Technical Bulletin

pGEM®-7Zf(+) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2251.

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pGEM®-7Zf(+) 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

The pGEM®-7Zf(+) Vector is a derivative of the pGEM®-3Zf(+) Vector and contains the origin of replication of the filamentous phage f1. The plasmid serves as a standard cloning vector, as a template for in vitro transcription and as a template for the production of circular ssDNA. The plasmid contains SP6 and T7 RNA polymerase promoters flanking a multiple cloning region within the α-peptide coding region of β-galactosidase (1). Insertional inactivation of the α-peptide allows recombinant clones to be directly identified by color screening on indicator plates. The multiple cloning region is unique and includes restriction sites for ApaI, AatII, SphI, XbaI, XhoI, EcoRI, KpnI, SmaI, Csp45I, ClaI, HindIII, BamHI, SacI, BstXI and NsiI. This arrangement is designed specifically for generation of unidirectional deletions with the Erase-a-Base® System. The polylinker contains restriction enzyme sites that produce 5´ overhangs or blunt ends (sensitive to Exonuclease III) flanked on both sides by blocks of restriction sites that generate 3´ overhangs (resistant to Exonuclease III).

For induction of ssDNA, bacterial cells containing pGEM®-7Zf(+) Vector recombinants 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-like particle. The sequence of the ssDNA rescued upon infection with helper phage is complementary to the sequence shown in Figure 1. The exported ssDNA can be used for mutagenesis in vitro or can be sequenced using the T7 Promoter Primer (Cat.# Q5021) or pUC/M13 Forward Primer (Cat.# Q5391, Q5601).

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

II. Product Components and Storage Conditions

Product	Size	Cat.#
pGEM®-7Zf(+) Vector	20µg	P2251

The pGEM®-7Zf(+) 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®-7Zf(+) Vector at –20°C and the glycerol stock of JM109 cells at –70°C.

III. pGEM®-7Zf(+) Vector Multiple Cloning Region and Circle Map

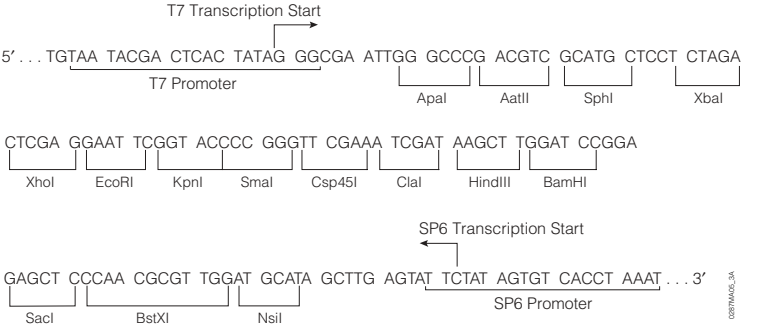

Figure 1. pGEM®-7Zf(+) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The strand shown is complementary to the ssDNA strand produced by this vector.

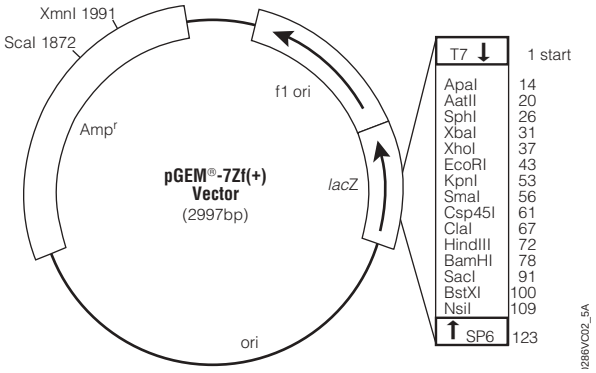

Figure 2. pGEM®-7Zf(+) Vector circle map and sequence reference points. The pGEM®-7Zf(+) and pGEM®-7Zf(–) Vectors are identical except for the orientation of the f1 origin. Use the T7 Promoter Primer or pUC/M13 Forward Primer to sequence ssDNA produced by the pGEM®-7Zf(+) Vector.

pGEM®-7Zf(+) Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
SP6 RNA polymerase transcription initiation site	123
T7 RNA polymerase promoter (–17 to +3)	2981–3
SP6 RNA polymerase promoter (–17 to +3)	121–140
multiple cloning region	10–110
binding site of pUC/M13 Reverse Sequencing Primer	158–174
*lac*Z start codon	162
lac operon sequences	2818–2978; 148–377
lac operator	182–198
β-lactamase (Amp^r) coding region	1319–2179
phage f1 region	2362–2817
binding site of pUC/M13 Forward Sequencing Primer	2938–2954

Specialized applications of the pGEM®-7Zf(+) Vector:

- Used with the Erase-a-Base® System
- ssDNA production
- Blue/white screening for recombinants
- Transcription in vitro from dual-opposed promoters (For protocol information, please request the *Riboprobe® in vitro Transcription Systems Technical Manual*, #TM016.)
- Translation in vitro (For protocol information, please request the *TNT® Quick Coupled Transcription/Translation System Technical Manual*, #TM045.)

