

# pGEM<sup>®</sup>-4Z Vector



Technical Bulletin No. 036

INSTRUCTIONS FOR USE OF PRODUCT P2161. PLEASE DISCARD PREVIOUS VERSIONS.

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## I. Description

The pGEM<sup>®</sup>-4Z Vector<sup>(a)</sup> is intended for use as a standard cloning vector, as well as for the highly efficient synthesis of RNA in vitro. The vector carries the *lacZ*  $\alpha$ -peptide and multiple cloning region arrangement from pUC18 (1). In addition, the vector contains both the SP6 and T7 RNA polymerase promoters flanking the multiple cloning region. This arrangement gives rise to a functional  $\alpha$ -peptide that is capable of complementing the product of the *lacZ* $\Delta$ M15 gene to produce functional  $\beta$ -galactosidase. Cells with the genotype, *lacZ* $\Delta$ M15, and also containing the pGEM<sup>®</sup>-4Z Vector will be blue in color when plated on indicator media containing IPTG and X-Gal.

However, when the *lacZ*  $\alpha$ -peptide is disrupted by cloning into the pGEM<sup>®</sup>-4Z multiple cloning region, complementation does not occur and no  $\beta$ -galactosidase activity is produced. Therefore, bacterial colonies harboring recombinant pGEM<sup>®</sup>-4Z Vector constructs remain white.

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

## II. Product Components

Product	Size	Cat.#
pGEM <sup>®</sup> -4Z Vector	20 $\mu$ g	P2161

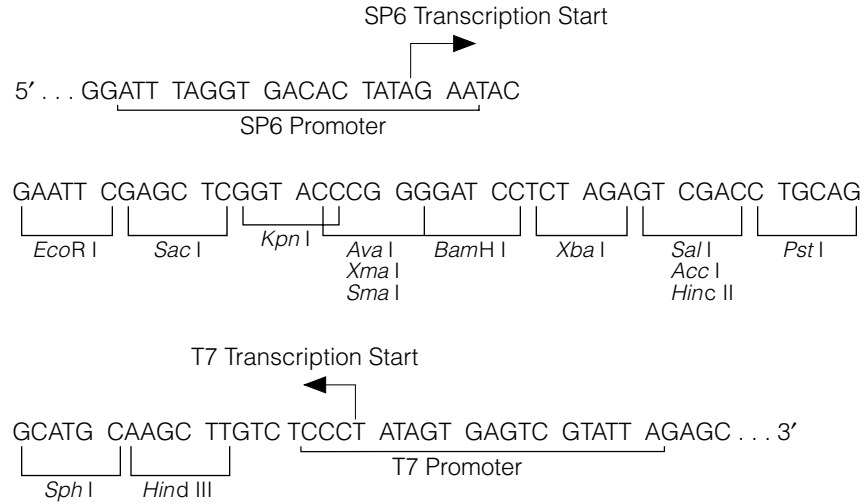
The pGEM<sup>®</sup>-4Z Vector is provided with a glycerol stock of bacterial strain JM109.

**Storage Conditions:** Store the pGEM<sup>®</sup>-4Z Vector at  $-20^{\circ}\text{C}$  and the glycerol stock of JM109 cells at  $-70^{\circ}\text{C}$ .

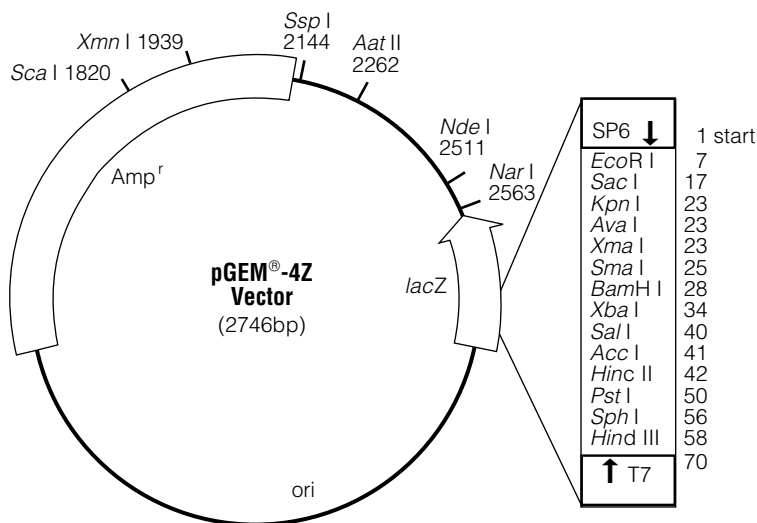


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### III. pGEM<sup>®</sup>-4Z Vector Multiple Cloning Region and Circle Map



