Technical Bulletin

pSP64 Poly(A) Vector

INSTRUCTIONS FOR USE OF PRODUCT P1241.

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pSP64 Poly(A) Vector

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I. Description

The Riboprobe® System Vector pSP64 Poly(A) can be used as a standard cloning vector and can also be used for in vitro transcription from the SP6 promoter. The pSP64 Poly(A) Vector can also be used to generate poly(A)+ transcripts in vitro. The vector has a stretch of 30 dA:dT residues inserted between the *Sac* I and *Eco*R I sites. Therefore, when foreign DNA is cloned into any polylinker site other than *Eco*R I (*Hin*d III, *Pst* I, *Sal* I, *Acc* I, *Hin*c II, *Xba* I, *Bam*H I, *Ava* I, *Sma* I or *Sac* I), linearization of the recombinant plasmid with *Eco*R I allows the use of SP6 RNA polymerase in vitro to prepare RNA copies of the inserted sequences that contain a synthetic 3´ "polyA" tail of 30 residues.

The sequence of the pSP64 Poly(A) Vector is available online at **www.promega.com/vectors/** and also from the GenBank® database. (Accession No. X65328).

Specialized applications of the pSP64 Poly(A) Vector.

- Transcription in vitro from SP6 promoter (For protocol information, please see the *Riboprobe® in vitro Transcription Systems Technical Manual*, #TM016, available online at: **www.promega.com/tbs/**)
- Generation of poly(A)+ transcripts in vitro.

II. Product Components and Storage Conditions

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

Storage Conditions: Store the pSP64 Poly(A) Vector at –20°C.

III. pSP64 Poly(A) Vector Multiple Cloning Region and Circle Map

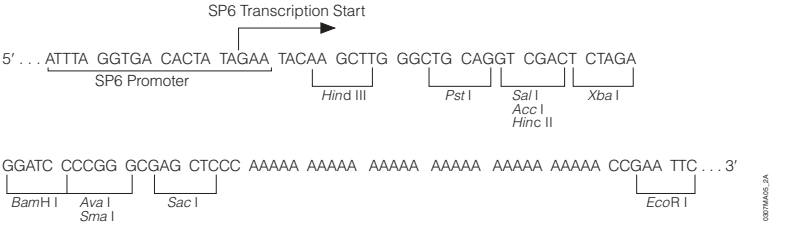

Figure 1. pSP64 Poly(A)Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase.

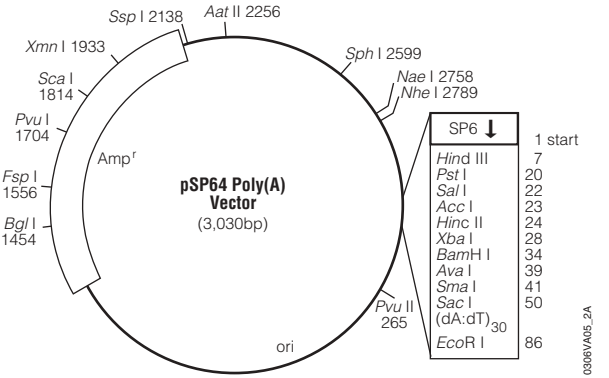

Figure 2. pSP64 Poly(A)Vector circle map and sequence reference points.

pSP64 Poly(A) Vector sequence reference points.

SP6 RNA polymerase transcription initiation site	1
SP6 RNA polymerase promoter	3014–3
multiple cloning region	7–91
poly(A) region	54–83
β-lactamase (Amp^r) coding region	1261–2121
binding site of pUC/M13 Reverse Primer	101–127

Note: The pSP64 Poly(A) Vector can be sequenced using the pUC/M13 Reverse Primer (Cat.# Q5421) or the SP6 Promoter Primer (Cat.# Q5011).

Blue/white screening of recombinants is not possible with the pSP64 Poly(A) Vector

IV. pSP64 Poly(A) 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. The pSP64 Poly(A) Vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number X65328) and on the Internet at **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pSP64 Vector 1–5 Times.

