



Promega

Technical Bulletin

pGEM[®]-13Zf(+) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2541.



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pGEM[®]-13Zf(+) Vector

All technical literature is available on the Internet at: www.promega.com/tbs/
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I. Description

The pGEM[®]-13Zf(+) 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 TM, XL1-Blue). The multiple cloning region contains unique restriction sites for SfiI, NotI and HindIII.

For induction of ssDNA, bacterial cells with the F' episome (e.g., JM109, XL1-Blue, DH5 α TMF') containing pGEM[®]-13Zf(+) 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 in vitro mutagenesis or can be sequenced using the T7 Promoter Primer or pUC/M13 Forward Primer.

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

Specialized Applications of the pGEM[®]-13Zf(+) Vector

- Excision of insert containing SP6 and T7 promoters.
- 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/tbs/

II. Product Components and Storage Conditions

Product	Size	Cat.#
pGEM [®] -13Zf(+) Vector	20µg	P2541

The pGEM[®]-13Zf(+) 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[®]-13Zf(+) Vector at -20°C and the glycerol stock of JM109 cells at -70°C.

III. pGEM[®]-13Zf(+) Vector Multiple Cloning Site and Circle Map

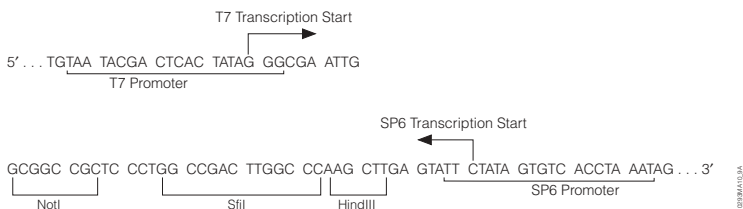


Figure 1. pGEM[®]-13Zf(+) Vector promoter and multiple cloning region sequence.

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 upon infection with an appropriate helper phage.

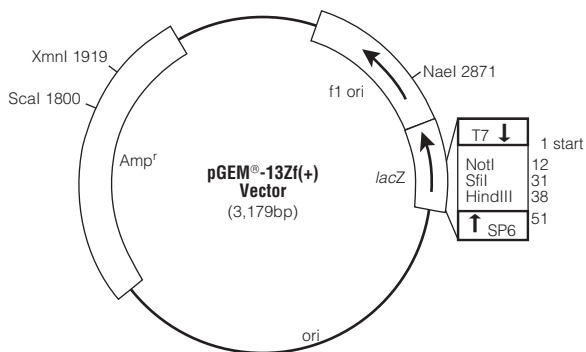


Figure 2. pGEM®-13Zf(+) Vector circle map and sequence reference points.

! Use the T7 or pUC/M13 Forward Primer to sequence ssDNA produced by the pGEM®-13Zf(+) Vector.

pGEM®-13Zf(+) Vector sequence reference points.

T7 RNA Polymerase transcription initiation site	1
SP6 RNA Polymerase transcription initiation site	51
T7 RNA Polymerase promoter (-17 to +3)	3163-3
SP6 RNA Polymerase promoter (-17 to +3)	49-68
multiple cloning region	11-43
<i>lacZ</i> start codon	90
<i>lac</i> operon sequences	3000-3160, 76-323
<i>lac</i> operator	110-126
β -lactamase coding region	1247-2107
phage f1 region	2544-2999
binding site of pUC/M13 Forward Sequencing Primer	3120-3136
binding site of pUC/M13 Reverse Sequencing Primer	86-102

IV. pGEM[®]-13Zf(+) 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 pGEM[®]-13Zf(+) Vector sequence is available in the GenBank[®] database (GenBank[®]/EMBL Accession Number X65315) and on the Internet at: www.promega.com/vectors/

