pGEM®-5Zf(–) Vector

Promega

Technical Bulletin No. 068

INSTRUCTIONS FOR USE OF PRODUCT P2351. *PLEASE DISCARD PREVIOUS VERSIONS.*
All technical literature is available on the Internet at www.promega.com
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I. Description

The pGEM®-5Zf(–) Vector[a] is a derivative of the pGEM®-5Zf(+) Vector[a] and differs from it by the orientation of the origin of replication of filamentous phage f1. The plasmid serves as a standard cloning vector, as a template for in vitro transcription, and can be used for the production of circular 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. The multiple cloning region is unique and includes restriction sites for *Apa* I, *Aat* II, *Sph* I, *Nco* I, *Sac* II, *Eco*R V, *Spe* I, *Not* I, *Pst* I, *Sal* I, *Nde* I, *Sac* I, *Bst*X I and *Nsi* I. This arrangement is designed specifically for generating unidirectional deletions with Promega's Erase-a-Base® System. The polylinker contains restriction enzyme sites that produce 5´ overhangs or blunt ends (sensitive to Exonuclease III) flanked on both sides by blocks of restriction sites that generate 3´ overhangs (resistant to Exonuclease III).

For induction of ssDNA, bacterial cells containing pGEM®-5Zf(–) 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 identical to the sequence shown in Figure 1. The exported ssDNA can be used for mutagenesis in vitro or can be sequenced using Promega's SP6 Promoter Primer (Cat.# Q5011) and pUC/M13 Reverse Primer (Cat.# Q5401, Q5421).

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

II. Product Components

Product	Size	Cat.#
pGEM®-5Zf(–) Vector	20µg	P2351

The pGEM®-5Zf(–) 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®-5Zf(–) Vector at –20°C and the glycerol stock of JM109 cells at –70°C.

III. pGEM®-5Zf(–) Vector Multiple Cloning Region and Circle Map

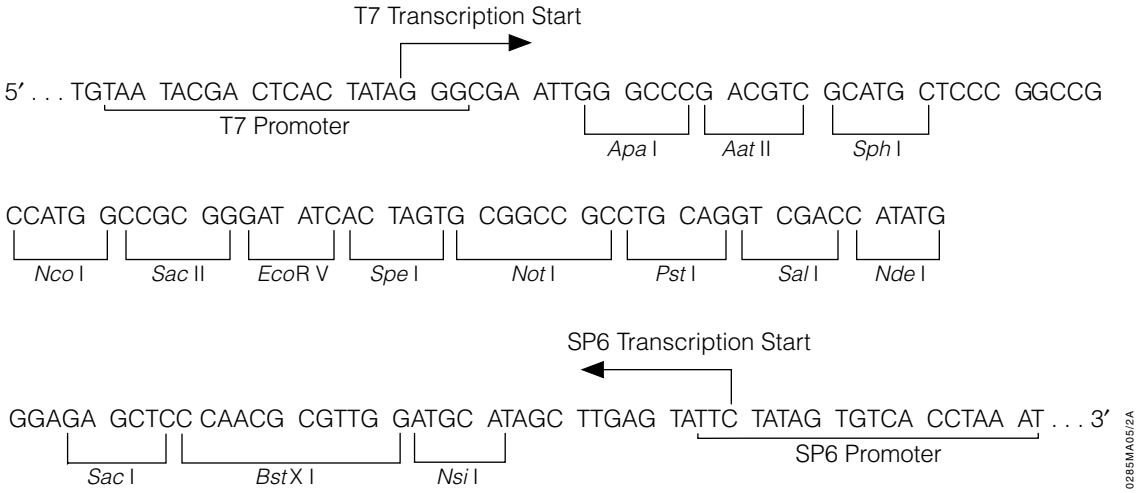

Figure 1. pGEM®-5Zf(–) Vector promoter and multiple cloning site sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase. The strand shown is the same as the ssDNA strand produced by this vector.

