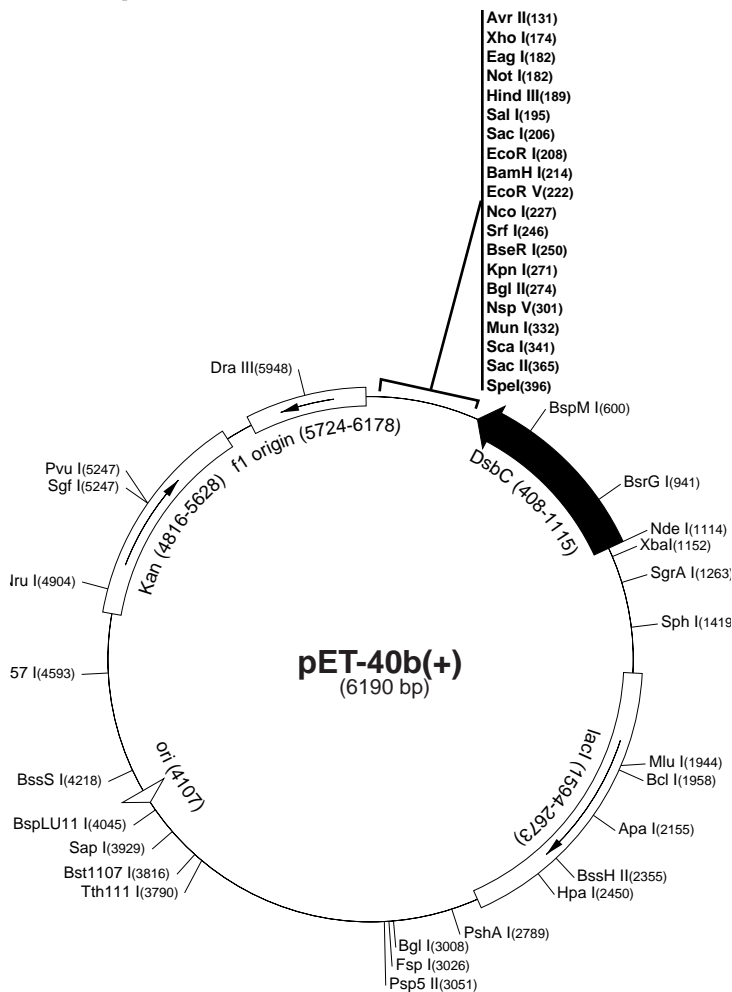


pET-40b(+)⁺ Vector

The pET-40b(+)⁺ vector (Cat. No. 70091-3) is designed for expression of DsbC fusion proteins. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the coding strand. Therefore, single stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

pET-40b(+)⁺ sequence landmarks

T7 promoter	1187-1203
T7 transcription start	1186
DsbC•Tag™ coding seq.	408-1115
His•Tag® coding sequence	369-386
S•Tag™ coding sequence	282-326
Multiple cloning sites (<i>Srf</i> I - <i>Xho</i> I)	174-250
His•Tag coding sequence	150-173
T7 terminator	26-72
<i>lac</i> I coding sequence	1594-2673
pBR322 origin	4107
Kan coding sequence	4816-5628
f1 origin	5724-6178



pET upstream primer #69214-3

ATGCGTCCGGCGTAGAGGATCGAGATCGATCTCGATCCCGCGAAATTAACGACTCACTATAGGGGAATTTGAGCGGATAACAATTCCTCTAGAAATAATTTTGTAACTTTAAGAAGGAGATATACAT

DsbC S-DsbC•Tag primer #70178-3

ATGAAGAAAGGTTTTATGTTTACTTTGTTAGCGCGCTTTTCAGGCTTTGCTCAGGCTGAT...606bp... GAATTTCTCGACGAACACCAAAAAATGACCAGCGGTAAGGATCAACTAGTGGTTCTGGT
Met Lys Lys Gly Phe Met Leu Phe Thr Leu Leu Ala Ala Phe Ser Gly Phe Ala Gln Ala Asp ...202aa... Glu Phe Leu Asp Glu His Gln Lys Met Thr Ser Gly Lys Gly Ser Thr Ser Gly Ser Gly
signal peptidase ↑