Table 1. Restriction Enzymes That Cut the pGEM[®]-13Zf(+) Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AatII	1	2242	EclHKI	1	1320
AcyI	2	1857, 2239	Eco52I	1	12
AflIII	1	427	FokI	5	1286, 1467, 1754, 2397, 3095
Alw26I	4	1381, 2157, 2310, 2352	FspI	2	1542, 3019
Alw44I	3	741, 1987, 2484	HaeII	4	305, 675, 2919, 2927
AlwNI	1	843	HgaI	5	538, 1116, 1846, 2404, 2985
AspHI	4	745, 1906, 1991, 2488	HindIII	1	38
Avall	2	1458, 1680	Hsp92I	2	1857, 2239
BanI	3	171, 1268, 2805	MaeI	4	922, 1175, 1510, 2919
BanII	1	2843	NaeI	1	2871
BglI	3	31, 1440, 3012	NciI	5	807, 1503, 1854, 2355, 2390
BsaI	1	1381	NdeI	1	2491
BsaAI	1	2768	NgoMIV	1	2869
BsaHI	2	1857, 2239	NotI	1	12
BsaJI	4	20, 166, 587, 3115	NspI	2	431, 2348
Bsp1286I	5	745, 1906, 1991, 2488, 2843	PvuI	2	1690, 3040
BspHI	3	1147, 2155, 2260	PvuII	2	251, 3069
BssSI	3	600, 1984, 2291	RsaI	2	1800, 2476
BstZI	1	12	ScaI	1	1800
Cfr10I	2	1400, 2869	SfiI	1	31
DraI	3	1186, 1205, 1897	SinI	2	1458, 1680
DraII	1	2296	SspI	2	2124, 2560
DraIII	1	2768	TaqI	3	527, 1971, 2801
DrdI	3	535, 2404, 2723	TfiI	2	262, 402
EaeI	5	12, 23, 266, 1708, 3149	VspI	3	198, 257, 1492
EagI	1	12	XmnI	1	1919
EarI	3	311, 2115, 3057			

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-13Zf(+) Vector.

AccB7I	BlpI	Eco47III	NheI	SgfI
AccI	Bpu1102I	Eco72I	NruI	SgrAI
AccIII	BsaBI	Eco81I	NsiI	SmaI
Acc65I	BsaMI	EcoICRI	PacI	SnaBI
AflII	BsmI	EcoNI	PfiMI	SpeI
AgeI	Bsp120I	EcoRI	PinAI	SphI
Apal	BspMI	EcoRV	PmeI	SpII
AscI	BsrGI	EheI	PmlI	SrfI
AvaI	BssHIII	FseI	Ppu10I	Sse8387I
AvrII	Bst1107I	HincII	PpuMI	StuI
BalI	Bst98I	HindII	PshAI	StyI
BamHI	BstEII	HpaI	Psp5II	Swal
BbeI	BstXI	I-PpoI	PspAI	Tth111I
BbrPI	Bsu36I	KasI	PstI	XbaI
BbsI	Clal	KpnI	RsrII	XcmI
BbuI	CspI	MluI	SacI	XhoI
BclI	Csp45I	NarI	SacII	XmaI
BglII	DsaI	NcoI	Sall	

Table 3. Restriction Enzymes That Cut the pGEM®-13Zf(+) Vector 6 or More Times.

AcI	BstUI	HinfI	MnlI	Sau3AI
AluI	CfoI	HpaII	MseI	Sau96I
BbvI	DdeI	HphI	MspI	ScrFI
BsaOI	DpnI	Hsp92II	MspAII	SfaNI
BsrI	DpnII	MaeII	NdeII	Tru9I
BsrSI	Fnu4HI	MaeIII	NlaIII	XhoII
Bst7II	HaeIII	MboI	NlaIV	
BstOI	HhaI	MboII	PleI	

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 [®] -11Zf(-) Vector	20µg	P2421

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 – T3	P1430
Riboprobe [®] System – T7	P1440

For Laboratory Use.

VI. Reference

1. Yanisch-Perron, C. *et al.* (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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