

# pSP73 Vector Sequence and Map

Technical Bulletin No. 041

INSTRUCTIONS FOR USE OF PRODUCT P2221. *PLEASE DISCARD PREVIOUS VERSIONS.*

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I. Description .....	1
II. Product Components .....	1
III. pSP73 Vector Multiple Cloning Site and Circle Map .....	2
IV. pSP73 Vector Restriction Sites .....	4
V. pSP73 Vector Sequence .....	5
VI. Related Products .....	7
VII. Reference .....	7

## I. Description

The pSP73 Vector<sup>(a)</sup> (1) offers a wide range of restriction sites, providing greater versatility in cloning and transcription of RNA *in vitro*. The pSP73 Vector contains the SP6 and T7 RNA polymerase promoters and a unique multiple cloning region which includes restriction sites for *Bgl* II, *EcoR* V, *Cla* I, *EcoR* I, *Sac* I, *Kpn* I, *Sma* I, *Bam*HI, *Xba* I, *Acc* I, *Sal* I, *Pst* I, *Sph* I, *Hind* III, *Pvu* II and *Xho* I.

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.

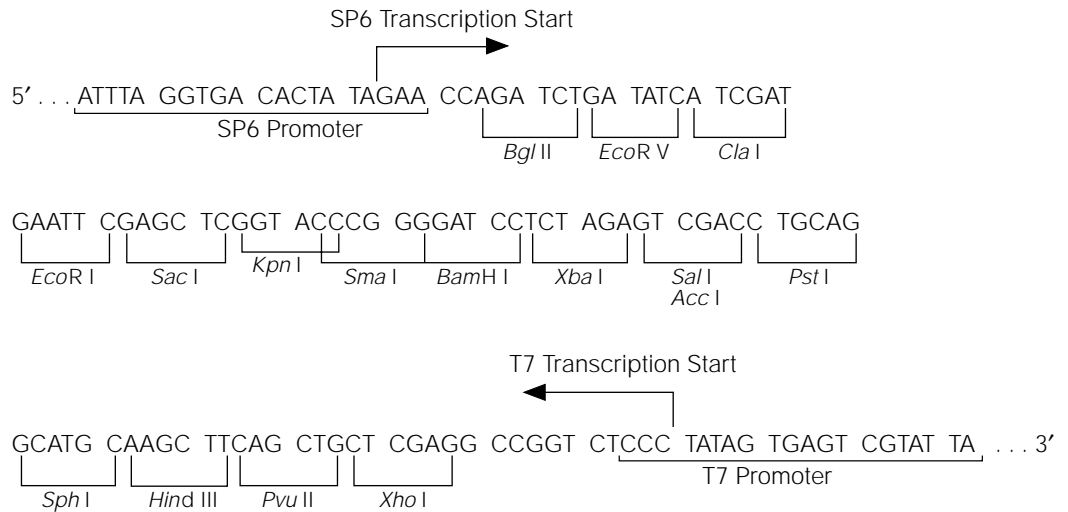
## II. Product Components

Product	Size	Cat.#
pSP73 Vector	20µg	<a href="#">P2221</a>

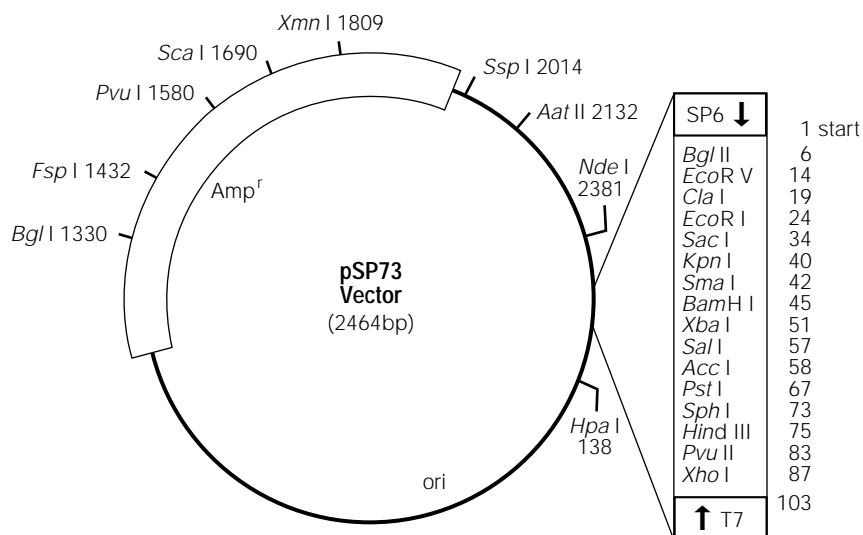
**Storage Conditions:** Store the pSP73 Vector at –20°C.