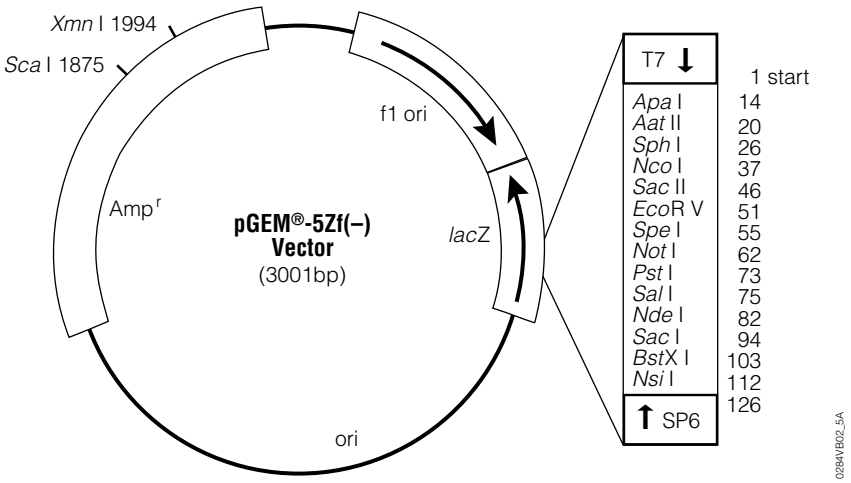

Figure 2. pGEM®-5Zf(–) Vector circle map and sequence reference points.

pGEM®-5Zf(–) Vector sequence reference points.

T7 RNA polymerase transcription initiation site	1
SP6 RNA polymerase transcription initiation site	126
T7 RNA polymerase promoter (–17 to +3)	2985–3
SP6 RNA polymerase promoter (–17 to +3)	124–143
multiple cloning region	10–113
*lac*Z start codon	165
lac operon sequences	2822–2982; 151–380
lac operator	185–201
β-lactamase (Amp^r) coding region	1322–2182
phage f1 region	2366–2821
binding site of pUC/M13 Forward Sequencing Primer	2942–2958
binding site of pUC/M13 Reverse Sequencing Primer	161–177

Specialized applications of the pGEM®-5Zf(–) Vector.

- Used with the Erase-a-Base® System.
- 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*[b,c] *Technical Manual*, #TM016.)
- Translation in vitro (For protocol information, please request Promega's *TNT® Quick Coupled Transcription/Translation System*[b,c,d,e,f] *Technical Manual*, #TM045.)

Promega's pGEM®-5Zf(–) and pGEM®-5Zf(+) Vectors are identical except for the orientation of the f1 origin.

Use the SP6 or pUC/M13 Reverse Primer to sequence ssDNA produced by the pGEM®-5Zf(–) Vector.

Note: All Promega technical literature is available on the Internet at www.promega.com.

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IV. pGEM®-5Zf(–) 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 in the GenBank® database (GenBank®/EMBL Accession Number X65309) and on the Internet at www.promega.com/vectors/.

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

Enzyme	# of Sites	Location
***Aat* II**	1	20
***Acc* I**	1	76
***Acy* I**	2	17, 1932
Afl III	2	99, 502
***Alw*26 I**	2	1456, 2232
***Alw*44 I**	2	816, 2062
*Alw*N I	1	918
***Apa* I**	1	14
*Asp*H I	4	94, 820, 1981, 2066
***Ava* II**	2	1533, 1755
***Ban* I**	3	246, 1343, 2555
***Ban* II**	3	14, 94, 2525
***Bbu* I**	1	26
***Bgl* I**	3	39, 1515, 2834
Bsa I	1	1456
*Bsa*A I	1	2596
*Bsa*H I	2	17, 1932
*Bsa*J I	5	37, 43, 241, 662, 2937
*Bsp*120 I	1	10
*Bsp*H I	2	1222, 2230
*Bsp*M I	1	62
*Bss*S I	2	675, 2059
***Bst*O I**	5	242, 530, 651, 664, 2938
***Bst*X I**	1	103
***Bst*Z I**	2	31, 62
*Cfr*10 I	2	1475, 2491
***Dde* I**	4	777, 1186, 1352, 1892
***Dra* I**	3	1261, 1280, 1972
Dra III	1	2599
Drd I	2	610, 2643
Dsa I	2	37, 43
Eag I	2	31, 62
Ear I	3	386, 2190, 2879
***Ecl*HK I**	1	1395
***Eco*52 I**	2	31, 62
***Eco*ICR I**	1	92
***Eco*R V**	1	51
***Fok* I**	5	119, 1361, 1542, 1829, 2917
Fsp I	2	1617, 2841
***Hae* II**	4	380, 750, 2441, 2449
Hga I	4	613, 1191, 1921, 2374
***Hinc* II**	1	77
*Hin*d II	1	77
***Hsp*92 I**	2	17, 1932
Mae I	5	56, 997, 1250, 1585, 2443
***Mlu* I**	1	99
***Nae* I**	1	2493
***Nci* I**	4	30, 882, 1578, 1929
***Nco* I**	1	37
***Nde* I**	1	82
***Ngo*M IV**	1	2491
***Not* I**	1	62
***Nsi* I**	1	112
Nsp I	2	26, 506
*Pae*R7 I	1	2560
*Ppu*10 I	1	108
***Pst* I**	1	73
***Pvu* I**	2	1765, 2862
***Pvu* II**	2	326, 2891
***Rsa* I**	1	1875
***Sac* I**	1	94
***Sac* II**	1	46
***Sal* I**	1	75
***Sca* I**	1	1875
***Sfi* I**	1	39
***Sin* I**	2	1533, 1755
***Spe* I**	1	55
***Sph* I**	1	26
*Sse*8387 I	1	73
***Ssp* I**	2	2199, 2804
***Sty* I**	1	37
***Taq* I**	4	76, 602, 2046, 2561
Tfi I	2	337, 477
***Vsp* I**	3	273, 332, 1567
***Xmn* I**	1	1994

