted by cloning into the pGEM®-4Z multiple cloning region, complementation does not occur, and no β-galactosidase activity is produced. Therefore, bacterial colonies harboring recombinant pGEM®-4Z Vector constructs remain white.

The sequences of Promega vectors are available at: **www.promega.com/vectors/** and from the GenBank® database.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
pGEM®-4Z Vector	**20µg**	**P2161**

The pGEM®-4Z Vector is provided with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain the vector and are not competent.

Storage Conditions: Store the pGEM®-4Z Vector at –20°C and the glycerol stock of JM109 cells at –70°C.

3. pGEM®-4Z Vector Multiple Cloning Region and Circle Map

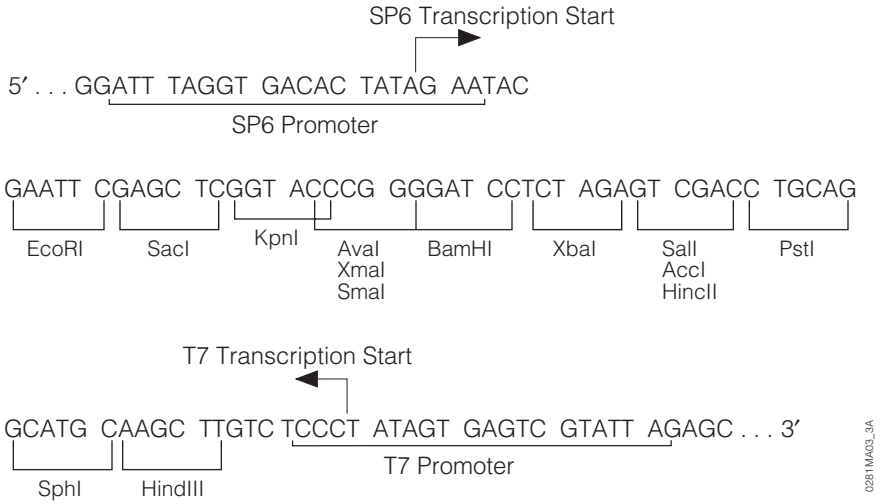

Figure 1. pGEM®-4Z Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase and is complementary to RNA synthesized by T7 RNA polymerase.

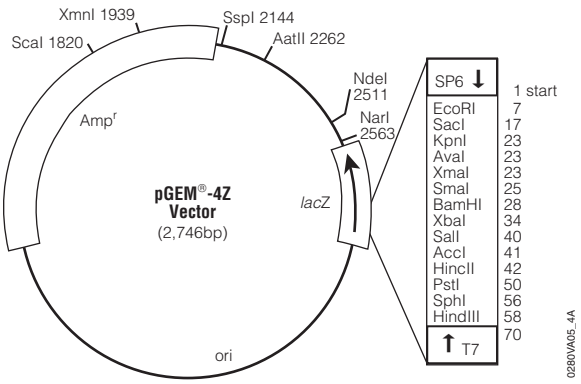

Figure 2. pGEM®-4Z Vector circle map and sequence reference points. The pGEM®-3Z and pGEM®-4Z Vectors are identical except for the orientation of the SP6 and T7 promoters.

pGEM®-4Z Vector sequence reference points:

SP6 RNA polymerase transcription initiation site	1
multiple cloning region	7–63
T7 RNA polymerase promoter (–17 to +3)	68–87
T7 RNA polymerase transcription initiation site	70
lac operon sequences	96–325; 2566–2726
*lac*Z start codon	110
*lac*Z operator	130–146
β-lactamase (Amp^r) coding region	1267–2127
SP6 RNA polymerase promoter (–17 to +3)	2730–3

Specialized applications of the pGEM®-4Z Vector:

- Blue/white screening for recombinants.
- Transcription in vitro from dual-opposed promoters (For protocol information, please see the *Riboprobe® in vitro Transcription Systems Technical Manual,* #TM016.)