**Figure 1. pGEM<sup>®</sup>-4Z 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.



**Note:** The pGEM®-3Z and pGEM®-4Z Vectors are identical except for the orientation of the SP6 and T7 promoters.

**Figure 2. pGEM®-4Z Vector circle map and sequence reference points.**

**pGEM®-4Z Vector sequence reference points.**

SP6 RNA polymerase transcription initiation site	1
T7 RNA polymerase transcription initiation site	70
SP6 RNA polymerase promoter (-17 to +3)	2730-3
T7 RNA polymerase promoter (-17 to +3)	68-87
multiple cloning region	7-63
<i>lacZ</i> start codon	110
<i>lac</i> operon sequences	2563-2726; 96-325
<i>lacZ</i> operator	130-146
β-lactamase ( <i>Amp<sup>r</sup></i> ) coding region	1267-2127
binding site of pUC/M13 Forward Sequencing Primer	2686-2702
binding site of pUC/M13 Reverse Sequencing Primer	106-122

**Specialized applications of the pGEM®-4Z Vector.**

- Blue/white screening for recombinants.
- Transcription in vitro from dual opposed promoters. (For protocol information, please request Promega's *Riboprobe® in vitro Transcription Systems*<sup>(b,c)</sup> *Technical Manual*, #TM016.)

**Note:** All Promega technical literature is available on the Internet at [www.promega.com](http://www.promega.com).

#### IV. pGEM<sup>®</sup>-4Z Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR<sup>®</sup> 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 in the GenBank<sup>®</sup> database (GenBank<sup>®</sup>/EMBL Accession Number X65305) and on the Internet at [www.promega.com/vectors/](http://www.promega.com/vectors/).

**Table 1. Restriction Enzymes That Cut the pGEM<sup>®</sup>-4Z Vector Between 1 and 5 Times.**

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
<b>Aat II</b>	1	2262	<b>EcoR I</b>	1	7
<b>Acc I</b>	1	41	<i>Ehe I</i>	1	2564
<b>Acc65 I</b>	1	19	<b>Fok I</b>	5	1306, 1487, 1774, 2417, 2661
<b>Acy I</b>	3	1877, 2259, 2563	<i>Fsp I</i>	2	1562, 2585
<i>Afl III</i>	1	447	<b>Hae II</b>	3	325, 695, 2566
<b>Alw26 I</b>	5	69, 1401, 2177, 2330, 2372	<i>Hga I</i>	4	558, 1136, 1866, 2424
<b>Alw44 I</b>	3	761, 2007, 2504	<b>Hinc II</b>	1	42
<i>AlwN I</i>	1	863	<i>Hind II</i>	1	42
<i>AspH I</i>	5	17, 765, 1926, 2011, 2508	<b>Hind III</b>	1	58
<b>Ava I</b>	1	23	<b>Hsp92 I</b>	3	1877, 2259, 2563
<b>Ava II</b>	2	1478, 1700	<i>Kas I</i>	1	2562
<b>BamH I</b>	1	28	<b>Kpn I</b>	1	23
<b>Ban I</b>	4	19, 191, 1288, 2562	<i>Mae I</i>	4	35, 942, 1195, 1530
<b>Ban II</b>	1	17	<i>Mae II</i>	5	1150, 1566, 1939, 2259, 2701
<i>Bbe I</i>	1	2566	<b>Nar I</b>	1	2563
<b>Bbu I</b>	1	56	<b>Nde I</b>	1	2511
<b>Bgl I</b>	2	1460, 2578	<i>Nsp I</i>	3	56, 451, 2368
<i>Bsa I</i>	1	1401	<i>Ple I</i>	5	46, 85, 341, 826, 1329
<b>BsaO I</b>	5	363, 787, 1710, 1859, 2606	<i>PspA I</i>	1	23
<i>BsaH I</i>	3	1877, 2259, 2563	<b>Pst I</b>	1	50
<i>BsaJ I</i>	5	23, 24, 186, 607, 2681	<b>Pvu I</b>	2	1710, 2606
<b>Bsp1286 I</b>	5	17, 765, 1926, 2011, 2508	<b>Pvu II</b>	2	271, 2635
<i>BspH I</i>	3	1167, 2175, 2280	<b>Rsa I</b>	3	21, 1820, 2496
<i>BspM I</i>	1	53	<b>Sac I</b>	1	17
<i>BssS I</i>	3	620, 2004, 2311	<b>Sal I</b>	1	40
<b>BstO I</b>	5	187, 475, 596, 609, 2682	<b>Sca I</b>	1	1820
<b>BstX I</b>	1	2725	<b>Sin I</b>	2	1478, 1700
<i>Cfr10 I</i>	1	1420	<b>Sma I</b>	1	25
<b>Dra I</b>	3	1206, 1225, 1917	<b>Sph I</b>	1	56
<i>Dra II</i>	1	2316	<i>Sse8387 I</i>	1	50
<i>Drd I</i>	2	555, 2424	<b>Ssp I</b>	1	2144
<i>Eae I</i>	3	286, 1728, 2715	<b>Taq I</b>	4	11, 41, 547, 1991
<i>Ear I</i>	3	331, 2135, 2623	<i>Tfi I</i>	2	282, 422
<b>EclHK I</b>	1	1340	<b>Vsp I</b>	3	218, 277, 1512
<b>EcoCR I</b>	1	15	<b>Xba I</b>	1	34
			<b>Xma I</b>	1	23
			<b>Xmn I</b>	1	1939

