Technical Bulletin

pGEM®-3Zf(+) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2271.

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pGEM®-3Zf(+) Vector

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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 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 EcoRI, SacI, KpnI, AvaI, SmaI, BamHI, XbaI, SalI, AccI, HincII, PstI, SphI and HindIII.

For induction of ssDNA, bacterial cells with the F´ episome (e.g., JM109, XL-1 Blue, DH5α™) containing pGEM®-3Zf(+) 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 or pUC/M13 Forward Primer.

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

II. Product Components and Storage Conditions

Product	Size	Cat.#
pGEM®-3Zf(+) Vector	20µg	P2271

Storage Conditions: Store the pGEM®-3Zf(+) Vector at –20°C.

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

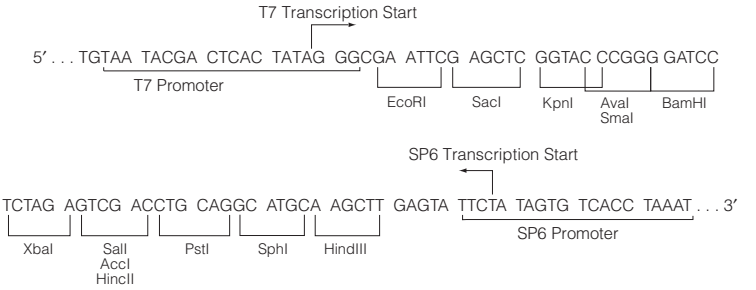

Figure 1. pGEM®-3Zf(+) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The strand shown is complementary to the ssDNA strand produced by this vector.

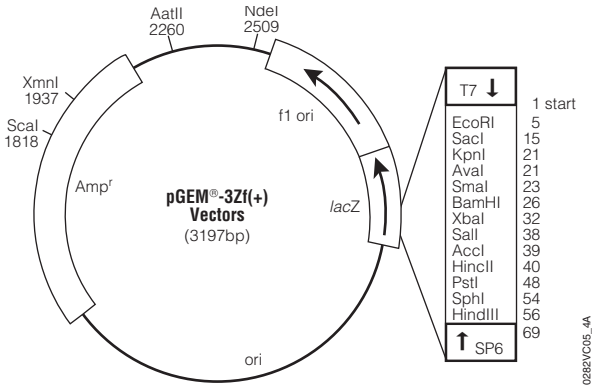

Figure 2. pGEM®-3Zf(+) Vector circle map and sequence reference points. The pGEM®-3Zf(+) and pGEM®-3Zf(–) Vectors are identical except for the orientation of the f1 origin. Use the T7 Promoter Primer or pUC/M13 Forward Primer to sequence ssDNA produced by the pGEM®-3Zf(+) Vector.

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

T7 RNA polymerase transcription initiation site	1
SP6 RNA polymerase transcription initiation site	69
T7 RNA polymerase promoter (–17 to +3)	3181–3
SP6 RNA polymerase promoter (–17 to +3)	67–86
multiple cloning region	5–61
*lac*Z start codon	108
lac operon sequences	3018–3178; 94–323
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
binding site of pUC/M13 Reverse Sequencing Primer	104–120

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).

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. The vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number X65306) 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, 2823
BanII	2	15, 2861
BbuI	1	54
BglI	2	1458, 3030
BsaI	1	1399
BsaOI	5	361, 785, 1708, 1857, 3058
BsaAI	1	2786
BsaHI	2	1875, 2257
BsaJI	5	21, 22, 184, 605, 3133
BspHI	3	1165, 2173, 2278
BspMI	1	51
BssSI	3	618, 2002, 2309
BstOI	5	185, 473, 594, 607, 3134
Cfr10I	2	1418, 2887
DraI	3	1204, 1223, 1915
DraII	1	2314
DraIII	1	2786
DrdI	3	553, 2422, 2741
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, 2937, 2945
HgaI	5	556, 1134, 1864, 2422, 3003
HincII	1	40
HindII	1	40
HindIII	1	56
Hsp92I	2	1875, 2257
KpnI	1	21
MaeI	5	33, 940, 1193, 1528, 2937
NaeI	1	2889
NdeI	1	2509
NgoMIV	1	2887
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, 2578
RsaI	3	19, 1818, 2494	**TaqI**	5	9, 39, 545, 1989, 2819
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.

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

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

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

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

V. Related Products

Product	Size	Cat.#
pGEM®-3Z Vector	20µg	P2151
pGEM®-4Z Vector	20µg	P2161
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

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.

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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