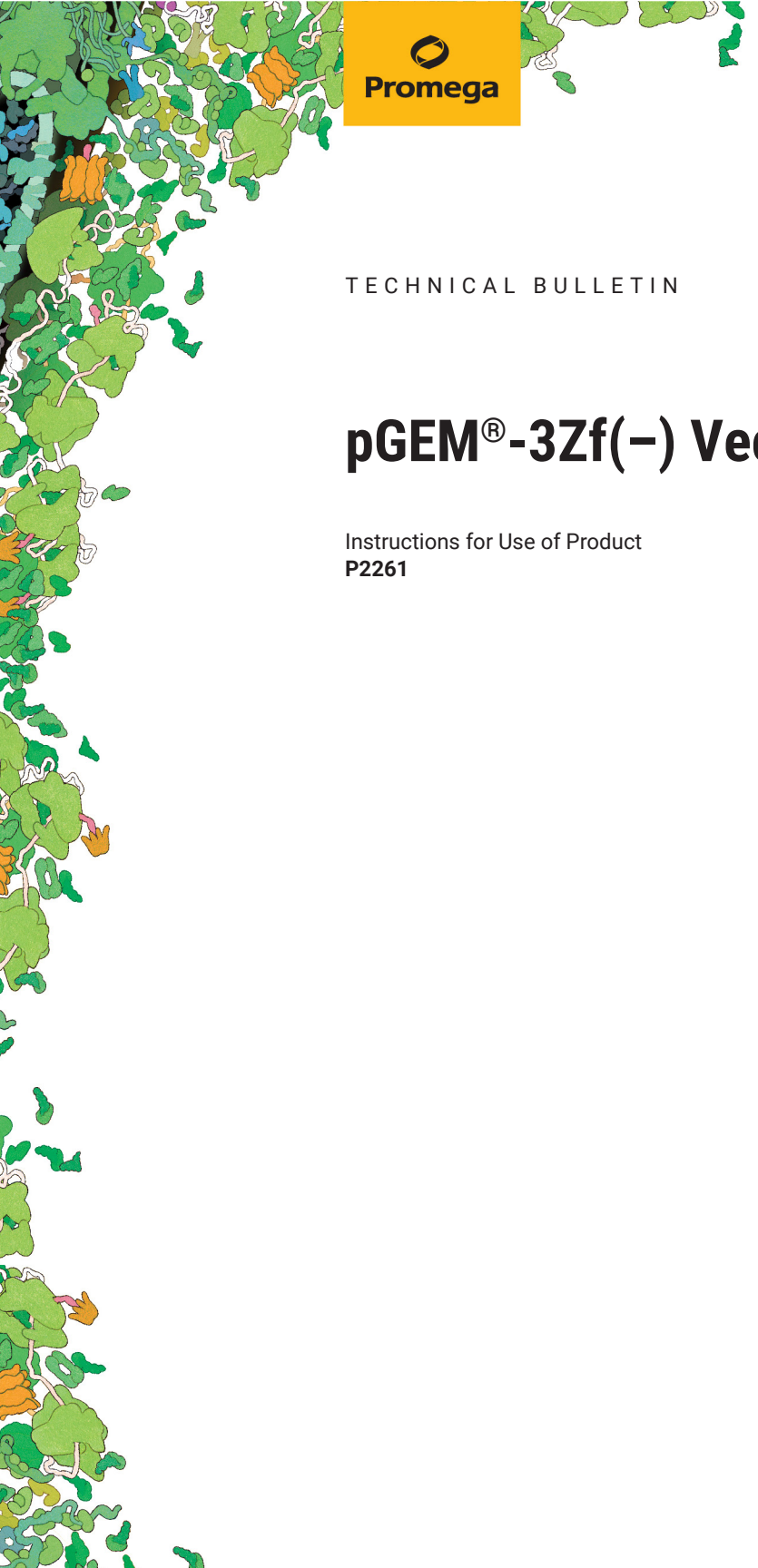

TECHNICAL BULLETIN

pGEM®-3Zf(–) Vector

Instructions for Use of Product
P2261

Revised 2/23
TB045

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

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

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
pGEM®-3Zf(–) Vector	**20µg**	**P2261**

Storage Conditions: Store the pGEM®-3Zf(–) Vector at –30°C to –10°C.

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

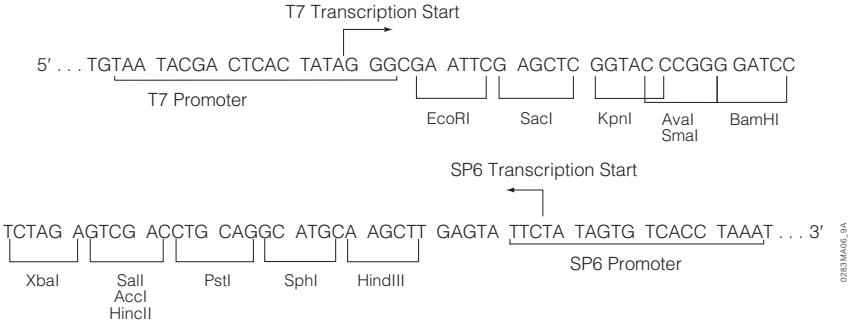

Figure 1. pGEM®-3Zf(–) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase.

