



Promega

Technical Bulletin

pGEM[®]-11Zf(-) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2421.



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pGEM[®]-11Zf(-) Vector

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of this system. E-mail: techserv@promega.com

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I. Description

The pGEM[®]-11Zf(-) Vector can be used as a standard cloning vector, as a template for in vitro transcription and for the production of 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 when using appropriate *E. coli* strains (e.g., JM109, DH5 α [™], XL1-Blue). The multiple cloning region contains unique restriction sites for SfiI, SacI, EcoRI, Sall, XhoI, BamHI, ApaI, XbaI, NotI, SphI, NsiI and HindIII.

For induction of ssDNA, bacterial cells with the F' episome (e.g., JM109, XL1-Blue, DH5 α [™]F') containing pGEM[®]-11Zf(-) 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 the same strand as that shown in Figure 1. The exported ssDNA can be used for in vitro mutagenesis or can be sequenced using the SP6 Promoter Primer or pUC/M13 Reverse 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 [®] -11Zf(-) Vector	20µg	P2421

The pGEM[®]-11Zf(-) Vector is supplied 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[®]-11Zf(-) Vector at -20°C and the glycerol stock of JM109 cells at -70°C.

III. pGEM[®]-11Zf(-) Vector Multiple Cloning Site and Circle Map

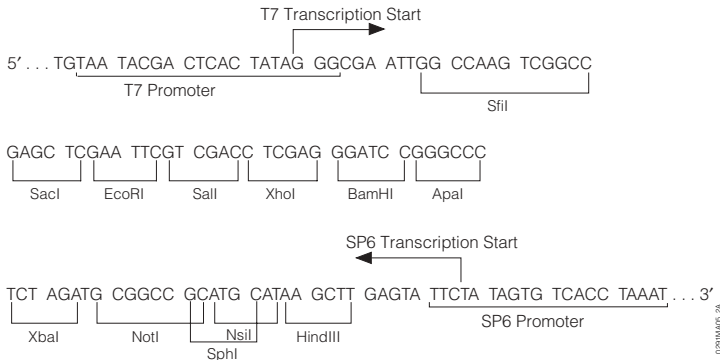


Figure 1. pGEM[®]-11Zf(-) Vector promoter and multiple cloning site sequence.

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 produced by this vector upon infection with an appropriate helper phage.

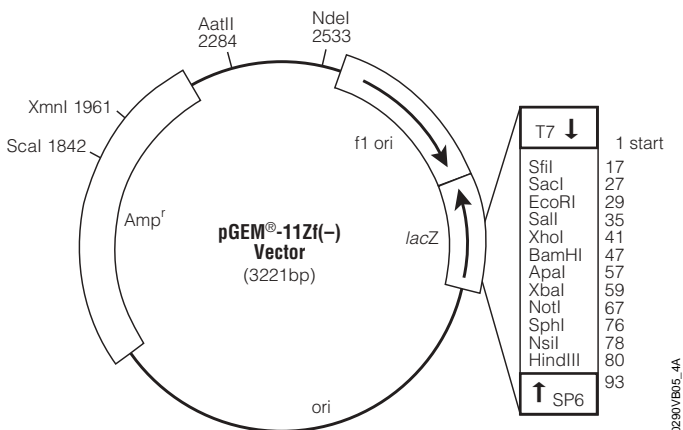


Figure 2. pGEM®-11Zf(-) Vector circle map and sequence reference points. The pGEM®-11Zf(-) and pGEM®-11Zf(+) Vectors are identical except for the orientation of the f1 origin. Use the SP6 or pUC/M13 Reverse Primer to sequence ssDNA produced by the pGEM®-11Zf(-) Vector.

pGEM®-11Zf(-) Vector sequence reference points:

T7 RNA Polymerase transcription initiation site	1
SP6 RNA Polymerase transcription initiation site	93
T7 RNA Polymerase promoter (-17 to +3)	3205-3
SP6 RNA Polymerase promoter (-17 to +3)	91-110
multiple cloning region	10-85
<i>lacZ</i> start codon	132
<i>lac</i> operon sequences	118-347, 3042-3202
<i>lac</i> operator	152-168
β-lactamase coding region	1289-2149
phage f1 region	2586-3041
binding site of pUC/M13 Forward Sequencing Primer	3162-3178
binding site of pUC/M13 Reverse Sequencing Primer	128-144

