Technical Bulletin

pGEM®-3Zf(–) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2261.

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pGEM®-3Zf(–) 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

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I. Description

The pGEM®-3Zf(–) Vector is a derivative of the pGEM®-3Z 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 pGEM®-3Zf(–) Vector contains SP6 and T7 RNA polymerase promoters flanking the 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. For induction of ssDNA, bacterial cells containing pGEM®-3Zf(–) 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, Q5421).

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®-3Zf(–) Vector	20µg	P2261

The pGEM®-3Zf(–) 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®-3Zf(–) Vector at –20°C and the glycerol stock of JM109 cells at –70°C.

III. pGEM®-3Zf(–) Vector Multiple Cloning Region and Circle Map

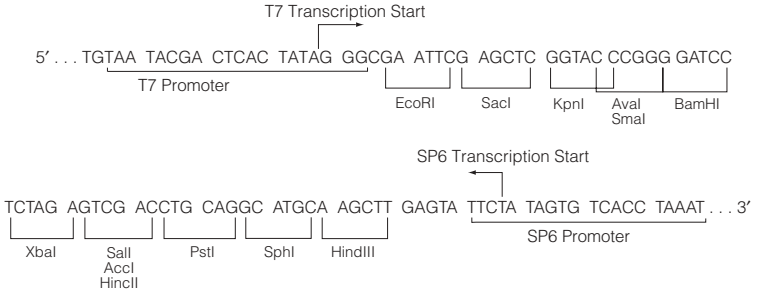

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Figure 1. pGEM®-3Zf(–) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The strand shown is the same as the ssDNA strand produced by this vector.

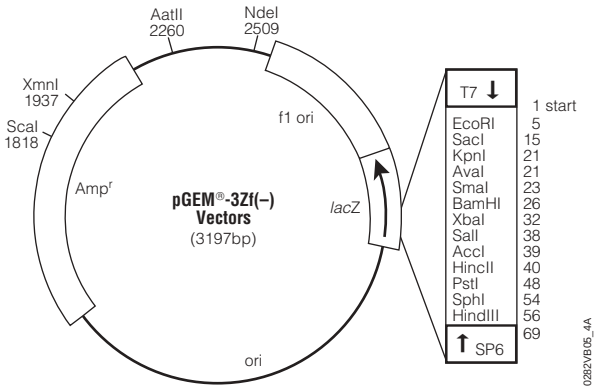

Figure 2. pGEM®-3Zf(–) Vector circle map and sequence reference points. The pGEM®-3Zf(–) and pGEM®-3Zf(+) Vectors are identical except for the orientation of the f1 orign. Use the SP6 or pUC/M13 Reverse prime to sequence ssDNA produced by the pGEM®-3Zf(–) Vector.

pGEM®-3Zf(–) Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	5–61
SP6 RNA polymerase promoter (–17 to +3)	67–86
SP6 RNA polymerase transcription initiation site	69
lac operon sequences	94–323; 3018–3178
binding site of pUC/M13 Reverse Sequencing Primer	104–120
*lac*Z start codon	108
lac operator	128–144
β-lactamase (Amp^r) coding region	1265–2125
phage f1 region	2562–3017
binding site of pUC/M13 Forward Sequencing Primer	3138–3154
T7 RNA polymerase promoter (–17 to +3)	3181–3

Specialized applications of the pGEM®-3Zf(–) 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.)
- 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®-3Zf(–) 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 X65307) and on the Internet at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pGEM®-3Zf(–) Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	2260
AccI	1	39
Acc65I	1	17
AcyI	2	1875, 2257
AflIII	1	445
Alw26I	4	1399, 2175, 2328, 2370
Alw44I	3	759, 2005, 2502
AlwNI	1	861
AspHI	5	15, 763, 1924, 2009, 2506
AvaI	1	21
AvaII	2	1476, 1698
BamHI	1	26
BanI	4	17, 189, 1286, 2751
BanII	2	15, 2721
BbuI	1	54
BglI	2	1458, 3030
Bsa I	1	1399
BsaAI	1	2792
BsaHI	2	1875, 2257
BsaJI	5	21, 22, 184, 605, 3133
BsaOI	5	361, 785, 1708, 1857, 3058
BspHI	3	1165, 2173, 2278
BspMI	1	51
BssSI	3	618, 2002, 2309
BstOI	5	185, 473, 594, 607, 3134
Cfr10I	2	1418, 2687
DraI	3	1204, 1223, 1915
DraII	1	2314
DraIII	1	2795
DrdI	3	553, 2422, 2839
EaeI	3	284, 1726, 3167
EarI	3	329, 2133, 3075
EclHKI	1	1338
EcoICRI	1	13
EcoRI	1	5
FokI	5	1304, 1485, 1772, 2415, 3113
FspI	2	1560, 3037
HaeII	4	323, 693, 2637, 2645
HgaI	5	556, 1134, 1864, 2422, 2570
HincII	1	40
HindII	1	40
HindIII	1	56
Hsp92I	2	1875, 2257
KpnI	1	21
MaeI	5	33, 940, 1193, 1528, 2639
NaeI	1	2689
NdeI	1	2509
NgoMIV	1	2687
NspI	3	54, 449, 2366
PspAI	1	21
PstI	1	48
PvuI	2	1708, 3058

Table 1. Restriction Enzymes That Cut the pGEM®-3Zf(–) Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
PvuII	2	269, 3087	**SspI**	2	2142, 3000
RsaI	3	19, 1818, 2494	**TaqI**	5	9, 39, 545, 1989, 2757
SacI	1	15			
SalI	1	38	TfiI	2	280, 420
ScaI	1	1818	**VspI**	3	216, 275, 1510
SinI	2	1476, 1698	**XbaI**	1	32
SmaI	1	23	**XmaI**	1	21
SphI	1	54	**XmnI**	1	1937
Sse8387I	1	48			

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-3Zf(–) Vector.

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

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

AciI	**CfoI**	**HpaII**	MseI	**Sau3AI**
AluI	**DdeI**	HphI	**MspI**	Sau96I
BbvI	**DpnI**	**Hsp92II**	**MspA1I**	ScrFI
Bsp1286I	DpnII	MaeII	**NciI**	SfaNI
BsrI	Fnu4HI	MaeIII	**NdeII**	**Tru9I**
BsrSI	**HaeIII**	**MboI**	NlaIII	**XhoII**
Bst71I	**HhaI**	**MboII**	NlaIV	
BstUI	**HinfI**	MnlI	PleI	

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®-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®-Zf 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—T3	P1430
Riboprobe® System—T7	P1440

For Laboratory Use.

TNT® Quick Coupled Transcription/Translation Systems

Product	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System	L1170
TNT® T7 Quick Coupled Transcription/Translation System, Trial Size	L1171
TNT® SP6 Quick Coupled Transcription/Translation System	L2080
TNT® SP6 Quick Coupled Transcription/Translation System, Trial Size	L2081

For Laboratory Use.

VI. 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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