

# pGEM<sup>®</sup>-3Zf(+)<sup>™</sup> Vector

Technical Bulletin No. 086

INSTRUCTIONS FOR USE OF PRODUCT P2271. PLEASE DISCARD PREVIOUS VERSIONS.  
All Technical Literature is Available on the Internet at [www.promega.com](http://www.promega.com)

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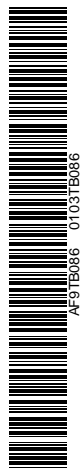
## I. Description

The pGEM<sup>®</sup>-3Zf(+)<sup>™</sup> Vector<sup>(a)</sup> is a derivative of the pGEM<sup>®</sup>-3Z Vector and contains the origin of replication of the filamentous phage f1. The plasmid serves as a standard cloning vector, as a template for in vitro transcription and as a template for the production of circular ssDNA.

The pGEM<sup>®</sup>-3Zf(+)<sup>™</sup> Vector contains SP6 and T7 RNA polymerase promoters flanking a multiple cloning site within the  $\alpha$ -peptide coding region of  $\beta$ -galactosidase (1). Insertional inactivation of the  $\alpha$ -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 *Eco*R I, *Sac* I, *Kpn* I, *Ava* I, *Sma* I, *Bam*H I, *Xba* I, *Sal* I, *Acc* I, *Hinc* II, *Pst* I, *Sph* I, and *Hind* III.

For induction of ssDNA, bacterial cells with the F' episome (e.g., JM109, XL-1 Blue, DH5 $\alpha$ <sup>®</sup>) containing pGEM<sup>®</sup>-3Zf(+)<sup>™</sup> Vector 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 mutagenesis in vitro or can be sequenced using Promega's T7 Promoter Primer (Cat.# Q5021) or pUC/M13 Forward Primer (Cat.# Q5601).

The sequences of Promega's Vectors are available online at [www.promega.com/vectors](http://www.promega.com/vectors) and are also available from the GenBank<sup>®</sup> database. The GenBank<sup>®</sup> accession number is X65306.



