Technical Bulletin

pSP73 Vector

INSTRUCTIONS FOR USE OF PRODUCT P2221.

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pSP73 Vector

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I. Description

The pSP73 Vector (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 BglII, EcoRV, ClaI, EcoRI, SacI, KpnI, SmaI, BamHI, XbaI, AccI, SalI, PstI, SphI, HindIII, PvuII and XhoI.

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

II. Product Components and Storage Conditions

Product	Size	Cat.#
pSP73 Vector	20µg	P2221

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

III. pSP73 Vector Multiple Cloning Region and Circle Map

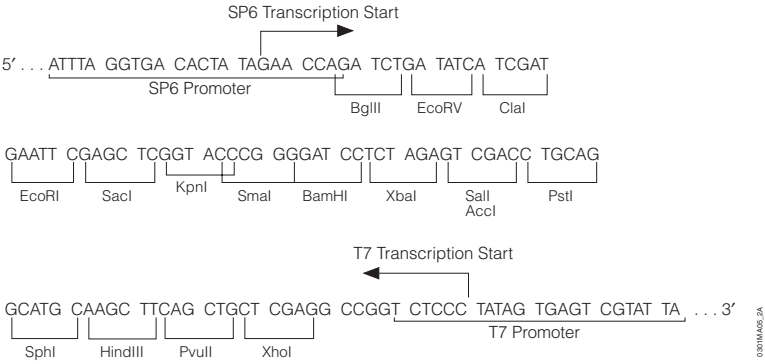

Figure 1. pSP73 Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase and is complementary to RNA synthesized by T7 RNA polymerase.

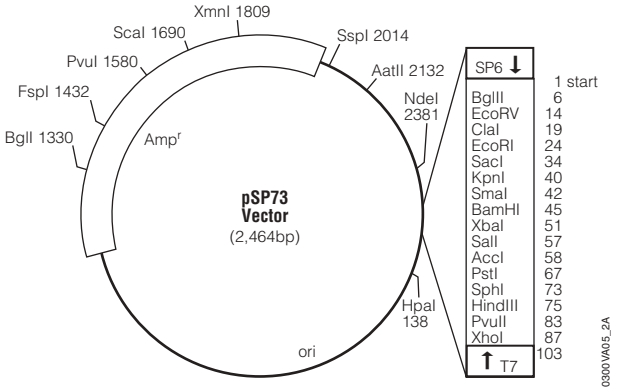

Figure 2. pSP73 Vector circle map and sequence reference points.

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

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

Note: Blue/white screening of recombinants is **not** possible with the pSP73 Vector.

Specialized application of the pSP73 Vector.

- Transcription in vitro from dual-opposed promoters (For protocol information, please request the Riboprobe® in vitro Transcription Systems Technical Manual, #TM016) available at: **www.promega.com/tbs/**

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/**

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

Enzyme	# of Sites	Location
AatII	1	2132
AccI	1	58
Acc65I	1	36
AcyI	2	1747, 2129
AflIII	1	317
Alw26I	5	102, 1271, 2047, 2200, 2242
Alw44I	3	631, 1877, 2374
AlwNI	1	733
AspHI	5	34, 635, 1796, 1881, 2378
AvaI	2	40, 87
AvaII	2	1348, 1570
BamHI	1	45
BanI	2	36, 1158
BanII	1	34
BbuI	1	73
BglI	1	1330
BglII	1	6
BsaI	2	102, 1271
BsaOI	4	233, 657, 1580, 1729
BsaHI	2	1747, 2129
BsaJI	3	40, 41, 477
Bsp1286I	5	34, 635, 1796, 1881, 2378
BspHI	3	1037, 2045, 2150
BspMI	2	70, 149
BssSI	3	490, 1874, 2181
BstOI	4	133, 345, 466, 479
Cfr10I	2	93, 1290
ClaI	1	19
DraI	3	1076, 1095, 1787
DraII	1	2186
DrdI	2	425, 2294
EaeI	2	156, 1598
EarI	2	201, 2005
EclHKI	2	1210, 2391
EcoICRI	1	32
EcoRI	1	24
EcoRV	1	14
FokI	4	1176, 1357, 1644, 2287
FspI	1	1432
HaeII	2	195, 565
HgaI	4	428, 1006, 1736, 2294
HincII	2	59, 138
HindII	2	59, 138
HindIII	1	75
HpaI	1	138
Hsp92I	2	1747, 2129
KpnI	1	40
MaeI	4	52, 812, 1065, 1400
MaeII	4	1020, 1436, 1809, 2129
MspA1I	5	83, 659, 904, 1845, 2311
NdeI	1	2381
NspI	3	73, 321, 2238
PaeR7I	1	87