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

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-5Zf(–) Vector.

***Acc* III**	***Bcl* I**	***Bsu*36 I**	***Hpa* I**	*Psh*A I	***Xba* I**
***Acc*B7 I**	***Bgl* II**	***Cla* I**	**I-*Ppo* I**	*Psp*5 II	*Xcm* I
***Acc*65 I**	*Blp* I	***Csp* I**	*Kas* I	*Psp*A I	***Xho* I**
Afl II	*Bpu*1102 I	***Csp*45 I**	***Kpn* I**	*Rsr* II	***Xma* I**
***Age* I**	*Bsa*B I	*Dra* II	***Nar* I**	***Sgf* I**(g)	
Asc I	***Bsa*M I**	***Eco*47 III**	***Nhe* I**	*Sgr*A I	
***Ava* I**	*Bsm* I	*Eco*72 I	***Nru* I**	***Sma* I**	
Avr II	***Bsr*BR I**	*Eco*81 I	*Pac* I	***Sna*B I**	
***Bal* I**	*Bsr*G I	*Eco*N I	*Pfl*M I	*Spl* I	
***Bam*H I**	***Bss*H II**	***Eco*R I**	*Pin*A I	*Srf* I	
Bbe I	*Bst*1107 I	*Ehe* I	*Pme* I	***Stu* I**	
*Bbr*P I	***Bst*98 I**	*Fse* I	*Pml* I	*Swa* I	
Bbs I	***Bst*E II**	***Hin*d III**	*Ppu*M I	***Tth*111 I**	

Table 3. Restriction Enzymes That Cut the pGEM®-5Zf(–) Vector 6 or More Times.

Aci I	*Bst*U I	***Hinf* I**	*Mnl* I	***Sau*3A I**
***Alu* I**	***Cfo* I**	***Hpa* II**	*Mse* I	***Sau*96 I**
Bbv I	***Dpn* I**	*Hph* I	***Msp* I**	*Scr*F I
***Bsa*O I**	*Dpn* II	***Hsp*92 II**	***Msp*A1 I**	*Sfa*N I
***Bsp*1286 I**	*Eae* I	*Mae* II	***Nde* II**	***Tru*9 I**
Bsr I	*Fnu*4H I	*Mae* III	*Nla* III	***Xho* II**
***Bsr*S I**	***Hae* III**	***Mbo* I**	*Nla* IV	
***Bst*71 I**	***Hha* I**	***Mbo* II**	*Ple* I	