**Note:** The enzymes listed in boldface are available from Promega.

**Table 2. Restriction Enzymes That Do Not Cut the pGEM®-4Z Vector.**

<b>AccB7 I</b>	<i>Bpu1102 I</i>	<b>Bsu36 I</b>	<i>Fse I</i>	<i>PfiM I</i>	<b>SnaB I</b>
<b>Acc III</b>	<i>BsaA I</i>	<b>Cla I</b>	<b>Hpa I</b>	<i>PinA I</i>	<b>Spe I</b>
<i>Afl II</i>	<i>BsaB I</i>	<b>Csp I</b>	<b>I-Ppo I</b>	<i>Pme I</i>	<i>Spl I</i>
<b>Age I</b>	<b>BsaM I</b>	<b>Csp45 I</b>	<b>Mlu I</b>	<i>Pml I</i>	<i>Srf I</i>
<b>Apa I</b>	<i>Bsm I</i>	<i>Dra III</i>	<b>Nae I</b>	<i>Ppu10 I</i>	<b>Stu I</b>
<i>Asc I</i>	<i>Bsp120 I</i>	<i>Dsa I</i>	<b>Nco I</b>	<i>PpuM I</i>	<b>Sty I</b>
<i>Avr II</i>	<b>BsrBR I</b>	<i>Eag I</i>	<b>NgoM IV</b>	<i>PshA I</i>	<i>Swa I</i>
<b>Bal I</b>	<i>BsrG I</i>	<b>Eco47 III</b>	<b>Nhe I</b>	<i>Psp5 II</i>	<b>Tth111 I</b>
<i>BbrP I</i>	<b>BssH II</b>	<b>Eco52 I</b>	<b>Not I</b>	<i>Rsr II</i>	<i>Xcm I</i>
<i>Bbs I</i>	<i>Bst1107 I</i>	<i>Eco72 I</i>	<b>Nru I</b>	<b>Sac II</b>	<b>Xho I</b>
<b>Bcl I</b>	<b>Bst98 I</b>	<i>Eco81 I</i>	<b>Nsi I</b>	<b>Sfi I</b>	
<b>Bgl II</b>	<b>BstE II</b>	<i>EcoN I</i>	<i>Pac I</i>	<b>Sgf I<sup>(d)</sup></b>	
<i>Blp I</i>	<b>BstZ I</b>	<b>EcoR V</b>	<i>PaeR7 I</i>	<i>SgrA I</i>	