Note: All Promega technical literature is available on the Internet at: **www.promega.com**

IV. pGEM®-7Zf(+) 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 X65310) and on the Internet at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pGEM®-7Zf(+) Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	20
AccIII	1	81
Acc65I	1	49
AcyI	2	17, 1929
AflIII	2	96, 499
Alw26I	2	1453, 2229
Alw44I	2	813, 2059
AlwNI	1	915
ApaI	1	14
AspHI	4	91, 817, 1978, 2063
AvaI	2	37, 54
AvaII	2	1530, 1752
BamHI	1	78
BanI	4	49, 243, 1340, 2623
BanII	3	14, 91, 2661
BbuI	1	26
BglI	2	1512, 2830
BsaI	1	1453
BsaAI	1	2586
BsaHI	2	17, 1929
BsaJI	5	53, 54, 238, 659, 2933
BsaOI	5	415, 839, 1762, 1911, 2858
Bsp120I	1	10
BspHI	2	1219, 2227
BssSI	2	672, 2056
BstOI	5	239, 527, 648, 661, 2934
BstXI	1	100
Cfr10I	2	1472, 2687
ClaI	1	67
Csp45I	1	61
DdeI	4	774, 1183, 1349, 1889
DraI	3	1258, 1277, 1969
DraIII	1	2586
DrdI	2	607, 2541
EaeI	3	338, 1780, 2967
EarI	3	383, 2187, 2875
EclHKI	1	1392
EcoICRI	1	89
EcoRI	1	43
FokI	5	116, 1358, 1539, 1826, 2913
FspI	2	1614, 2837
HaeII	4	377, 747, 2737, 2745
HgaI	4	610, 1188, 1918, 2803
HindIII	1	72
Hsp92I	2	17, 1929
KpnI	1	53
MaeI	5	32, 994, 1247, 1582, 2737
MluI	1	96
MspA1I	5	323, 841, 1086, 2027, 2887
NaeI	1	2689

Table 1. Restriction Enzymes That Cut the pGEM®-7Zf(+) Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NciI	5	55, 56, 879, 1575, 1926	**ScaI**	1	1872
NgoMIV	1	2687	**SinI**	2	1530, 1752
NsiI	1	109	**SmaI**	1	56
NspI	2	26, 503	**SphI**	1	26
PaeR7I	1	37	**SspI**	2	2196, 2378
Ppu10I	1	105	TfiI	2	334, 474
PspAI	1	54	**VspI**	3	270, 329, 1564
PvuI	2	1762, 2858	**XbaI**	1	31
PvuII	2	323, 2887	**XhoI**	1	37
RsaI	2	51, 1872	**XmaI**	1	54
SacI	1	91	**XmnI**	1	1991

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-7Zf(+) Vector.

AccI	BsmI	Eco81I	PacI	**SpeI**
AccB7I	BspMI	EcoNI	PflMI	SplI
AflII	BsrGI	**EcoRV**	PinAI	SrfI
AgeI	**BssHII**	EheI	PmeI	Sse8387I
AscI	Bst1107I	FseI	PmlI	**StuI**
AvrII	**Bst98I**	**HincII**	PpuMI	**StyI**
BalI	**BstEII**	HindII	PshAI	SwaI
BbeI	**BstZI**	**HpaI**	Psp5II	**Tth111I**
BbrPI	**Bsu36I**	**I-PpoI**	**PstI**	XcmI
BbsI	**CspI**	KasI	RsrII	
BclI	DraII	**NarI**	**SacII**	
BglII	DsaI	**NcoI**	**SalI**	
BlpI	EagI	**NdeI**	**SfiI**	
Bpu1102I	**Eco47III**	**NheI**	**SgfI**	
BsaBI	Eco52I	**NotI**	SgrAI	
BsaMI	Eco72I	**NruI**	**SnaBI**	

Table 3. Restriction Enzymes That Cut the pGEM®-7Zf(+) Vector 6 or More Times.

AciI	**CfoI**	HphI	**MspI**	SfaNI
AluI	**DpnI**	**Hsp92II**	**NdeII**	**TaqI**
BbvI	DpnII	MaeII	NlaIII	**Tru9I**
Bsp1286I	Fnu4HI	MaeIII	NlaIV	**XhoII**
BsrI	**HaeIII**	**MboI**	PleI	
BsrSI	**HhaI**	**MboII**	**Sau3AI**	
Bst71I	**HinfI**	MnlI	Sau96I	
BstUI	**HpaII**	MseI	ScrFI	

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

V. Related Products

pGEM® Vectors

Product	Size	Cat.#
pGEM®-3Z Vector	20µg	P2151
pGEM®-4Z Vector	20µg	P2161
pGEM®-3Zf(+) Vector	20µg	P2271
pGEM®-3Zf(–) Vector	20µg	P2261
pGEM®-5Zf(+) Vector	20µg	P2241
pGEM®-5Zf(–) Vector	20µg	P2351
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 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

Related Systems

Product	Cat.#
Erase-a-Base® System	E5750
Riboprobe® System—SP6*	P1420
Riboprobe® System—T7*	P1440
TNT® T7 Quick Coupled Transcription/Translation System*	L1170
TNT® SP6 Quick Coupled Transcription/Translation System*	L2080

*For Laboratory Use.

VI. Reference

1. Yanish-Perron, C. *et al.* (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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