Specialized applications of the pGEM®-11Zf(-) 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[®]-11Zf(-) 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 X65314) and on the Internet at: www.promega.com/vectors/

Table 1. Restriction Enzymes That Cut the pGEM[®]-11Zf(-) Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AatII	1	2284	DraII	2	54, 2338
AccI	1	36	DraIII	1	2819
Acyl	2	1899, 2281	DrdI	3	577, 2446, 2863
AflIII	1	469	EagI	2	18, 67
Alw26I	4	1423, 2199, 2352, 2394	EarI	3	353, 2157, 3099
Alw44I	3	783, 2029, 2526	EclHKI	1	1362
AlwNI	1	885	Eco52I	2	18, 67
ApaI	1	57	EcoICRI	1	25
AspHI	5	27, 787, 1948, 2033, 2530	EcoRI	1	29
AvaI	1	41	FokI	5	1328, 1509, 1796, 2439, 3137
AvaII	2	1500, 1722	FspI	2	1584, 3061
BalI	1	11	HaeII	4	347, 717, 2661, 2669
BamHI	1	47	HgaI	5	580, 1158, 1888, 2446, 2594
BanI	3	213, 1310, 2775	HincII	1	37
BanII	3	27, 57, 2745	HindII	1	37
BbuI	1	76	HindIII	1	80
BglI	3	17, 1482, 3054	MaeI	5	60, 964, 1217, 1552, 2663
BsaI	1	1423	NaeI	1	2713
BsaAI	1	2816	NdeI	1	2533
BsaHI	2	1899, 2281	NgoMIV	1	2711
BsaJI	3	208, 629, 3157	NotI	1	67
Bsp120I	1	53	NsiI	1	78
BspHI	3	1189, 2197, 2302	NspI	3	76, 473, 2390
BssSI	3	642, 2026, 2333	PaeR7I	1	41
BstOI	5	209, 497, 618, 631, 3158	Ppu10I	1	74
BstZI	2	18, 67	PvuI	2	1732, 3082
Cfr10I	2	1442, 2711	PvuII	2	293, 3111
DraI	3	1228, 1247, 1939			

Table 1. Restriction Enzymes That Cut the pGEM[®]-11Zf(-) Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
RsaI	2	1842, 2518	SspI	2	2166, 3024
SacI	1	27	TfiI	2	304, 444
SalI	1	35	VspI	3	240, 299, 1534
ScaI	1	1842	XbaI	1	59
SfiI	1	17	XhoI	1	41
SinI	2	1500, 1722	XmnI	1	1961
SphI	1	76			

Table 2. Restriction Enzymes That Do Not Cut the pGEM[®]-11Zf(-) Vector.

AccB7I	BsaMI	Eco72I	PacI	SnaBI
AccIII	BsmI	Eco81I	PfiMI	SpeI
Acc65I	BspMI	EcoNI	PinAI	SplI
AflII	BsrGI	EcoRV	PmeI	SrfI
AgeI	BssHIII	EheI	PmlI	Sse8387I
AscI	Bst98I	FseI	PpuMI	StuI
AvrII	Bst1107I	HpaI	PshAI	StyI
BbeI	BstEII	I-PpoI	Psp5II	Swal
BbrPI	BstXI	KasI	PspAI	Tth111I
BbsI	Bsu36I	KpnI	PstI	XcmI
BclI	Clal	MluI	RsrII	XmaI
BglIII	CspI	NarI	SacII	
BlpI	Csp45I	NcoI	SgfI	
Bpu1102I	DsaI	NheI	SgrAI	
BsaBI	Eco47III	NruI	SmaI	

Table 3. Restriction Enzymes That Cut the pGEM[®]-11Zf(-) Vector 6 or More Times.

AccI	CfoI	HinfI	MseI	Sau96I
AluI	DdeI	HpaII	MspI	ScrFI
BbvI	DpnI	HphI	MspAII	SfaNI
BsaOI	DpnII	Hsp92II	NciI	TaqI
Bsp1286I	EaeI	MaeII	NdeII	Tru9I
BsrI	Fnu4HI	MaeIII	NlaIII	XhoII
BsrSI	HaeIII	MboI	NlaIV	
Bst7II	HhaI	MbolI	PleI	
BstUI	HinPI	MnlI	Sau3AI	

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	P2271
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 [®] -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.

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 – T7	P1440

VI. Reference

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