Technical Bulletin

pGEM®-5Zf(–) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2351.

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pGEM®-5Zf(–) Vector

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I. Description 1

II. Product Components and Storage Conditions 2

III. pGEM®-5Zf(–) Vector Multiple Cloning Region and Map 2

IV. pGEM®-5Zf(–) Vector Restriction Enzyme Sites 4

V. Related Products 6

VI. Reference 7

I. Description

The pGEM®-5Zf(–) Vector is a derivative of the pGEM®-5Zf(+) Vector and differs from it by the orientation of the origin of replication of filamentous phage f1. The plasmid serves as a standard cloning vector, as a template for in vitro transcription, and can be used for the production of circular ssDNA. The plasmid contains T7 and SP6 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, NcoI, SacII, EcoRV, SpeI, NotI, PstI, SalI, NdeI, SacI, BstXI and NsiI. This arrangement is designed specifically for generating unidirectional deletions with the Erase-a-Base® System (Cat.# E5750). 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®-5Zf(–) 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) and pUC/M13 Reverse Primers (Cat.# Q5401, Q5421).

Promega vector sequences 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®-5Zf(–) Vector	20µg	P2351

The pGEM®-5Zf(–) Vector is provided with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain the vector and are not competent cells.

Storage Conditions: Store the pGEM®-5Zf(–) Vector at –20°C and the glycerol stock of JM109 cells at –70°C.

III. pGEM®-5Zf(–) Vector Multiple Cloning Region and Map

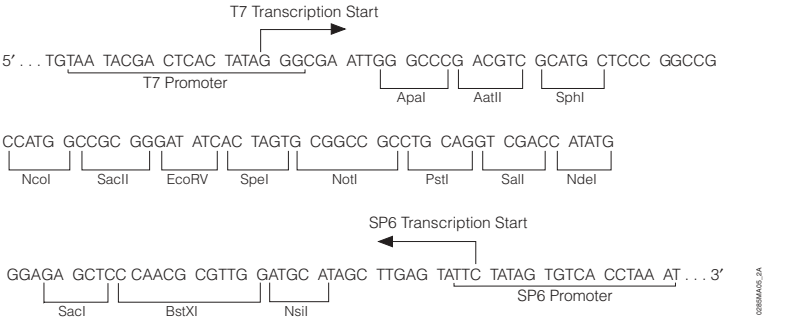

Figure 1. pGEM®-5Zf(–) Vector promoter and multiple cloning site sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The strand shown is the same as the ssDNA strand produced by this vector.

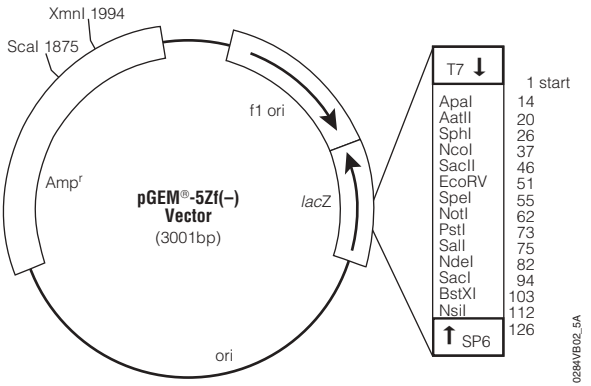

Figure 2. pGEM®-5Zf(–) Vector map.

pGEM®-5Zf(–) and pGEM®-5Zf(+) Vectors are identical except for the orientation of the f1 origin.

pGEM®-5Zf(–) Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
Multiple cloning region	10–113
SP6 RNA polymerase promoter (–17 to +3)	124–143
SP6 RNA polymerase transcription initiation site	126
pUC/M13 Reverse Sequencing Primer binding region	161–177
lacZ start codon	165
lac operator	185–201
β-lactamase (Amp^r) coding region	1322–2182
Phage f1 region	2366–2821
lac operon sequences	2822–2982; 151–380
pUC/M13 Forward Sequencing Primer binding region	2942–2958
T7 RNA polymerase promoter (–17 to +3)	2985–3

Use the SP6 or pUC/M13 Reverse Primer to sequence ssDNA produced by the pGEM®-5Zf(–) Vector.

Specialized applications of the pGEM®-5Zf(–) 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.)