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

V. pGEM®-5Zf(–) 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 the same as the ssDNA strand produced by this vector.

```
  1  GGGCGAATTG GGCCCGACGT CGCATGCTCC CGGCCGCCAT GGCCGCGGGA
 51  TATCACTAGT GCGGCCGCCT GCAGGTCGAC CATATGGGAG AGCTCCCAAC
101  GCGTTGGATG CATAGCTTGA GTATTCTATA GTGTCACCTA AATAGCTTGG
151  CGTAATCATG GTCATAGCTG TTTCCTGTGT GAAATTGTTA TCCGCTCACA
201  ATTCCACACA ACATACGAGC CGGAAGCATA AAGTGTAAAG CCTGGGGTGC
251  CTAATGAGTG AGCTAACTCA CATTAATTGC GTTGCGCTCA CTGCCCGCTT
301  TCCAGTCGGG AAACCTGTCG TGCCAGCTGC ATTAATGAAT CGGCCAACGC
351  GCGGGGAGAG GCGGTTTGCG TATTGGGCGC TCTTCCGCTT CCTCGCTCAC
401  TGACTCGCTG CGCTCGGTCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT
451  CAAAGGCGGT AATACGGTTA TCCACAGAAT CAGGGGATAA CGCAGGAAAG
501  AACATGTGAG CAAAAGGCCA GCAAAAGGCC AGGAACCGTA AAAAGGCCGC
551  GTTGCTGGCG TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA
601  ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC
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pGEM®-5Zf(–) Vector Sequence (continued)

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 651  CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT
 701  GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC
 751  TTTCTCATAG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC
 801  TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCGC
 851  CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT
 901  CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC GAGGTATGTA
 951  GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG
1001  AAGAACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA
1051  AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC AAACCACCGC TGGTAGCGGT
1101  GGTTTTTTTG TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA
1151  AGAAGATCCT TTGATCTTTT CTACGGGGTC TGACGCTCAG TGGAACGAAA
1201  ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG GATCTTCACC
1251  TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA
1301  TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
1351  TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC
1401  GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
1451  AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA
1501  ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC
1551  GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC
1601  GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG
1651  TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA
1701  TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
1751  CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC
1801  TCATGGTTAT GGCAGCACTG CATAATTCTC TTACTGTCAT GCCATCCGTA
1851  AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA
1901  GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAATA CGGGATAATA
1951  CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT
2001  TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
2051  GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA
2101  GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA
2151  ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA
2201  TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG
2251  AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA
2301  AAAGTGCCAC CTGTATGCGG TGTGAAATAC CGCACAGATG CGTAAGGAGA
2351  AAATACCGCA TCAGGACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT
2401  GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC
2451  CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC GCCGGCTTTC
2501  CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT
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pGEM®-5Zf(–) Vector Sequence (continued)

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2551  TTACGGCACC TCGACCCCAA AAAACTTGAT TAGGGTGATG GTTCACGTAG
2601  TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA
2651  CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT
2701  ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA
2751  TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG AATTTTAACA
2801  AAATATTAAC GCTTACAATT TCCATTCGCC ATTCAGGCTG CGCAACTGTT
2851  GGGAAGGGCG ATCGGTGCGG GCCTCTTCGC TATTACGCCA GCTGGCGAAA
2901  GGGGGATGTG CTGCAAGGCG ATTAAGTTGG GTAACGCCAG GGTTTTCCCA
2951  GTCACGACGT TGTAAAACGA CGGCCAGTGA ATTGTAATAC GACTCACTAT
3001  A
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VI. Related Products

pGEM® Vectors

Product	Size	Cat.#
pGEM®-3Z Vector[a]	20µg	P2151
pGEM®-4Z Vector[a]	20µg	P2161
pGEM®-3Zf(+) Vector[a]	20µg	P2271
pGEM®-3Zf(–) Vector[a]	20µg	P2261
pGEM®-5Zf(+) Vector[a]	20µg	P2241
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. The JM109 cells do not contain the vector and are not competent.

Other Vectors

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

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Related Products (continued)

Erase-a-Base® Systems

Product	Cat.#
Erase-a-Base® System (plus vectors)	E5850
Erase-a-Base® System (minus vectors and bacterial strain)	E5750

Riboprobe® in vitro Transcription Systems

Product	Cat.#
Riboprobe® System - SP6[b,c]	P1420
Riboprobe® System - T3[b,c]	P1430
Riboprobe® System - T7[b,c]	P1440

TnT® Quick Coupled Transcription/Translation Systems

Product	Cat.#
TnT® T7 Quick Coupled Transcription/Translation System[b,c,d,e,f]	L1170
TnT® T7 Quick Coupled Transcription/Translation System, Trial Size[b,c,d,e,f]	L1171
TnT® SP6 Quick Coupled Transcription/Translation System[b,c,d,e,f]	L2080
TnT® SP6 Quick Coupled Transcription/Translation System, Trial Size[b,c,d,e,f]	L2081

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.

[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. Nos. 5,283,179, 5,641,641, 5,650,289, Australian Pat. No. 649289 and European Pat. No. 0 553 234 have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Certain applications of this product may require licenses from others.

[e]U.S. Pat. Nos. 5,324,637, 5,492,817 and 5,665,563, European Pat. No. 0 566 714 B1, Australian Pat. No. 660329 and Japanese Pat. No. 2904583 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.

[f]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.

[g]U.S. Pat. No. 5,391,487 has been issued to Promega Corporation for Restriction Endonuclease *Sgf* I.

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