Technical Bulletin

pGEM®-9Zf(–) Vector

INSTRUCTIONS FOR USE OF PRODUCT P2391.

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pGEM®-9Zf(–) Vector

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I. Description

The pGEM®-9Zf(–) Vector is a recombinant plasmid designed to provide a versatile range of cloning strategies, efficient synthesis of RNA in vitro and the production of single-stranded DNA. 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. The multiple cloning region is unique and includes restriction sites for NsiI, SpeI, HindIII, XbaI, EcoRI, SalI and SacI.

For induction of ssDNA, bacterial cells containing pGEM®-9Zf(–) 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 produced is identical to the sequence shown in Figure 1. The exported ssDNA can be used for mutagenesis in vitro or can be sequenced using the Promega T7 Promoter Primer or pUC/M13 Reverse Primer.

Promega vector sequences 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®-9Zf(–) Vector	20µg	P2391

The pGEM®-9Zf(–) 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®-9Zf(–) Vector at –20°C and the glycerol stock of JM109 cells at –70°C.

III. pGEM®-9Zf(–) Vector Multiple Cloning Region and Map

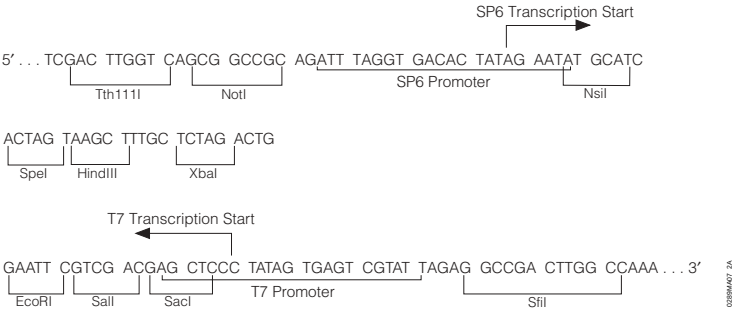

Figure 1. pGEM®-9Zf(–) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase and is complementary to RNA synthesized by T7 RNA polymerase. The strand shown is the same as the ssDNA strand produced by this vector.

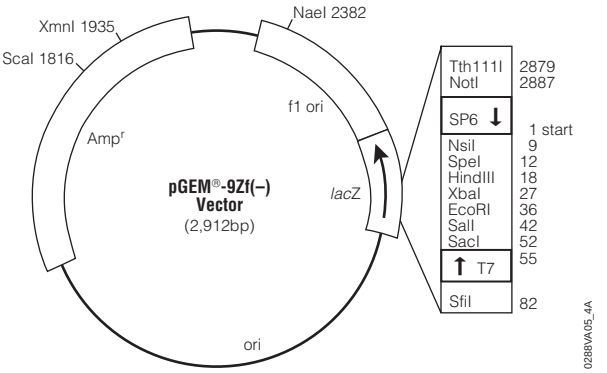

Figure 2. pGEM®-9Zf(–) Vector map and sequence reference points.

pGEM®-9Zf(–) Vector sequence reference points:

SP6 RNA polymerase transcription initiation site	1
T7 RNA polymerase promoter (–17 to +3)	53–72
T7 RNA polymerase transcription initiation site	55
lac operon sequences	93–321, 2712–2868
binding site of pUC / M13 Reverse Sequencing Primer	102–118
*lac*Z start codon	106
lac operator	126–142
β-lactamase (Amp^r) coding region	1263–2123
phage f1 region	2255–2710
binding site of pUC / M13 Forward Sequencing Primer	2831–2847
multiple cloning region	2876–87
SP6 RNA polymerase promoter (–17 to +3)	2896–3

Note: Use the T7 or pUC / M13 Reverse Primer to sequence ssDNA produced by the pGEM®-9Zf(–) Vector.

Specialized applications of the pGEM®-9Zf(–) Vector:

- Allows 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.)