### III. pSP73 Vector Multiple Cloning Site and Circle Map



**Figure 1. pSP73 Vector promoter and multiple cloning site sequence.** The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase and is complementary to RNA synthesized by T7 RNA polymerase.



**Figure 2. pSP73 Vector circle map and sequence reference points.**

**pSP73 Vector sequence reference points.**

SP6 RNA polymerase transcription initiation site	1
T7 RNA polymerase transcription initiation site	103
SP6 RNA polymerase promoter (-17 to +3)	2448-3
T7 RNA polymerase promoter (-17 to +3)	107-120
multiple cloning region	6-92
β-lactamase coding region	1137-1997

**Specialized application of the pSP73 Vector.**

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



**PROMEGA's**

... pSP72 and pSP73 Vectors are identical except for the orientation of the multiple cloning region.



**BLUE/WHITE**

... screening of recombinants is **not** possible with the pSP73 Vector.

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

#### IV. pSP73 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 X65333) and on the Internet at [www.promega.com/vectors/](http://www.promega.com/vectors/).

**Table 1. Restriction Enzymes That Cut the pSP73 Vector Between 1 and 5 Times.**

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
<b>Aaf II</b>	1	2132	<b>EcoR V</b>	1	14
<b>Acc I</b>	1	58	<b>Fok I</b>	4	1176, 1357, 1644, 2287
<b>Acc65 I</b>	1	36	<b>Fsp I</b>	1	1432
<b>Acy I</b>	2	1747, 2129	<b>Hae II</b>	2	195, 565
<b>Afl III</b>	1	317	<b>Hga I</b>	4	428, 1006, 1736, 2294
<b>Alw26 I</b>	5	102, 1271, 2047, 2200 2242	<b>Hinc II</b>	2	59, 138
<b>Alw44 I</b>	3	631, 1877, 2374	<b>Hind II</b>	2	59, 138
<b>AlwN I</b>	1	733	<b>Hind III</b>	1	75
<b>AspH I</b>	5	34, 635, 1796, 1881, 2378	<b>Hpa I</b>	1	138
<b>Ava I</b>	2	40, 87	<b>Hsp92 I</b>	2	1747, 2129
<b>Ava II</b>	2	1348, 1570	<b>Kpn I</b>	1	40
<b>BamH I</b>	1	45	<b>Mae I</b>	4	52, 812, 1065, 1400
<b>Ban I</b>	2	36, 1158	<b>Mae II</b>	4	1020, 1436, 1809, 2129
<b>Ban II</b>	1	34	<b>MspA1 I</b>	5	83, 659, 904, 1845, 2311
<b>Bbu I</b>	1	73	<b>Nde I</b>	1	2381
<b>Bgl I</b>	1	1330	<b>Nsp I</b>	3	73, 321, 2238
<b>Bgl II</b>	1	6	<b>Ple I</b>	5	63, 118, 211, 696, 1199
<b>Bsa I</b>	2	102, 1271	<b>PspA I</b>	1	40
<b>BsaO I</b>	4	233, 657, 1580, 1729	<b>Pst I</b>	1	67
<b>BsaH I</b>	2	1747, 2129	<b>Pvu I</b>	1	1580
<b>BsaJ I</b>	3	40, 41, 477	<b>Pvu II</b>	1	83
<b>Bsp1286 I</b>	5	34, 635, 1796, 1881, 2378	<b>Rsa I</b>	3	38, 1690, 2366
<b>BspH I</b>	3	1037, 2045, 2150	<b>Sac I</b>	1	34
<b>BspM I</b>	2	70, 149	<b>Sal I</b>	1	57
<b>BssS I</b>	3	490, 1874, 2181	<b>Sau96 I</b>	5	1252, 1331, 1348, 1570 2186
<b>BstO I</b>	4	133, 345, 466, 479	<b>Sca I</b>	1	1690
<b>Cfr10 I</b>	2	93, 1290	<b>Sin I</b>	2	1348, 1570
<b>Cla I</b>	1	19	<b>Sma I</b>	1	42
<b>Dra I</b>	3	1076, 1095, 1787	<b>Sph I</b>	1	73
<b>Dra II</b>	1	2186	<b>Sse8387 I</b>	1	67
<b>Drd I</b>	2	425, 2294	<b>Ssp I</b>	1	2014
<b>Eae I</b>	2	156, 1598	<b>Tfi I</b>	2	152, 292
<b>Ear I</b>	2	201, 2005	<b>Vsp I</b>	4	118, 147, 1382, 2415
<b>EclHK I</b>	2	1210, 2391	<b>Xba I</b>	1	51
<b>EcoCR I</b>	1	32	<b>Xho I</b>	1	87
<b>EcoR I</b>	1	24	<b>Xma I</b>	1	40
			<b>Xmn I</b>	1	1809