IV. pGEM®-5Zf(–) Vector Restriction Enzyme 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 also available in the GenBank® database (GenBank®/EMBL Accession Number X65309) and on the Internet at: **www.promega.com/vectors/**

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

Enzyme	# of Sites	Location
AatII	1	20
AccI	1	76
AcyI	2	17, 1932
AflIII	2	99, 502
Alw26I	2	1456, 2232
Alw44I	2	816, 2062
AlwNI	1	918
ApaI	1	14
AspHI	4	94, 820, 1981, 2066
AvaII	2	1533, 1755
BanI	3	246, 1343, 2555
BanII	3	14, 94, 2525
BbuI	1	26
BglI	3	39, 1515, 2834
BsaI	1	1456
BsaAI	1	2596
BsaHI	2	17, 1932
BsaJI	5	37, 43, 241, 662, 2937
Bsp120I	1	10
BspHI	2	1222, 2230
BspMI	1	62
BssSI	2	675, 2059
BstOI	5	242, 530, 651, 664, 2938
BstXI	1	103
BstZI	2	31, 62
Cfr10I	2	1475, 2491
DdeI	4	777, 1186, 1352, 1892
DraI	3	1261, 1280, 1972
DraIII	1	2599
DrdI	2	610, 2643
DsaI	2	37, 43
EagI	2	31, 62
EarI	3	386, 2190, 2879
EclHKI	1	1395
Eco52I	2	31, 62
EcoICRI	1	92
EcoRV	1	51
FokI	5	119, 1361, 1542, 1829, 2917
FspI	2	1617, 2841
HaeII	4	380, 750, 2441, 2449
HgaI	4	613, 1191, 1921, 2374
HincII	1	77
HindII	1	77
Hsp92I	2	17, 1932
MaeI	5	56, 997, 1250, 1585, 2443
MluI	1	99
NaeI	1	2493
NciI	4	30, 882, 1578, 1929
NcoI	1	37
NdeI	1	82
NgoMIV	1	2491
NotI	1	62
NsiI	1	112
NspI	2	26, 506

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

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

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Ppu10I	1	108	**SpeI**	1	55
PstI	1	73	**SphI**	1	26
PvuI	2	1765, 2862	Sse8387I	1	73
PvuII	2	326, 2891	**SspI**	2	2199, 2804
RsaI	1	1875	**StyI**	1	37
SacI	1	94	**TaqI**	4	76, 602, 2046, 2561
SacII	1	46			
SalI	1	75	TfiI	2	337, 477
ScaI	1	1875	**VspI**	3	273, 332, 1567
SfiI	1	39	**XmnI**	1	1994
SinI	2	1533, 1755			

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-5Zf(–) Vector.

AccB7I	BlpI	**Eco47III**	PacI	SplI
AccIII	Bpu1102I	Eco72I	PaeR7I	SrfI
Acc65I	BsaBI	Eco81I	PflMI	**StuI**
AflII	**BsaMI**	EcoNI	PinAI	SwaI
AgeI	BsmI	**EcoRI**	PmeI	**Tth111I**
AscI	BsrGI	EheI	PmlI	**XbaI**
AvaI	**BssHII**	FseI	PpuMI	XcmI
AvrII	Bst1107I	**HindIII**	PshAI	**XhoI**
BalI	**Bst98I**	**HpaI**	Psp5II	**XmaI**
BamHI	**BstEII**	**I-PpoI**	PspAI	
BbeI	**Bsu36I**	KasI	RsrII	
BbrPI	**ClaI**	**KpnI**	**SgfI**	
BbsI	**CspI**	**NarI**	SgrAI	
BclI	**Csp45I**	**NheI**	**SmaI**	
BglII	DraII	**NruI**	**SnaBI**	

Table 3. Restriction Enzymes That Cut the pGEM®-5Zf(–) Vector 6 or More Times.

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

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

V. Related Products

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	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 vector and are not competent cells.

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
Erase-a-Base® System (minus vectors and bacterial strain)	1 system	E5750

Competent Cells

Product	Size	Cat. #
Single Step (KRX) Competent Cells	5 × 200µl	L3001
	20 × 50µl	L3002
JM109 Competent Cells, >10^8cfu/µg*	1ml (5 × 200µl)	L2001
JM109 Competent Cells, >10^7cfu/µg	1ml (5 × 200µl)	L1001
HB101 Competent Cells, >10^8cfu/µg	1ml (5 × 200µl)	L2011
HB101 Competent Cells, >10^7cfu/µg	1ml (5 × 200µl)	L1011

*For Laboratory Use.

Riboprobe® in vitro Transcription Systems

Product	Size	Cat. #
Riboprobe® System—SP6	1 system	P1420
Riboprobe® System—T3	1 system	P1430
Riboprobe® System—T7	1 system	P1440

For Laboratory Use.

VI. Reference

1. Yanisch-Perron, C., Vieira, J., 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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