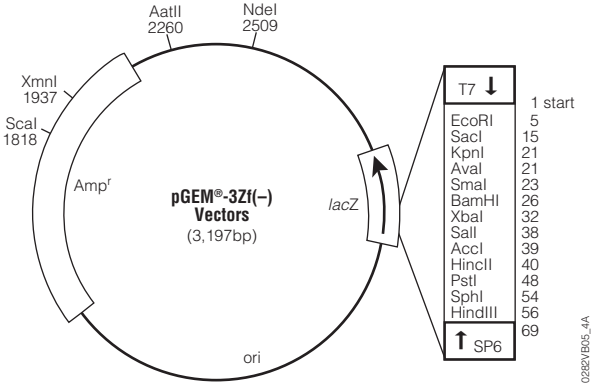

Figure 2. pGEM®-3Zf(–) Vector circle map.

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
lac operon sequences	94–323; 3018–3178
*lac*Z start codon	108
lac operator	128–144
β-lactamase (Amp®) 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 or **techserv@promega.com**

Vector sequences are available in the GenBank® database (GenBank®/EMBL Accession Number X65307) and on the Internet at: **www.promega.com/vectors/**

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

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

Enzyme	# of Sites	Location
AatII	1	2260
AccI	1	39
Acc65I	1	17
AcyI 2	2	1875, 2257
AflIII	1	445
Alw26I	4	1399, 2175, 2328, 2370
Alw44I	3	759, 2005, 2502
AlwNI	1	861
AspHI	5	15, 763, 1924, 2009, 2506
AvaI	1	21
AvaII	2	1476, 1698
BamHI	1	26
BanI	4	17, 189, 1286, 2751
BanII	2	15, 2721
BbuI	1	54
BglI	2	1458, 3030
Bsa I	1	1399
BsaAI	1	2792
BsaHI	2	1875, 2257
BsaJI	5	21, 22, 184, 605, 3133
BsaOI	5	361, 785, 1708, 1857, 3058
BspHI	3	1165, 2173, 2278
BspMI	1	51
BssSI	3	618, 2002, 2309

Enzyme	# of Sites	Location
BstOI	5	185, 473, 594, 607, 3134
Cfr10I	2	1418, 2687
DraI	3	1204, 1223, 1915
DraII	1	2314
DraIII	1	2795
DrdI	3	553, 2422, 2839
EaeI	3	284, 1726, 3167
EarI	3	329, 2133, 3075
EclHKI	1	1338
EcoICRI	1	13
EcoRI	1	5
FokI	5	1304, 1485, 1772, 2415, 3113
FspI	2	1560, 3037
HaeII	4	323, 693, 2637, 2645
HgaI	5	556, 1134, 1864, 2422, 2570
HincII	1	40
HindII	1	40
HindIII	1	56
Hsp92I	2	1875, 2257
KpnI	1	21
MaeI	5	33, 940, 1193, 1528, 2639
NaeI	1	2689
NdeI	1	2509
NgoMIV	1	2687
NspI	3	54, 449, 2366

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

Enzyme	# of Sites	Location
PspAI	1	21
PstI	1	48
PvuI	2	1708, 3058
PvuII	2	269, 3087
RsaI	3	19, 1818, 2494
SacI	1	15
SalI	1	38
ScaI	1	1818
SinI	2	1476, 1698
SmaI	1	23

Enzyme	# of Sites	Location
SphI	1	54
Sse8387I	1	48
SspI	2	2142, 3000
TaqI	5	9, 39, 545, 1989, 2757
TfiI	2	280, 420
VspI	3	216, 275, 1510
XbaI	1	32
XmaI	1	21
XmnI	1	1937

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

AccIII	BlpI	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	
BglI	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	MspA1I	ScrFI
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®-7Zf(+) Vector	20µg	P2251

For Research Use Only. Not for Use in Diagnostic Procedures.

Other Vectors

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

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Sequencing Primer

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011

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In Vitro Transcription Systems

Product	Cat.#
Riboprobe® System—SP6	P1420
Riboprobe® System—T3	P1430
Riboprobe® System—T7	P1440
RiboMAX™ Large Scale RNA Production Systems, SP6	P1280
RiboMAX™ Large Scale RNA Production Systems T7	P1300
T7 RiboMAX™ Express Large Scale RNA Production System	P1320

For Research Use Only. Not for Use in Diagnostic Procedures.

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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6. Summary of Changes

The following changes were made to the 2/23 revision of this document:

1. Removed JM109 cells due to discontinuation.
2. Updated Related Products.
3. Updated font and cover image.

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