4. pGEM®-4Z 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 X65305) and on the Internet at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pGEM®-4Z Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	2262
AccI	1	41
Acc65I	1	19
AcyI	3	1877, 2259, 2563
AflIII	1	447
Alw26I	5	69, 1401, 2177, 2330, 2372
Alw44I	3	761, 2007, 2504
AlwNI	1	863
AspHI	5	17, 765, 1926, 2011, 2508
AvaI	1	23
AvaII	2	1478, 1700
BamHI	1	28
BanI	4	19, 191, 1288, 2562
BanII	1	17
BbeI	1	2566
BbuI	1	56
BglI	2	1460, 2578
BsaI	1	1401
BsaHI	3	1877, 2259, 2563
BsaJI	5	23, 24, 186, 607, 2681
BsaOI	5	363, 787, 1710, 1859, 2606
Bsp1286I	5	17, 765, 1926, 2011, 2508
BspHI	3	1167, 2175, 2280
BspMI	1	53
BssSI	3	620, 2004, 2311
BstOI	5	187, 475, 596, 609, 2682
BstXI	1	2725
Cfr10I	1	1420
DraI	3	1206, 1225, 1917
DraII	1	2316
DrdI	2	555, 2424
EaeI	3	286, 1728, 2715
EarI	3	331, 2135, 2623
EclHKI	1	1340
EcoICRI	1	15
EcoRI	1	7
EheI	1	2564
FokI	5	1306, 1487, 1774, 2417, 2661
FspI	2	1562, 2585
HaeII	3	325, 695, 2566
HgaI	4	558, 1136, 1866, 2424
HincII	1	42
HindII	1	42
HindIII	1	58
Hsp92I	3	1877, 2259, 2563
KasI	1	2562
KpnI	1	23
MaeI	4	35, 942, 1195, 1530
MaeII	5	1150, 1566, 1939,2259, 2701
NarI	1	2563

Table 1. Restriction Enzymes That Cut the pGEM®-4Z Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location
NdeI	1	2511
NspI	3	56, 451, 2368
PleI	5	46, 85, 341, 826, 1329
PspAI	1	23
PstI	1	50
PvuI	2	1710, 2606
PvuII	2	271, 2635
RsaI	3	21, 1820, 2496
SacI	1	17
SalI	1	40
ScaI	1	1820

Enzyme	# of Sites	Location
SinI	2	1478, 1700
SmaI	1	25
SphI	1	56
Sse8387I	1	50
SspI	1	2144
TaqI	4	11, 41, 547, 1991
TfiI	2	282, 422
VspI	3	218, 277, 1512
XbaI	1	34
XmaI	1	23
XmnI	1	1939

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-4Z Vector.

AccIII	BsaBI	DsaI	NheI	SacII
AccB7I	BsaMI	EagI	NotI	SfiI
AflII	BsmI	Eco47III	NruI	SgfI
AgeI	Bsp120I	Eco52I	NsiI	SgrAI
ApaI	BsrGI	Eco72I	PacI	SnaBI
AscI	BssHII	Eco81I	PaeR7I	SpeI
AvrII	Bst1107I	EcoNI	PflMI	SplI
BalI	Bst98I	EcoRV	PinAI	SrfI
BbrPI	BstEII	FseI	PmeI	StuI
BbsI	BstZI	HpaI	PmlI	StyI
BclI	Bsu36I	I-PpoI	Ppu10I	SwaI
BglII	ClaI	MluI	PpuMI	Tth111I
BlpI	CspI	NaeI	PshAI	XcmI
Bpu1102I	Csp45I	NcoI	Psp5II	XhoI
BsaAI	DraIII	NgoMIV	RsrII	

4. pGEM®-4Z Vector Restriction Sites (continued)

Table 3. Restriction Enzymes That Cut the pGEM®-4Z Vector 6 or More Times.

AciI	CfoI	HinfI	MnlI	NlaIV
AluI	DdeI	HpaII	MseI	Sau3AI
BbvI	DpnI	HphI	MspI	Sau96I
BsrI	DpnII	Hsp92II	MspA1I	ScrFI
BsrSI	Fnu4HI	MaeIII	NciI	SfaNI
Bst71I	HaeIII	MboI	NdeII	Tru9I
BstUI	HhaI	MboII	NlaIII	XhoII

5. Related Products

pGEM® Vectors

Product	Size	Cat.#
pGEM®-3Z Vector	20µg	P2151
pGEM®-3Zf(+) Vector	20µg	P2271
pGEM®-3Zf(–) Vector	20µg	P2261
pGEM®-5Zf(+) Vector	20µg	P2241
pGEM®-5Zf(–) Vector	20µg	P2351
pGEM®-7Zf(+) Vector	20µg	P2251
pGEM®-7Zf(–) Vector	20µg	P2371
pGEM®-9Zf(–) Vector	20µg	P2391
pGEM®-11Zf(+) Vector	20µg	P2411
pGEM®-13Zf(+) Vector	20µg	P2541

All pGEM® Vectors are provided with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain the vector and are not competent.

Other Vectors

Product	Size	Cat.#
pSP64 Poly(A) Vector	20µg	P1241
pSP72 Vector	20µg	P2191
pSP73 Vector	20µg	P2221

Sequencing Primers

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011
T7 Promoter Primer	2µg	Q5021

Riboprobe® in vitro Transcription Systems

Product	Cat.#
Riboprobe® System—SP6	P1420
Riboprobe® System—T7	P1440

RiboMAX™ Large-Scale RNA Production Systems

Product	Cat.#
RiboMAX™ Large Scale RNA Production System—SP6	P1280
RiboMAX™ Large Scale RNA Production System—T7	P1300
T7 RiboMAX™ Express Large Scale RNA Production System	P1320