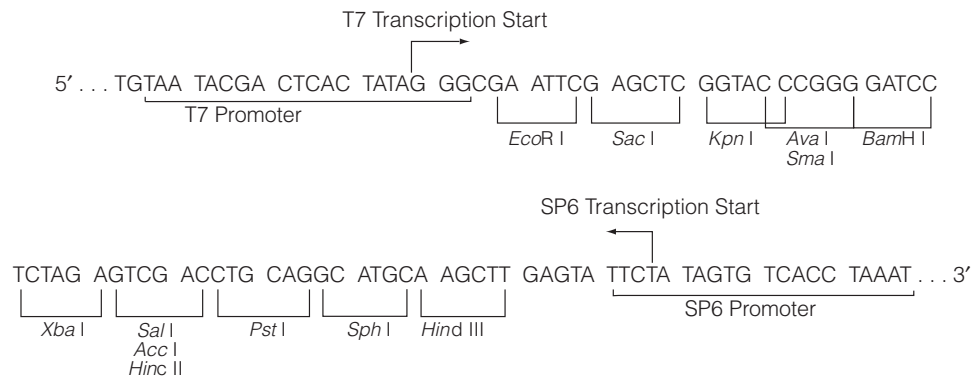
## II. Product Components

Product	Size	Cat.#
pGEM <sup>®</sup> -3Zf(+) Vector	20µg	P2271

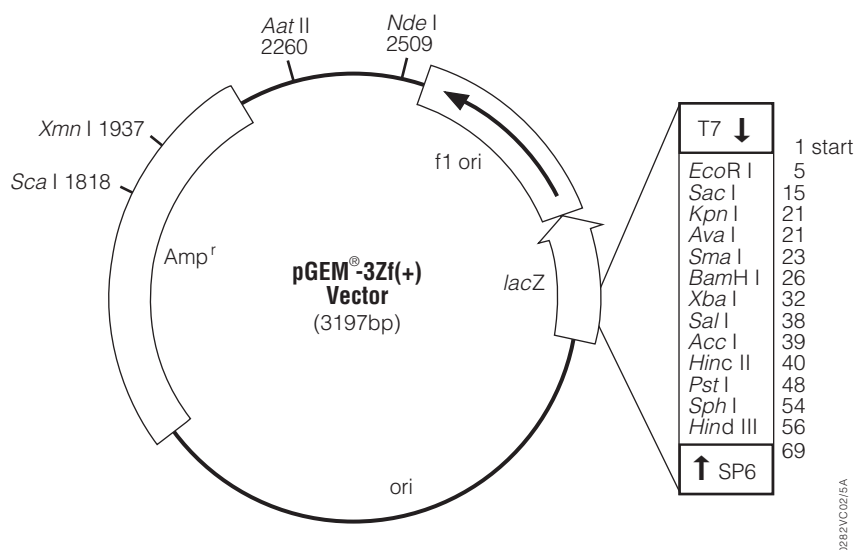
The pGEM<sup>®</sup>-3Zf(+) Vector is provided with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain vector and are not competent cells.

**Storage Conditions:** Store the pGEM<sup>®</sup>-3Zf(+) Vector at -20°C and the glycerol stock of JM109 cells at -70°C.

## III. Multiple Cloning Site and Circle Map



**Figure 1. pGEM<sup>®</sup>-3Zf(+) 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 complementary to the ssDNA strand produced by this vector.



**Figure 2. pGEM®-3Zf(+) Vector circle map and sequence reference points.**

**Sequence reference points:**

T7 RNA polymerase transcription initiation site	1
SP6 RNA polymerase transcription initiation site	69
T7 RNA polymerase promoter (-17 to +3)	3181-3
SP6 RNA polymerase promoter (-17 to +3)	67-86
multiple cloning region	5-61
<i>lacZ</i> start codon	108
<i>lac</i> operon sequences	3018-3178; 94-323
<i>lac</i> operator	128-144
$\beta$ -lactamase ( <i>Amp<sup>r</sup></i> ) coding region	1265-2125
phage f1 region	2562-3017
binding site of pUC/M13 Forward Sequencing Primer	3138-3154
binding site of pUC/M13 Reverse Sequencing Primer	104-120

**Specialized applications:**

- ssDNA production
- blue/white screening for recombinants
- transcription in vitro from dual opposed promoters (For protocol information, please request Promega's *Riboprobe*® in vitro *Transcription Systems Technical Manual*, #TM016.)



**USE the T7 Promoter Primer** (Cat.# Q5021) or **pUC/M13 Forward Primer** (Cat.# Q5601) to sequence ssDNA produced by the pGEM®-3Zf(+) Vector.

**Note:** The pGEM®-3Zf(+) and pGEM®-3Zf(-) Vectors are identical except for the orientation of the f1 origin.

#### IV. pGEM<sup>®</sup>-3Zf(+) 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 on the Internet at [www.promega.com/vectors](http://www.promega.com/vectors) and from GenBank<sup>®</sup> (GenBank<sup>®</sup>/EMBL Accession Number X65306).