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

**Table 2. Restriction Enzymes That Do Not Cut the pSP73 Vector.**

<b>Acc B7 I</b>	<i>Bpu 1102 I</i>	<b>BstZ I</b>	<i>Fse I</i>	<i>PaeR7 I</i>	<i>SgrA I</i>
<b>Acc III</b>	<i>BsaA I</i>	<b>Bsu36 I</b>	<b>I-Ppo I</b>	<i>PfiM I</i>	<b>SnaB I</b>
<i>Afl II</i>	<i>BsaB I</i>	<b>Csp I</b>	<i>Kas I</i>	<i>PinA I</i>	<b>Spe I</b>
<b>Age I</b>	<b>BsaM I</b>	<b>Csp45 I</b>	<b>Mlu I</b>	<i>Pme I</i>	<i>Spl I</i>
<b>Apa I</b>	<i>Bsm I</i>	<i>Dra III</i>	<b>Nae I</b>	<i>Pml I</i>	<i>Srf I</i>
<i>Asc I</i>	<i>Bsp120 I</i>	<i>Dsa I</i>	<b>Nar I</b>	<i>Ppu10 I</i>	<b>Stu I</b>
<i>Avr II</i>	<b>BsrBR I</b>	<i>Eag I</i>	<b>Nco I</b>	<i>PpuM I</i>	<b>Sty I</b>
<b>Bal I</b>	<i>BsrG I</i>	<b>Eco47 III</b>	<b>NgoM IV</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>Bcl I</b>	<b>BstE II</b>	<i>EcoN I</i>	<b>Nsi I</b>	<b>Sfi I</b>	
<i>Blp I</i>	<b>BstX I</b>	<i>Ehe I</i>	<i>Pac I</i>	<b>Sgf I(c)</b>	

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

**Table 3. Restriction Enzymes That Cut the pSP73 Vector 6 or More Times.**

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

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

## V. pSP73 Vector Sequence

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1  GAACCAGATC TGATATCATC GATGAATTCG AGCTCGGTAC CCGGGGATCC
51  TCTAGAGTCG ACCTGCAGGC ATGCAAGCTT CAGCTGCTCG AGGCCGGTCT
101 CCCTATAGTG AGTCGTATTA ATTTGATAA GCCAGGTTAA CCTGCATTAA
151 TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG GCGCTCTTC
201 CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGTTCCG CTGCGGCGAG
251 CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTTATCCAC AGAATCAGGG
301 GATAACGCAG GAAAGAACAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA
351 CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC CGCCCCCTG
401 ACGAGCATCA CAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA
451 GGACTATAAA GATACCAGGC GTTCCCCCT GGAAGCTCCC TCGTGCGCTC
501 TCCTGTTCGG ACCCTGCCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT
551 CGGGAAGCGT GCGCTTTCT CATAGCTCAC GCTGTAGGTA TCTCAGTTCCG
601 GTGTAGGTCG TTCGCTCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA
651 GCCCGACCGC TCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG
701 TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC
751 AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA

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801 CTACGGCTAC ACTAGAAGAA CAGTATTTGG TATCTGCGCT CTGCTGAAGC  
851 CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC  
901 ACCGCTGGTA GCGGTGGTTT TTTTGTGTTG AAGCAGCAGA TTACGCGCAG  
951 AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG  
1001 CTCAGTGGAA CGAAAACCTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA  
1051 AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC  
1101 AATCTAAAGT ATATATGAGT AAACCTGGTC TGACAGTTAC CAATGCTTAA  
1151 TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTTCGTTT ATCCATAGTT  
1201 GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC  
1251 TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG  
1301 ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT  
1351 CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC  
1401 TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG  
1451 CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTACAGC  
1501 TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA  
1551 AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG  
1601 CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT  
1651 GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA  
1701 GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGCGT  
1751 CAATACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC  
1801 ATTGGAAAAC GTTCTTCGGG GCGAAAACCTC TCAAGGATCT TACCGCTGTT  
1851 GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT  
1901 CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT  
1951 GCCGCAAAAA AGGGAATAAG GCGGACACGG AAATGTTGAA TACTCATACT  
2001 CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA  
2051 GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTCCG  
2101 CGCACATTTT CCGGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT  
2151 CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCC TTTTCGTCTCG  
2201 CGCGTTTTCG TGATGACGGT GAAAACCTCT GACACATGCA GCTCCCGGAG  
2251 ACGGTCACAG CTTGTCTGTA AGCGGATGCC GGGAGCAGAC AAGCCCGTCA  
2301 GGGCGCGTCA GCGGGTGTG GCGGGTGTG GGGCTGGCTT AACTATGCGG  
2351 CATCAGAGCA GATTGTACTG AGAGTGCACC ATATGGACAT ATTGTCGTTA  
2401 GAACGCGGCT ACAATTAATA CATAACCTTA TGTATCATA ACATACGATT  
2451 TAGGTGACAC TATA

## VI. Related Products

Product	Size	Cat.#
pSP64 Poly(A) Vector	20µg	P1241
pSP72 Vector <sup>(a)</sup>	20µg	P2191

Product	Size	Cat.#
pGEM <sup>®</sup> -3Z Vector <sup>(a)</sup>	20µg	P2151
pGEM <sup>®</sup> -4Z Vector <sup>(a)</sup>	20µg	P2161
pGEM <sup>®</sup> -3Zf(+) Vector <sup>(a)</sup>	20µg	P2271
pGEM <sup>®</sup> -3Zf(-) Vector <sup>(a)</sup>	20µg	P2261
pGEM <sup>®</sup> -5Zf(+) Vector <sup>(a)</sup>	20µg	P2241
pGEM <sup>®</sup> -5Zf(-) Vector <sup>(a)</sup>	20µg	P2351
pGEM <sup>®</sup> -7Zf(+) Vector <sup>(a)</sup>	20µg	P2251
pGEM <sup>®</sup> -7Zf(-) Vector <sup>(a)</sup>	20µg	P2371
pGEM <sup>®</sup> -9Zf(-) Vector <sup>(a)</sup>	20µg	P2391
pGEM <sup>®</sup> -11Zf(+) Vector <sup>(a)</sup>	20µg	P2411
pGEM <sup>®</sup> -11Zf(-) Vector <sup>(a)</sup>	20µg	P2421
pGEM <sup>®</sup> -13Zf(+) Vector <sup>(a)</sup>	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.

### 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 <sup>(b)</sup>	P1420
Riboprobe <sup>®</sup> System - T7 <sup>(b)</sup>	P1440

## VII. Reference

- Krieg, P.A. and Melton, D.A. (1987) In vitro RNA synthesis with SP6 RNA polymerase. *Meth. Enzymol.* **155**, 397.

<sup>(a)</sup>U.S. Pat. No. 4,766,072.

<sup>(b)</sup>U.S. Pat. No. 5,552,302 and other patents. *Inhibitors of Angiogenesis*, which comprises a segment of human PRI, is the subject of U.S. Pat. No. 4,966,964 and other patents assigned to the President and Fellows of Harvard College and exclusively licensed to Promega Corporation.

<sup>(c)</sup>U.S. Pat. No. 5,391,487 has been issued to Promega Corporation for Restriction Endonuclease *Sgf I*.

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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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