Enzyme	# of Sites	Location
***Aat* II**	1	2256
***Acc* I**	1	23
Acy I	5	1871, 2253, 2610, 2724, 2745
Afl III	1	441
***Alw*26 I**	4	1395, 2171, 2324, 2366
***Alw*44 I**	3	755, 2001, 2498
*Alw*N I	1	857
***Ava* I**	1	39
***Ava* II**	2	1472, 1694
***Bam*H I**	1	34
***Ban* I**	5	185, 1282, 2609, 2723, 2744
***Ban* II**	3	50, 2676, 2690
Bbe I	3	2613, 2727, 2748
***Bbu* I**	1	2599
***Bgl* I**	1	1454
Bsa I	1	1395
*Bsa*O I	4	357, 781, 1704, 1853
*Bsa*H I	5	1871, 2253, 2610, 2724, 2745
*Bsp*H I	4	1161, 2169, 2274, 2668
*Bsp*M I	1	9
*Bsr*G I	1	2950
*Bss*S I	3	614, 1998, 2305
***Bst*O I**	4	181, 469, 590, 603
*Cfr*10 I	3	1414, 2747, 2756
***Dra* I**	3	1200, 1219, 1911
Dra II	2	2310, 2634
Drd I	2	549, 2418
Dsa I	1	2629
Eae I	4	280, 1722, 2626, 2758
Ear I	2	325, 2129
***Ecl*HK I**	1	1334
***Eco*47 III**	1	2665
***Eco*ICR I**	1	48
*Eco*N I	1	2534
***Eco*R I**	1	86
Ehe I	3	2611, 2725, 2746
***Fok* I**	4	1300, 1481, 1768, 2411
Fsp I	1	1556
***Hinc* II**	1	24
*Hin*d II	1	24
***Hin*d III**	1	7
***Hsp*92 I**	5	1871, 2253, 2610, 2724, 2745
Kas I	3	2609, 2723, 2744
Mae I	5	29, 936, 1189, 1524, 2790
Mae II	4	1144, 1560, 1933, 2253
***Msp*A1 I**	5	265, 783, 1028, 1969, 2435
***Nae* I**	1	2758
***Nar* I**	3	2610, 2724, 2745
***Ngo*M IV**	1	2756
***Nhe* I**	1	2789
Nsp I	3	445, 2362, 2599
*Psp*A I	1	39
***Pst* I**	1	20
***Pvu* I**	1	1704
***Pvu* II**	1	265
***Rsa* I**	3	1814, 2490, 2952
***Sac* I**	1	50
***Sal* I**	1	22
***Sca* I**	1	1814

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

Table 1. Restriction Enzymes That Cut the pSP64 Vector 1–5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
*Sgr*A I	1	2747	*Tfi* I	2	276, 416
***Sin* I**	2	1472, 1694	***Vsp* I**	4	212, 271, 1506, 2981
***Sma* I**	1	41			
***Sph* I**	1	2599	***Xba* I**	1	28
***Ssp* I**	1	2138	***Xma* I**	1	39
***Taq* I**	4	23, 541, 1985, 2507	***Xmn* I**	1	1933

Table 2. Restriction Enzymes That Do Not Cut the pSP64 Vector.

***Acc*B7 I**	***Bgl* II**	***Bst*X I**	*Fse* I	*Pfl*M I	***Sna*B I**
***Acc* III**	*Blp* I	***Bst*Z I**	***Hpa* I**	*Pin*A I	***Spe* I**
***Acc*65 I**	*Bpu*1102 I	***Bsu*36 I**	***I-Ppo* I**	*Pme* I	*Spl* I
Afl II	*Bsa*A I	***Cla* I**	***Kpn* I**	*Pml* I	*Srf* I
***Age* I**	*Bsa*B I	***Csp* I**	***Mlu* I**	*Ppu*10 I	*Sse*8387 I
***Apa* I**	***Bsa*M I**	***Csp*45 I**	***Nco* I**	*Ppu*M I	***Stu* I**
Asc I	*Bsm* I	*Dra* III	***Nde* I**	*Psh*A I	***Sty* I**
Avr II	*Bsp*120 I	*Eag* I	***Not* I**	*Psp*5 II	*Swa* I
***Bal* I**	***Bss*H II**	*Eco*52 I	***Nru* I**	*Rsr* II	***Tth*111 I**
*Bbr*P I	*Bst*1107 I	*Eco*72 I	***Nsi* I**	***Sac* II**	*Xcm* I
Bbs I	***Bst*98 I**	*Eco*81 I	*Pac* I	***Sfi* I**	***Xho* I**
***Bcl* I**	***Bst*E II**	***Eco*R V**	*Pae*R7 I	***Sgf* I**	

Table 3. Restriction Enzymes That Cut the pSP64 Vector 6 or More Times.

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

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

V. Reference

1. Melton, D., *et al*. (1984) Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes form plasmids containing a bacteriophage SP6 promoter. *Nucl. Acids Res*. **12**, 7035–56.

VI. Related Products

Sequencing Primers

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011
pUC/M13 Primer, Reverse (22mer)	2µg	Q5421

Riboprobe® in vitro Transcription Systems

Product	Cat.#
Riboprobe® System—SP6	P1420

pGEM® Vectors

Product	Size	Cat.#
pSP72 Vector	20µg	P2191
pSP73 Vector	20µg	P2221
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.

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