**Table 3. Restriction Enzymes That Cut the pGEM®-4Z Vector 6 or More Times.**

<i>Aci I</i>	<b>Cfo I</b>	<b>Hinf I</b>	<i>Mnl I</i>	<i>Nla IV</i>
<b>Alu I</b>	<b>Dde I</b>	<b>Hpa II</b>	<i>Mse I</i>	<b>Sau3A I</b>
<i>BbvZ I</i>	<b>Dpn I</b>	<i>Hph I</i>	<b>Msp I</b>	<b>Sau96 I</b>
<i>Bsr I</i>	<i>Dpn II</i>	<b>Hsp92 II</b>	<b>MspA1 I</b>	<i>ScrF I</i>
<b>BsrS I</b>	<i>Fnu4H I</i>	<i>Mae III</i>	<b>Nci I</b>	<i>SfaN I</i>
<b>Bst71 I</b>	<b>Hae III</b>	<b>Mbo I</b>	<b>Nde II</b>	<b>Tru9 I</b>
<i>BstU I</i>	<b>Hha I</b>	<b>Mbo II</b>	<i>Nla III</i>	<b>Xho II</b>

**Note:** The enzymes listed in boldface are available from Promega.

## V. pGEM®-4Z Vector Sequence

The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase and is complementary to RNA synthesized by T7 RNA polymerase. The GenBank®/EMBL Accession Number is X65305.

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1  GAATACGAAT TCGAGCTCGG TACCCGGGGA TCCTCTAGAG TCGACCTGCA
51  GGCATGCAAG CTTGTCTCCC TATAGTGAGT CGTATTAGAG CTTGGCGTAA
101 TCATGGTCAT AGCTGTTTCC TG'TGTGAAAT TGTTATCCGC TCACAATTCC
151 ACACAACATA CGAGCCGGAA GCATAAAGTG TAAAGCCTGG GGTGCCTAAT
201 GAGTGAGCTA ACTCACATTA ATTGCGTTGC GCTCACTGCC CGCTTTCAG
251 TCGGGAAACC TGTCGTGCCA GCTGCATTAA TGAATCGGCC AACGCGCGGG
301 GAGAGGCGGT TTGCGTATTG GGCCTCTTTC CGCTTCCTCG CTCACTGACT
351 CGCTGCGCTC GGTCGTTCCG CTGCGGCGAG CGGTATCAGC TCACTCAAAG
401 GCGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT
451 GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC
501 TGGCGTTTTT CCATAGGCTC CGCCCCCTG ACGAGCATCA CAAAAATCGA
551 CGCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA GATACCAGGC
601 GTTTCCCCTT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG ACCCTGCCGC
651 TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT
701 CATAGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG TTCGCTCAA

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751 GCTGGGCTGT GTGCACGAAC CCCCCTTCA GCCCGACCGC TGCGCCTTAT
801 CCGGTA ACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA CTTATCGCCA
851 CTGGCAGCAG CCACTGGTAA CAGGATTAGC AGAGCGAGGT ATGTAGGCGG
901 TGCTACAGAG TTCTTGAAGT GGTGGCCTAA CTACGGCTAC ACTAGAAGAA
951 CAGTATTTGG TATCTGCGCT CTGCTGAAGC CAGTTACCTT CGGAAAAAGA
1001 GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT
1051 TTTTGTGGC AAGCAGCAGA TTACGCGCAG AAAAAAAGGA TCTCAAGAAG
1101 ATCCTTTGAT CTTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAA ACTCA
1151 CGTTAAGGGA TTTTGGTCAT GAGATTATCA AAAAGGATCT TCACCTAGAT
1201 CCTTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT ATATATGAGT
1251 AAACCTGGTC TGACAGTTAC CAATGCTTAA TCAGTGAGGC ACCTATCTCA
1301 GCGATCTGTC TATTTCTGTT ATCCATAGTT GCCTGACTCC CCGTCGTGTA
1351 GATAACTACG ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA
1401 TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC AATAAAC CAG
1451 CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT CCTGCAACTT TATCCGCTC
1501 CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCAG
1551 TTAATAGTTT GCGCAACGTT GTTGCCATTG CTACAGGCAT CGTGGTGTCA
1601 CGCTCGTCGT TTGGTATGGC TTCATT CAGC TCCGGTTCCC AACGATCAAG
1651 GCGAGTTACA TGATCCCCCA TGTTGTGCAA AAAAGCGGTT AGCTCCTTCG
1701 GTCCTCCGAT CGTTGT CAGA AGTAAGTTGG CCGCAGTGTT ATCACTCATG
1751 GTTATGGCAG CACTGCATAA TTCTCTTACT GTCATGCCAT CCGTAAGATG
1801 CTTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA
1851 TGCGGCGACC GAGTTGCTCT TGCCCGGCGT CAATACGGGA TAATACCGCG
1901 CCACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC GTTCTTCGGG
1951 GCGAAA ACTC TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC
2001 CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT CACCAGCGTT
2051 TCTGGGTGAG CAAAAACAGG AAGGCCAAAAT GCCGAAAAA AGGGAATAAG
2101 GGCGACACGG AAATGTTGAA TACTCATACT CTTCTTTTTT CAATATTATT
2151 GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAATGT
2201 ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTC CCCGAAAAGT
2251 GCCACCTGAC GTCTAAGAAA CCATTATTAT CATGACATTA ACCTATAAAA
2301 ATAGGCGTAT CACGAGGCC TTTCTGCTCG CGCGTTTCGG TGATGACGGT
2351 GAAAACCTCT GACACATGCA GCTCCCGGAG ACGGTCACAG CTTGTCTGTA
2401 AGCGGATGCC GGGAGCAGAC AAGCCCGTCA GGGCGCGTCA GCGGGTGTG
2451 GCGGGTGTG GGGCTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG
2501 AGAGTGCACC ATATGCGGTG TGA AATACCG CACAGATGCG TAAGGAGAAA
2551 ATACCGCATC AGGCGCCATT CGCCATT CAG GCTGCGCAAC TGTTGGGAAG
2601 GGCGATCGGT GCGGGCTCT TCGCTATTAC GCCAGCTGGC GAAAGGGGGA
2651 TGTGCTGCAA GGCGATTAAG TTGGGTAACG CCAGGGTTTT CCCAGTCACG
2701 ACGTTGTAAA ACGACGGCCA GTGAATTGGA TTTAGGTGAC ACTATA