**Table 1. Restriction Enzymes That Cut the pGEM<sup>®</sup>-3Zf(+) Vector Between 1 and 5 Times.**

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
<b>Aat II</b>	1	2260	<b>EcoR I</b>	1	5
<b>Acc I</b>	1	39	<b>Fok I</b>	5	1304, 1485, 1772, 2415, 3113
<b>Acc65 I</b>	1	17	<i>Fsp I</i>	2	1560, 3037
<b>Acy I</b>	2	1875, 2257	<b>Hae II</b>	4	323, 693, 2937, 2945
<i>Afl III</i>	1	445	<i>Hga I</i>	5	556, 1134, 1864, 2422, 3003
<b>Alw26 I</b>	4	1399, 2175, 2328, 2370	<b>Hinc II</b>	1	40
<b>Alw44 I</b>	3	759, 2005, 2502	<i>Hind II</i>	1	40
<i>AlwN I</i>	1	861	<b>Hind III</b>	1	56
<i>AspH I</i>	5	15, 763, 1924, 2009, 2506	<b>Hsp92 I</b>	2	1875, 2257
<b>Ava I</b>	1	21	<b>Kpn I</b>	1	21
<b>Ava II</b>	2	1476, 1698	<i>Mae I</i>	5	33, 940, 1193, 1528, 2937
<b>BamH I</b>	1	26	<b>Nae I</b>	1	2889
<b>Ban I</b>	4	17, 189, 1286, 2823	<b>Nde I</b>	1	2509
<b>Ban II</b>	2	15, 2861	<b>NgoM IV</b>	1	2887
<b>Bbu I</b>	1	54	<i>Nsp I</i>	3	54, 449, 2366
<b>Bgl I</b>	2	1458, 3030	<i>PspA I</i>	1	21
<i>Bsa I</i>	1	1399	<b>Pst I</b>	1	48
<b>BsaO I</b>	5	361, 785, 1708, 1857, 3058	<b>Pvu I</b>	2	1708, 3058
<i>BsaA I</i>	1	2786	<b>Pvu II</b>	2	269, 3087
<i>BsaH I</i>	2	1875, 2257	<b>Rsa I</b>	3	19, 1818, 2494
<i>BsaJ I</i>	5	21, 22, 184, 605, 3133	<b>Sac I</b>	1	15
<i>BspH I</i>	3	1165, 2173, 2278	<b>Sal I</b>	1	38
<i>BspM I</i>	1	51	<b>Sca I</b>	1	1818
<i>BssS I</i>	3	618, 2002, 2309	<b>Sin I</b>	2	1476, 1698
<b>BstO I</b>	5	185, 473, 594, 607, 3134	<b>Sma I</b>	1	23
<i>Cfr10 I</i>	2	1418, 2887	<b>Sph I</b>	1	54
<b>Dra I</b>	3	1204, 1223, 1915	<i>Sse8387 I</i>	1	48
<i>Dra II</i>	1	2314	<b>Ssp I</b>	2	2142, 2578
<i>Dra III</i>	1	2786	<b>Taq I</b>	5	9, 39, 545, 1989, 2819
<i>Drd I</i>	3	553, 2422, 2741	<i>Tfi I</i>	2	280, 420
<i>Eae I</i>	3	284, 1726, 3167	<b>Vsp I</b>	3	216, 275, 1510
<i>Ear I</i>	3	329, 2133, 3075	<b>Xba I</b>	1	32
<b>Ecl/HK I</b>	1	1338	<b>Xma I</b>	1	21
<b>EcoICR I</b>	1	13	<b>Xmn I</b>	1	1937

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

**Table 2. Restriction Enzymes That Do Not Cut the pGEM<sup>®</sup>-3Zf(+) Vector.**

<b>Acc B7 I</b>	<i>Blp I</i>	<b>BstZ I</b>	<i>Ehe I</i>	<i>Pac I</i>	<i>SgrA I</i>
<b>Acc III</b>	<i>Bpu1102 I</i>	<b>Bsu36 I</b>	<i>Fse I</i>	<i>PfiM I</i>	<b>SnaB I</b>
<i>Afl II</i>	<i>BsaB I</i>	<b>Cla I</b>	<b>Hpa I</b>	<i>PinA I</i>	<b>Spe I</b>
<b>Age I</b>	<b>BsaM I</b>	<b>Csp I</b>	<b>I-Ppo I</b>	<i>Pme I</i>	<i>Spl I</i>
<b>Apa I</b>	<i>Bsm I</i>	<b>Csp45 I</b>	<i>Kas I</i>	<i>Pml I</i>	<i>Srf I</i>
<i>Asc I</i>	<i>Bsp120 I</i>	<i>Dsa I</i>	<b>Mlu I</b>	<i>Ppu10 I</i>	<b>Stu I</b>
<i>Avr II</i>	<b>BsrBR I</b>	<i>Eag I</i>	<b>Nar I</b>	<i>PpuM I</i>	<b>Sty I</b>
<b>Bal I</b>	<i>BsrG I</i>	<b>Eco47 III</b>	<b>Nco I</b>	<i>PshA I</i>	<i>Swa I</i>
<i>Bbe I</i>	<b>BssH II</b>	<b>Eco52 I</b>	<b>Nhe I</b>	<i>Psp5 II</i>	<b>Tth111 I</b>
<i>BbrP I</i>	<i>Bst1107 I</i>	<i>Eco72 I</i>	<b>Not I</b>	<i>Rsr II</i>	<i>Xcm I</i>
<i>Bbs I</i>	<b>Bst98 I</b>	<i>Eco81 I</i>	<b>Nru I</b>	<b>Sac II</b>	<b>Xho I</b>
<b>Bcl I</b>	<b>BstE II</b>	<i>EcoN I</i>	<b>Nsi I</b>	<b>Sfi I</b>	
<b>Bgl II</b>	<b>BstX I</b>	<b>EcoR V</b>	<i>PaeR7 I</i>	<b>Sgf I</b>	

