

TECHNICAL BULLETIN

pGEM[®]-3Zf(-) Vector

Instructions for Use of Product
P2261



pGEM[®]-3Zf(-) 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

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1. Description

The pGEM[®]-3Zf(-) Vector is a derivative of the pGEM[®]-3Z Vector. The plasmid serves as a standard cloning vector and as a template for in vitro transcription.

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.

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



2. 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.

3. pGEM[®]-3Zf(-) Vector Multiple Cloning Region and Circle Map

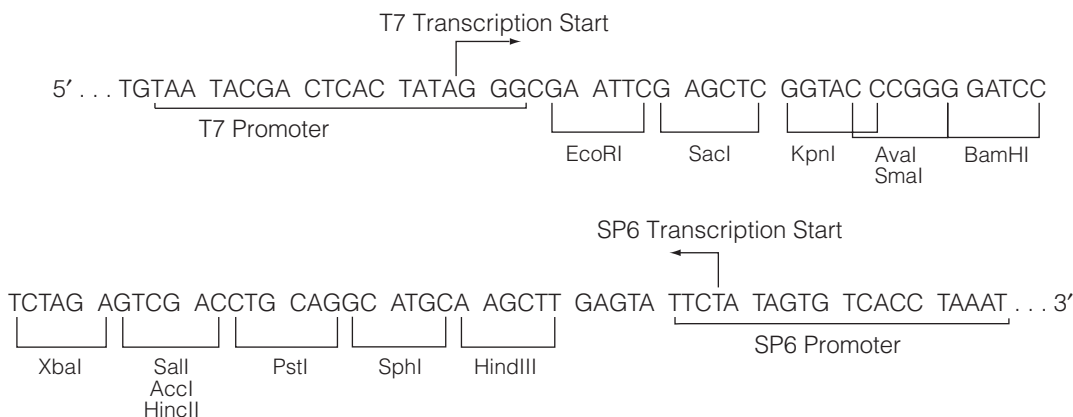
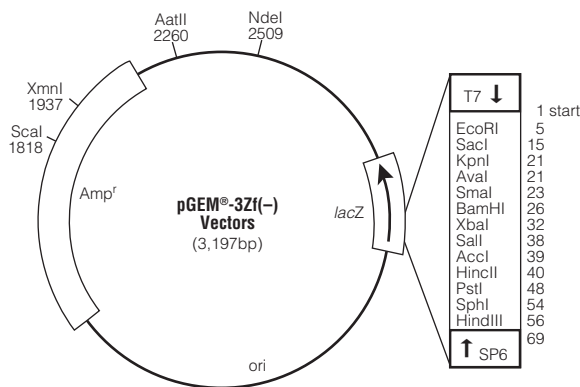


Figure 1. pGEM[®]-3Zf(-) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase.



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Figure 2. pGEM®-3Zf(-) Vector circle map and sequence reference points.

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
<i>lac</i> operon sequences	94-323; 3018-3178
<i>lacZ</i> start codon	108
<i>lac</i> operator	128-144
β -lactamase (<i>Amp^r</i>) coding region	1265-2125
T7 RNA polymerase promoter (-17 to +3)	3181-3

Specialized applications of the pGEM®-3Zf(-) 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.)
- Translation *in vitro* (For protocol information, please request the *TnT® Quick Coupled Transcription/ Translation System Technical Manual*, #TM045.)



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

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

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
PspAI	1	21	SphI	1	54
PstI	1	48	Sse8387I	1	48
PvuI	2	1708, 3058	SspI	2	2142, 3000
PvuII	2	269, 3087	TaqI	5	9, 39, 545, 1989, 2757
RsaI	3	19, 1818, 2494	TfiI	2	280, 420
SacI	1	15	VspI	3	216, 275, 1510
SalI	1	38	XbaI	1	32
ScaI	1	1818	XmaI	1	21
SinI	2	1476, 1698	XmnI	1	1937
SmaI	1	23			

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

AccIII	BlnI	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	



4. pGEM[®]-3Zf(-) Vector Restriction Sites (continued)

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	MspAII	SerFI
Bsp1286I	DpnII	MaeII	NciI	SfaNI
BsrI	Fnu4HI	MaeIII	NdeII	Tru9I
BsrSI	HaeIII	MboI	NlaIII	XhoII
Bst71I	HhaI	MboII	NlaIV	
BstUI	HinfI	MnlI	PleI	

5. 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 [®] -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	P222

Sequencing Primers

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

Riboprobe® in vitro Transcription Systems

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

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

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