Technical Bulletin

pGEM®-7Zf(–) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2371.

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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®-7Zf(+) Vector and differs from it by the orientation of the filamentous phage f1 origin of replication. 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. 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 with the F´ episome (e.g., JM109, XL-1 Blue, DH5α™) 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 identical to the sequence shown in Figure 1. The exported ssDNA can be used for mutagenesis in vitro or can be sequenced using the SP6 Promoter Primer (Cat.# Q5011) or pUC/M13 Reverse Primer (Cat.# Q5401).

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	P2371

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

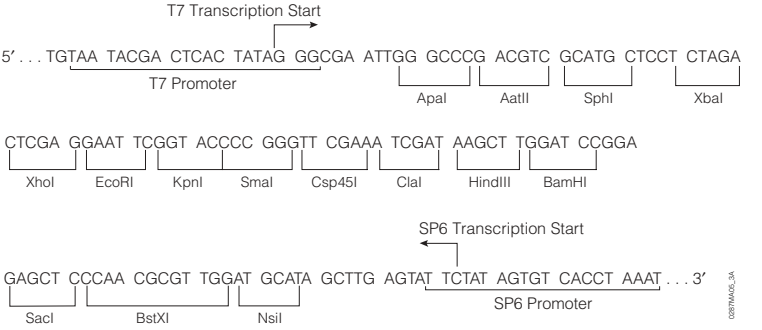

Figure 1. pGEM®-7Zf(–) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The strand shown is the same as the ssDNA produced by this vector.

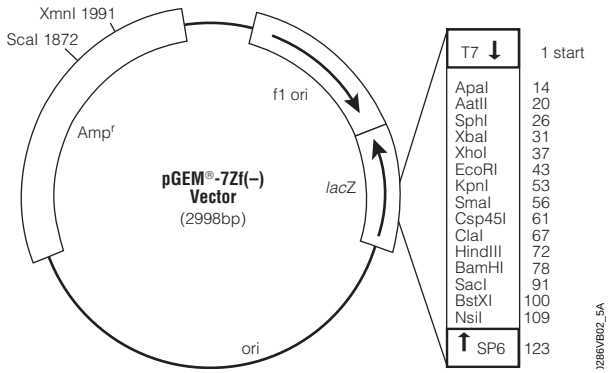

Figure 2. pGEM®-7Zf(–) Vector circle map and sequence reference points. The pGEM®-7Zf(+) and pGEM®-7Zf(–) Vectors are identical except for the orientation of the f1 origin. Use the SP6 Promoter Primer (Cat.# Q5011) or pUC/M13 Reverse Primer (Cat.# Q5401) to sequence ssDNA produced by the pGEM®-7Zf(–) 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)	2982–3
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	2819–2979; 148–377
lac operator	182–198
β-lactamase (Amp^r) coding region	1319–2179
phage f1 region	2363–2818
binding site of pUC/M13 Forward Sequencing Primer	2939–2955

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

- 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.)

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 X65311) 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, 2552
BanII	3	14, 91, 2522
BbuI	1	26
BglI	2	1512, 2831
BsaI	1	1453
BsaAI	1	2593
BsaHI	2	17, 1929
BsaJI	5	53, 54, 238, 659, 2934
BsaOI	5	415, 839, 1762, 1911, 2859
Bsp120I	1	10
BspHI	2	1219, 2227
BssSI	2	672, 2056
BstOI	5	239, 527, 648, 661, 2935
BstXI	1	100
Cfr10I	2	1472, 2488
ClaI	1	67
Csp45I	1	61
DdeI	4	774, 1183, 1349, 1889
DraI	3	1258, 1277, 1969
DraIII	1	2596
DrdI	2	607, 2640
EaeI	3	338, 1780, 2968
EarI	3	383, 2187, 2876
EclHKI	1	1392
EcoICRI	1	89
EcoRI	1	43
FokI	5	116, 1358, 1539, 1826, 2914
FspI	2	1614, 2838
HaeII	4	377, 747, 2438, 2446
HgaI	4	610, 1188, 1918, 2371
HindIII	1	72
Hsp92I	2	17, 1929
KpnI	1	53
MaeI	5	32, 994, 1247, 1582, 2440
MluI	1	96
MspA1I	5	323, 841, 1086, 2027, 2888
NaeI	1	2490

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	2488	**SinI**	2	1530, 1752
NsiI	1	109	**SmaI**	1	56
NspI	2	26, 503	**SphI**	1	26
PaeR7I	1	37	**SspI**	2	2196, 2801
Ppu10I	1	105	TfiI	2	334, 474
PspAI	1	54	**VspI**	3	270, 329, 1564
PvuI	2	1762, 2859	**XbaI**	1	31
PvuII	2	323, 2888	**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	Eco72I	**NruI**	**SnaBI**
AccB7I	BspMI	Eco81I	PacI	**SpeI**
AflII	**BsrBRI**	EcoNI	PflMI	SplI
AgeI	BsrGI	**EcoRV**	PinAI	SrfI
AscI	**BssHII**	EheI	PmeI	Sse8387I
AvrII	Bst1107I	FseI	PmlI	**StuI**
BalI	**Bst98I**	**HincII**	PpuMI	**StyI**
BbeI	**BstEII**	HindII	PshAI	SwaI
BbrPI	**BstZI**	**HpaI**	Psp5II	**Tth111I**
BbsI	**Bsu36I**	**I-PpoI**	**PstI**	XcmI
BclI	**CspI**	KasI	RsrII	
BglII	DraII	**NarI**	**SacII**	
BlpI	DsaI	**NcoI**	**SalI**	
Bpu1102I	EagI	**NdeI**	**SfiI**	
BsaBI	**Eco47III**	**NheI**	**SgfI**	
BsaMI	Eco52I	**NotI**	SgrAI	

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	P2251
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.#
Riboprobe® System—SP6	P1420
Riboprobe® System—T3	P1430
Riboprobe® System—T7	P1440

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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