**Table 3. Restriction Enzymes That Cut the pGEM<sup>®</sup>-3Zf(+) Vector 6 or More Times.**

<i>Aci I</i>	<i>BstU I</i>	<b>Hha I</b>	<b>Mbo I</b>	<b>Nde II</b>	<i>SfaN I</i>
<b>Alu I</b>	<b>Cfo I</b>	<b>Hinf I</b>	<b>Mbo II</b>	<i>Nla III</i>	<b>Tru9 I</b>
<i>Bbv I</i>	<b>Dde I</b>	<b>Hpa II</b>	<i>Mnl I</i>	<i>Nla IV</i>	<b>Xho II</b>
<b>Bsp1286 I</b>	<b>Dpn I</b>	<i>Hph I</i>	<i>Mse I</i>	<i>Ple I</i>	
<i>Bsr I</i>	<i>Dpn II</i>	<b>Hsp92 II</b>	<b>Msp I</b>	<b>Sau3A I</b>	
<b>BsrS I</b>	<i>Fnu4H I</i>	<i>Mae II</i>	<b>MspA1 I</b>	<b>Sau96 I</b>	
<b>Bst71 I</b>	<b>Hae III</b>	<i>Mae III</i>	<b>Nci I</b>	<i>ScrF I</i>	

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

## V. pGEM<sup>®</sup>-3Zf(+) Vector 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 strand produced by this vector. The GenBank/EMBL Accession Number is X65306.

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1  GGGCGAATTC GAGCTCGGTA CCCGGGGATC CTCTAGAGTC GACCTGCAGG
51  CATGCAAGCT TGAGTATTCT ATAGTGTAC CTAATAGCT TGGCGTAATC
101 ATGGTCATAG CTGTTTCTG TGTGAAATTG TTATCCGCTC ACAATTCCAC
151 ACAACATACG AGCCGGAAGC ATAAAGTGTA AAGCCTGGGG TGCCTAATGA
201 GTGAGCTAAC TCACATTAAT TGC GTTGC GC TCACTGCCCG CTTTCCAGTC
251 GGGAAACCTG TCGTGCCAGC TGCATTAATG AATCGGCCAA CGCGCGGGGA
301 GAGGCGGTTT GCGTATTGGG CGCTCTTCCG CTTCTCGCT CACTGACTCG
351 CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC
401 GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT
451 GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG
501 GCGTTTTTCC ATAGGCTCCG CCCCCTGAC GAGCATCACA AAAATCGACG
551 CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGGCGT

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601 TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT  
651 ACCGGATACC TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA  
701 TAGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC  
751 TGGGCTGTGT GCACGAACCC CCCGTTACGC CCGACCGCTG CGCCTTATCC  
801 GGTA ACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT  
851 GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGGT  
901 CTACAGAGTT CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGAACA  
951 GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT  
1001 TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT  
1051 TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT  
1101 CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAAACG AAAACTCACG  
1151 TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC  
1201 TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA  
1251 ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC  
1301 GATCTGTCTA TTTTCGTTTCA CCATAGTTGC CTGACTCCCC GTCGTGTAGA  
1351 TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC TGCAATGATA  
1401 CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC  
1451 AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA  
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1551 AATAGTTTGC GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTACAG  
1601 CTCGTCGTTT GGTATGGCTT CATT CAGCTC CGGTTCCCAA CGATCAAGGC  
1651 GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT  
1701 CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTAT CACTCATGGT  
1751 TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT  
1801 TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG  
1851 CGGCGACCGA GTTGCTCTTG CCCGGCGTCA ATACGGGATA ATACCGCGCC  
1901 ACATAGCAGA ACTTTAAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGGC  
1951 GAAA ACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC  
2001 ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC  
2051 TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAG GGAATAAGGG  
2101 CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA  
2151 AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT  
2201 TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC CGAAAAGTGC  
2251 CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC CTATAAAAAT  
2301 AGGCGTATCA CGAGGCCCTT TCGTCTCGCG CGTTTCGGTG ATGACGGTGA  
2351 AAACCTCTGA CACATGCAGC TCCCGGAGAC GGTCACAGCT TGTCTGTAAG  
2401 CGGATGCCGG GAGCAGACAA GCCCGTCAGG GCGCGTCAGC GGGTGTGGC  
2451 GGGTGTCCGG GCTGGCTTAA CTATGCGGCA TCAGAGCAGA TTGTACTGAG  
2501 AGTGCACCAT ATGCGGTGTG AAATACCGCA CAGATGCGTA AGGAGAAAAT

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2551 ACCGCATCAG GAAATTGTAA GCGTTAATAT TTTGTTAAAA TTCGCGTTAA
2601 ATTTTTGTTA AATCAGCTCA TTTTTTAACC AATAGGCCGA AATCGGCAAA
2651 ATCCCTTATA AATCAAAAGA ATAGACCGAG ATAGGGTTGA GTGTTGTTCC
2701 AGTTTGGAAC AAGAGTCCAC TATTAAAGAA CGTGGACTCC AACGTCAAAG
2751 GGCGAAAAAC CGTCTATCAG GGCGATGGCC CACTACGTGA ACCATCACCC
2801 TAATCAAGTT TTTTGGGGTC GAGGTGCCGT AAAGCACTAA ATCGGAACCC
2851 TAAAGGGAGC CCCCATTATA GAGCTTGACG GGGAAAGCCG GCGAACGTGG
2901 CGAGAAAGGA AGGGAAGAAA GCGAAAGGAG CGGGCGCTAG GGCGCTGGCA
2951 AGTGTAGCGG TCACGCTGCG CGTAACCACC ACACCCGCCG CGCTTAATGC
3001 GCCGCTACAG GGCGCGTCCA TTCGCCATTC AGGCTGCGCA ACTGTTGGGA
3051 AGGGCGATCG GTGCGGGCCT CTTCGCTATT ACGCCAGCTG GCGAAAGGGG
3101 GATGTGCTGC AAGGCGATTA AGTTGGGTAA CGCCAGGGTT TTCCAGTCA
3151 CGACGTTGTA AAACGACGGC CAGTGAATTG TAATACGACT CACTATA

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## VI. Related Products

Product	Size	Cat.#
pGEM®-3Z Vector(a)	20µg	P2151
pGEM®-4Z Vector(a)	20µg	P2161
pGEM®-3Zf(-) Vector(a)	20µg	P2261
pGEM®-5Zf(+) Vector(a)	20µg	P2241
pGEM®-5Zf(-) Vector(a)	20µg	P2351
pGEM®-7Zf(+) Vector(a)	20µg	P2251
pGEM®-7Zf(-) Vector(a)	20µg	P2371
pGEM®-9Zf(-) Vector(a)	20µg	P2391
pGEM®-11Zf(+) Vector(a)	20µg	P2411
pGEM®-11Zf(-) Vector(a)	20µg	P2421
pGEM®-13Zf(+) Vector(a)	20µg	P2541

All pGEM®-Zf Vectors are provided with a glycerol stock of bacterial strain JM109.

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



## Riboprobe® in vitro Transcription Systems

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

### VII. 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.

<sup>(a)</sup>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

<sup>(b)</sup>U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803 and Japanese Pat. No. 3009458 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

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All prices and specifications are subject to change without prior notice.

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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