Promega

Technical Bulletin

pGEM®-4Z Vector

INSTRUCTIONS FOR USE OF PRODUCT P2161.

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Part# TB036

pGEM®-4Z Vector

All technical literature is available on the Internet at: www.promega.com/tbs/
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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*ZΔM15 gene to produce functional β-galactosidase. Cells with the genotype, *lac*ZΔM15, 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 online at: **www.promega.com/vectors/** and from the GenBank® database.

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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

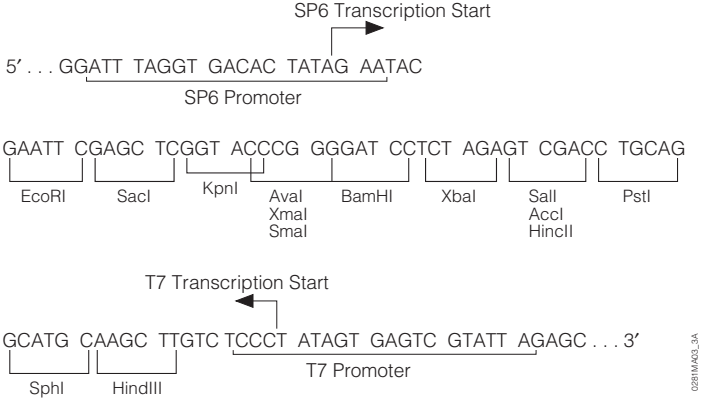

Figure 1. pGEM®-4Z Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase and is complementary to RNA synthesized by T7 RNA polymerase.

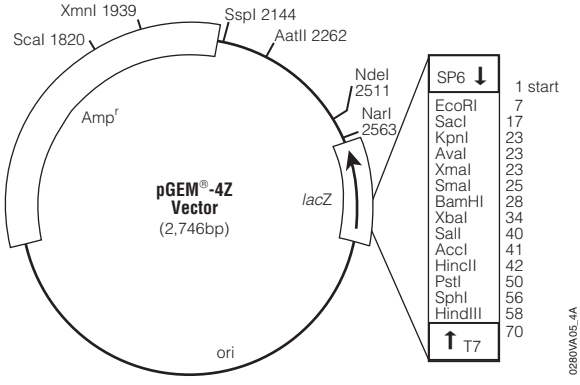

Figure 2. pGEM®-4Z Vector circle map and sequence reference points. The pGEM®-3Z and pGEM®-4Z Vectors are identical except for the orientation of the SP6 and T7 promoters.

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
binding site of pUC/M13 Reverse Sequencing Primer	106–122
*lac*Z start codon	110
*lac*Z operator	130–146
β-lactamase (Amp^r) coding region	1267–2127
binding site of pUC/M13 Forward Sequencing Primer	2686–2702
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.)

Note: All Promega technical literature is available at: **www.promega.com/tbs**

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
Bsa I	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
NdeI	1	2511

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

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NspI	3	56, 451, 2368	**SinI**	2	1478, 1700
PleI	5	46, 85, 341, 826, 1329	**SmaI**	1	25
			SphI	1	56
PspAI	1	23	Sse8387I	1	50
PstI	1	50	**SspI**	1	2144
PvuI	2	1710, 2606	**TaqI**	4	11, 41, 547, 1991
PvuII	2	271, 2635	TfiI	2	282, 422
RsaI	3	21, 1820, 2496	**VspI**	3	218, 277, 1512
SacI	1	17	**XbaI**	1	34
SalI	1	40	**XmaI**	1	23
ScaI	1	1820	**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	

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**

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

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®-11Zf(–) Vector	20µg	P2421
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
pUC/M13 Primer, Reverse (17mer)	2µg	Q5401
pUC/M13 Primer, Forward (17mer)	2µg	Q5391
pUC/M13 Primer, Forward (24mer)	2µg	Q5601
pUC/M13 Primer, Reverse (22mer)	2µg	Q5421

Riboprobe® in vitro Transcription Systems

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

For Laboratory Use.

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

For Laboratory Use.

6. Reference

1. Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103–19.

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