TECHNICAL BULLETIN

pGEM®-5Zf(+) Vector

Instructions for Use of Product
P2241

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TB047

pGEM®-5Zf(+) Vector

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The pGEM®-5Zf(+) Vector is a derivative of the pGEM®-3Zf(+) Vector. The plasmid serves as a standard cloning vector and as a template for in vitro transcription. pGEM®-5Zf(+) contains T7 and SP6 RNA polymerase promoters flanking a multiple cloning region within the a-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. 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).

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

2. 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.

3. pGEM®-5Zf(+) Vector Multiple Cloning Region and Map

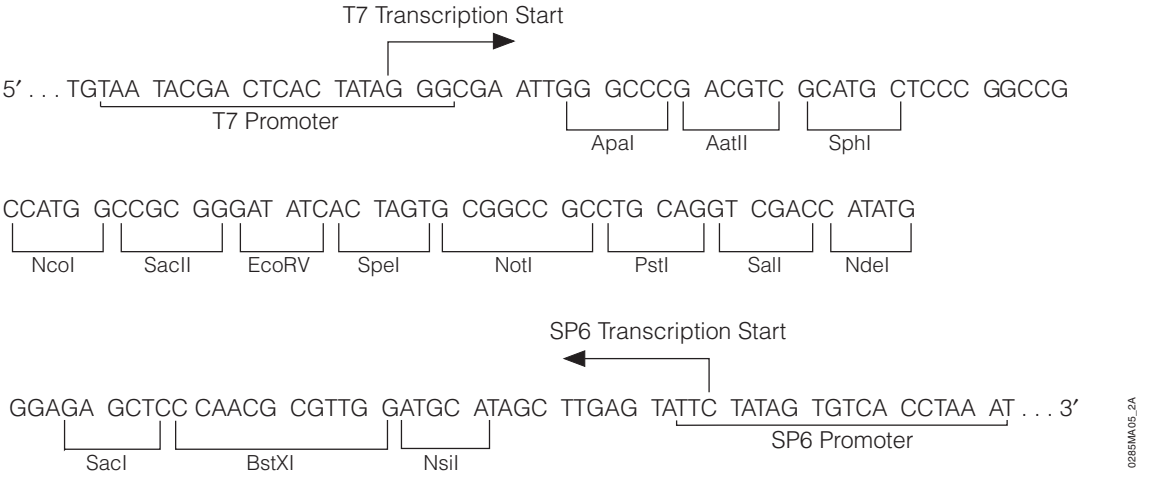

Figure 1. pGEM®-5Zf(+) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase.

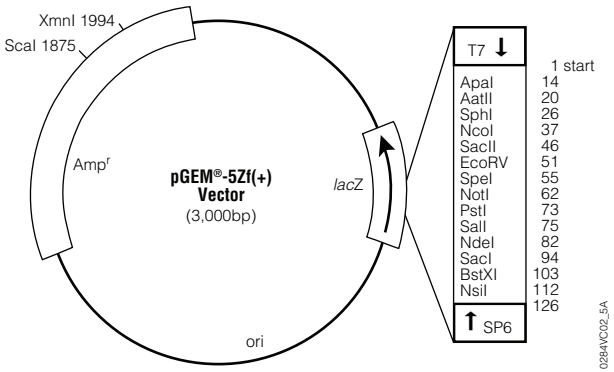

Figure 2. pGEM®-5Zf(+) Vector map.

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
*lac*Z start codon	165
lac operon sequences	151–380; 2821–2981
lac operator	185–201
β-lactamase (Amp^r) coding region	1322–2182
T7 RNA polymerase promoter (–17 to +3)	2984–3

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

- 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.)

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4. 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, 1456
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

Enzyme	# of Sites	Location
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

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

Enzyme	# of Sites	1875Location39
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
RsaI	1	1875
SacI	1	94
SacII	1	46
ScaI	1	75

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

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	

4. pGEM®-5Zf(+) Vector Restriction Enzyme Sites (continued)

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	

5. 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®-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.

Other Vectors

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

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

Sequencing Primers

Product	Size	Cat. #
SP6 Promoter Primer	2µg	Q5011
T7 Promoter Primer	2µg	Q5021

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

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