IV. pGEM®-9Zf(–) 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 also available through GenBank® (GenBank®/EMBL Accession Number X65312) and on the Internet at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pGEM®-9Zf(–) Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AccI	1	43	**HaeII**	4	321, 691, 2330, 2338
AflIII	1	443			
Alw26I	2	1397, 2173	HgaI	4	554, 1132, 1862, 2263
Alw44I	2	757, 2003			
AlwNI	1	859	**HincII**	1	44
AvaII	2	1474, 1696	**HindIII**	1	18
BalI	1	85	**Hsp92I**	1	1873
BanI	3	187, 1284, 2444	**NaeI**	1	2382
BanII	2	52, 2414	**NciI**	3	823, 1519, 1870
BglI	3	82, 1456, 2723	**NgoMIV**	1	2380
BsaI	1	1397	**NotI**	1	2887
BsaAI	1	2485	**NsiI**	1	9
BsaJI	3	182, 603, 2826	NspI	1	447
Bsp1286I	5	52, 761, 1922, 2007, 2414	**PvuI**	2	1706, 2751
			PvuII	2	267, 2780
BspHI	2	1163, 2171	**RsaI**	1	1816
BssSI	2	616, 2000	**SacI**	1	52
BstOI	5	183, 471, 592, 605, 2827	**SalI**	1	42
			ScaI	1	1816
BstZI	1	2887	SfaNI	5	16, 540, 1592, 1783, 2032
DdeI	4	718, 1127, 1293, 1833	**SfiI**	1	82
DraI	3	1202, 1221, 1913	**SinI**	2	1474, 1696
DraIII	1	2488	**SpeI**	1	12
DrdI	2	551, 2532	**SspI**	2	2140, 2693
EaeI	5	83, 282, 1724, 2860, 2887	**TaqI**	4	43, 543, 1987, 2450
EarI	3	327, 2131, 2768	TfiI	2	278, 418
EclHKI	1	1336	**Tth111I**	1	2879
EcoICRI	1	50	**VspI**	3	214, 273, 1508
EcoRI	1	36	**XbaI**	1	27
FokI	4	1302, 1483, 1770, 2806	**XmnI**	1	1935
FspI	2	1558, 2730			

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-9Zf(–) Vector.

AatII	**BclI**	**Csp45I**	**NdeI**	**SnaBI**
Acc65I	**BglII**	**CspI**	**NheI**	**SphI**
AccB7I	BlpI	**Eco47III**	**NruI**	**StuI**
AccIII	BsaBI	EcoNI	PacI	**StyI**
AgeI	**BsaMI**	**EcoRV**	PmeI	SwaI
ApaI	BspMI	FseI	PmlI	XcmI
AscI	BsrGI	**HpaI**	PpuMI	**XhoI**
AvaI	**BssHII**	**I-PpoI**	PshAI	**XmaI**
AvrII	**Bst98I**	KasI	**PstI**	
BamHI	**BstEII**	**KpnI**	**SacII**	
BbeI	**BstXI**	**MluI**	**SgfI**	
BbsI	**Bsu36I**	**NarI**	SgrAI	
BbuI	**ClaI**	**NcoI**	**SmaI**	

Table 3. Restriction Enzymes That Cut the pGEM®-9Zf(–) Vector 6 or More Times.

AciI	**DpnI**	HphI	**MspA1I**	Sau96I
AluI	Fnu4HI	**Hsp92II**	**MspI**	ScrFI
BbvI	**HaeIII**	MaeIII	**NdeII**	**Tru9I**
BsrSI	**HhaI**	**MboI**	NlaIV	**XhoII**
BstUI	**HinfI**	**MboII**	PleI	
CfoI	**HpaII**	MnlI	**Sau3AI**	

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®-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 the vector and are not competent.

Product	Size	Cat.#
pSP64 Poly(A) Vector	20µg	P1241
pSP72 Vector	20µg	P2191
pSP73 Vector	20µg	P2221

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

VI. Reference

1. Yanish-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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