Technical Bulletin

pGEM®-5Zf(+) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2241.

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Part# TB047

pGEM®-5Zf(+) 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®-5Zf(+) 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 can be used for the production of circular ssDNA. pGEM®-5Zf(+) 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 contains unique 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. 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 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) and pUC/M13 Forward Primer (Cat.# Q5391, Q5601).

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

II. Product Components and Storage Conditions

Product	Size	Cat. #
pGEM®-5Zf(+) Vector	20µg	P2241

The pGEM®-5Zf(+) Vector is supplied 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

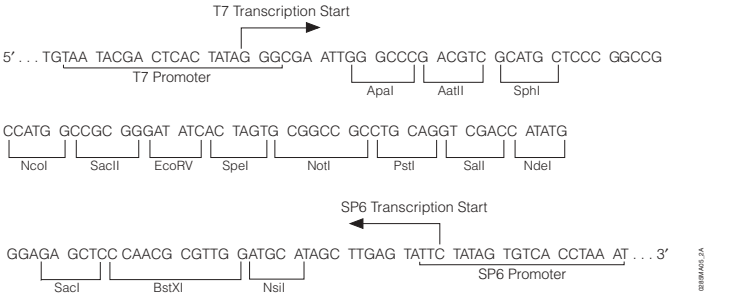

Figure 1. pGEM®-5Zf(+) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The strand shown is complementary to the ssDNA produced by this vector.

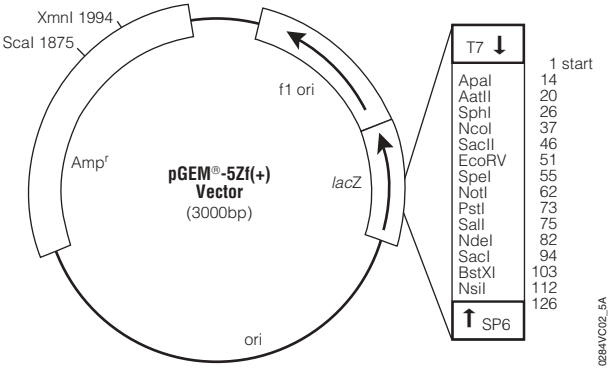

Figure 2. pGEM®-5Zf(+) Vector map.

The 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
Binding site of pUC/M13 Reverse Sequencing Primer	161–177
*lac*Z start codon	165
lac operon sequences	151–380; 2821–2981
lac operator	185–201
β-lactamase coding region	1322–2182
phage f1 region	2365–2820
binding site of pUC/M13 Forward Sequencing Primer	2941–2957
T7 RNA Polymerase promoter (–17 to +3)	2984–3

Use the T7 or pUC/M13 Forward Primer to sequence ssDNA produced by the pGEM®-5Zf(+) Vector.

Specialized applications of the pGEM®-5Zf(+) Vector:

- used with the Erase-a-Base® System (For information, please request Technical Manual #TM006.)
- 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.)

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 X65308) 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, 2626
BanII	3	14, 94, 2664
BbuI	1	26
BglI	3	39, 1515, 2833
BsaI	1	1456
BsaAI	1	2589
BsaHI	2	17, 1932
BsaJI	5	37, 43, 241, 662, 2936
Bsp120I	1	10
BspHI	2	1222, 2230
BspMI	1	62
BssSI	2	675, 2059
BstOI	5	242, 530, 651, 664, 2937
BstXI	1	103
BstZI	2	31, 62
Cfr10I	2	1475, 2690
DdeI	4	777, 1186, 1352, 1892
DraI	3	1261, 1280, 1972
DraIII	1	2589
DrdI	2	610, 2544
DsaI	2	37, 43
EagI	2	31, 62
EarI	3	386, 2190, 2878
EclHKI	1	1395
Eco52I	2	31, 62
EcoICRI	1	92
EcoRV	1	51
FokI	5	119, 1361, 1542, 1829, 2916
FspI	2	1617, 2840
HaeII	4	380, 750, 2740, 2748
HgaI	4	613, 1191, 1921, 2806
HincII	1	77
HindII	1	77
Hsp92I	2	17, 1932
MaeI	5	56, 997, 1250, 1585, 2740
MluI	1	99
NaeI	1	2692
NciI	4	30, 882, 1578, 1929
NcoI	1	37
NdeI	1	82
NgoMIV	1	2690
NotI	1	62
NsiI	1	112
NspI	2	26, 506
Ppu10I	1	108
PstI	1	73
PvuI	2	1765, 2861
PvuII	2	326, 2890

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

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
RsaI	1	1875	Sse8387I	1	73
SacI	1	94	**SspI**	2	2199, 2381
SacII	1	46	**StyI**	1	37
SalI	1	75	**TaqI**	4	76, 602, 2046, 2622
ScaI	1	1875			
SfiI	1	39	TfiI	2	337, 477
SinI	2	1533, 1755	**VspI**	3	273, 332, 1567
SpeI	1	55	**XmnI**	1	1994
SphI	1	26			

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

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

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

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

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

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.

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

RiboMAX™ Large Scale RNA Production Systems

Product	Size	Cat.#
RiboMAX™ Large Scale RNA Production System—SP6	1 system	P1280
RiboMAX™ Large Scale RNA Production System—T7	1 system	P1300

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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