Table 1. Restriction Enzymes That Cut the pSP73 Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
PleI	5	63, 118, 211, 696, 1199	**SinI**	2	1348, 1570
PspAI	1	40	**SmaI**	1	42
PstI	1	67	**SphI**	1	73
PvuI	1	1580	Sse8387I	1	67
PvuII	1	83	**SspI**	1	2014
RsaI	3	38, 1690, 2366	TfiI	2	152, 292
SacI	1	34	**VspI**	4	118, 147, 1382, 2415
SalI	1	57	**XbaI**	1	51
Sau96I	5	1252, 1331, 1348, 1570, 2186	**XhoI**	1	87
			XmaI	1	40
ScaI	1	1690	**XmnI**	1	1809

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

AccB7I	BsaBI	DraIII	**NcoI**	RsrII
AccIII	**BsaMI**	DsaI	**NgoMIV**	**SacII**
AflII	BsmI	EagI	**NheI**	**SfiI**
AgeI	Bsp120I	**Eco47III**	**NotI**	**SgfI**
ApaI	BsrBRI	Eco52I	**NruI**	SgrAI
AscI	BsrGI	Eco72I	**NsiI**	**SnaBI**
AvrII	**BssHII**	Eco81I	PacI	**SpeI**
BalI	Bst1107I	EcoNI	PflMI	SplI
BbeI	**Bst98I**	EheI	PinAI	SrfI
BbrPI	**BstEII**	FseI	PmeI	**StuI**
BbsI	**BstXI**	**I-PpoI**	PmlI	**StyI**
BclI	**BstZI**	KasI	Ppu10I	SwaI
BlpI	**Bsu36I**	**MluI**	PpuMI	**Tth111I**
Bpu1102I	**CspI**	**NaeI**	PshAI	XcmI
BsaAI	**Csp45I**	**NarI**	Psp5II	

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

IV. pSP73 Vector Restriction Sites (continued)

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

AciI	**CfoI**	**HinfI**	MnlI	**Sau3AI**
AluI	**DdeI**	**HpaII**	MseI	ScrFI
BbvI	**DpnI**	HphI	**MspI**	SfaNI
BsrI	DpnII	**Hsp92II**	**NciI**	**TaqI**
BsrSI	Fnu4HI	MaeIII	**NdeII**	**Tru9I**
Bst71I	**HaeIII**	MboI	NlaIII	**XhoII**
BstUI	**HhaI**	**MboII**	NlaIV	

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

V. Related Products

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

Product	Size	Cat.#
pGEM®-3Z Vector	20µg	P2151
pGEM®-4Z Vector	20µg	P2161
pGEM®-3Zf(+) Vector	20µg	P2271
pGEM®-3Zf(-) Vector	20µg	P2261
pGEM®-5Zf(+) Vector	20µg	P2241
pGEM®-5Zf(-) Vector	20µg	P2351
pGEM®-7Zf(+) Vector	20µg	P2251
pGEM®-7Zf(-) Vector	20µg	P2371
pGEM®-9Zf(-) Vector	20µg	P2391
pGEM®-11Zf(+) Vector	20µg	P2411
pGEM®-11Zf(-) Vector	20µg	P2421
pGEM®-13Zf(+) Vector	20µg	P2541

All pGEM® 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

Riboprobe® in vitro Transcription Systems

Product	Cat.#
Riboprobe® System - SP6	P1420
Riboprobe® System - T7	P1440

For Laboratory Use.

VI. Reference

1. Krieg, P.A. and Melton, D.A. (1987) In vitro RNA synthesis with SP6 RNA polymerase. *Methods Enzymol.* **155**, 397–415.

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