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## VI. Related Products

Product	Size	Cat.#
pGEM <sup>®</sup> -3Z Vector(a)	20µg	P2151
pGEM <sup>®</sup> -3Zf(+) Vector(a)	20µg	P2271
pGEM <sup>®</sup> -3Zf(-) Vector(a)	20µg	P2261
pGEM <sup>®</sup> -5Zf(+) Vector(a)	20µg	P2241
pGEM <sup>®</sup> -5Zf(-) Vector(a)	20µg	P2351
pGEM <sup>®</sup> -7Zf(+) Vector(a)	20µg	P2251
pGEM <sup>®</sup> -7Zf(-) Vector(a)	20µg	P2371
pGEM <sup>®</sup> -9Zf(-) Vector(a)	20µg	P2391
pGEM <sup>®</sup> -11Zf(+) Vector(a)	20µg	P2411
pGEM <sup>®</sup> -11Zf(-) Vector(a)	20µg	P2421
pGEM <sup>®</sup> -13Zf(+) Vector(a)	20µg	P2541

All pGEM<sup>®</sup> 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(a)	20µg	P2191
pSP73 Vector(a)	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<sup>®</sup> in vitro Transcription Systems

Product	Cat.#
Riboprobe <sup>®</sup> System - SP6(b,c)	P1420
Riboprobe <sup>®</sup> System - T3(b,c)	P1430
Riboprobe <sup>®</sup> System - T7(b,c)	P1440

### RiboMAX<sup>™</sup> Large Scale RNA Production Systems

Product	Cat.#
RiboMAX <sup>™</sup> Large Scale RNA Production System - SP6(b,c,e)	P1280
RiboMAX <sup>™</sup> Large Scale RNA Production System - T3(b,c,e,f)	P1290
RiboMAX <sup>™</sup> Large Scale RNA Production System - T7(b,c,e,f)	P1300

## VII. 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.



- (a) U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.
- (b) U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217 and Australian Pat. No. 646803 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.
- (c) U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.
- (d) U.S. Pat. No. 5,391,487 has been issued to Promega Corporation for Restriction Endonuclease *Sgf I*.
- (e) The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.
- (f) The RiboMAX™ Large Scale RNA Production Systems - T7 and T3 (Cat.# P1290 and P1300) are covered by U.S. Pat. No. 5,256,555 and are sold under a license from Ambion, Inc. They are intended for research use only. Parties wishing to use these products for other applications should contact Ambion, Inc.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.



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