His•Tag

thrombin ↓

CATCACCATCACCATCACTCCGCGGGTCTGGTGCCACGCGGTAGTACTGCAATTTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACAGCCAGATCTGGGTACC
His His His His His His Ser Ala Gly Leu Val Pro Arg Gly Ser Thr Ala Ile Ser Met Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser Pro Asp Leu Gly Thr

enterokinase ↓

GATGACGACGACAAGACCCGGGCTTCTCCTCAACCATGGCGATATCGGATCCGAATTCGAGCTCGCAAGCTTGGCGGCCGCACTCGAGCACCACCACCACCACCACCACCCTAA
Asp Asp Asp Asp Lys Ser Pro Gly Phe Ser Ser Thr Met Ala Ile Ser Asp Pro Asn Ser Ser Val Asp Lys Leu Ala Ala Ala Leu Glu His His His His His His End

TTGATTAATACCTAGGCTGCTAAACAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCCTGAGCAATAACTAGCATAACCCTTGGGGCTCTAAACGGGCTTGAAGGGTTTTTTTG

Avr II
Bpu1102 I
T7 terminator primer #69337-3

pET-40b(+)⁺ cloning/expression regions

pET-40b(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
AccI	2	196 3815	DrdI	3	3738 4153 5903	SfiI	5	983 1186 4310 4501 6167
AcII	86		DsaI	4	227 362 1381 3017	SgfI	1	5247
AflIII	3	790 1944 4045	EaeI	4	182 1252 1384 2618	SgrAI	1	1263
AluI	25		EagI	1	182	SmaI	2	246 5121
AlwI	14		EarI	3	1562 3929 5060	SpeI	1	396
Alw26I	6	1641 2046 2172 2559 3686 5263	Eco47III	3	1349 2850 3299	SphI	1	1419
AlwNI	2	677 4461	Eco57I	1	4593	SrfI	1	246
ApaI	1	2155	EcoNI	2	1479 5159	SspI	2	5172 5740
ApaLI	3	1924 3859 4359	EcoO109I	3	53 1377 3051	StyI	3	57 131 227
ApoI	8	208 303 441 2219 4860 5044 5750 5761	EcoRI	1	208	TalI	18	
AvaI	3	174 244 5119	EcoRII	10	676 1667 1982 2522 2579 4071 4192 4205 5135 5492	TaqI	18	
Avall	5	2496 2872 2960 3051 3330	EcoRV	1	222	TfiI	9	2623 2925 3095 3599 4020 5158 5214 5386 5477
AvrII	1	131	FauI	20		ThaI	41	
BamHI	1	214	Fnu4HI	49		TseI	26	
BanI	10	267 352 1266 1287 1401 1864 2583 2713 2839 5985	FokI	9	1990 1999 3264 3326 3404 3590 3731 4885 5491	Tsp45I	8	867 2125 2953 3484 3697 3792 5394 6121
BanII	7	206 251 1328 1342 2155 4902 6023	FspI	1	3026	Tsp509I	25	
BbsI	5	963 2090 2429 2803 3163	HaeII	15		TspRI	12	
BbvI	26		HaeIII	24		Tth111I	1	3790
Bcgl	4	210 2236 2804 3656	HgaI	14		VspI	6	139 1201 2629 2688 5446 5635
Bcgl'	4	176 2270 2770 3622	HhaI	51		XbaI	1	1152
BclI	1	1958	HincII	2	197 2450	XcmI	5	542 567 1800 2316 2334
Bfal	8	70 132 397 1153 3059 4540 4847 6099	HindIII	1	189	XhoI	1	174
BglI	1	3008	HinfI	18		XmnI	2	3603 5636
BglII	1	274	HpaI	1	2450			
BpmI	4	1782 2271 2905 3572	HphI	22				
Bpu10I	3	1059 3151 5264	KpnI	1	271			
Bpu1102I	2	80 493	MaeIII	19				
BsaAI	2	3797 5948	MbolI	14				
BsaBI	3	1217 1227 3242	MluI	1	1944			
BsaHI	6	532 1267 1288 1402 1901 2584	MnlI	26				
BsaJI	14		MseI	27				
BsaWI	8	2 877 2263 2766 3234 4251 4398 5382	MslI	7	784 1996 2284 2314 3032 3227 3618			
BseRI	1	250	MspI	32				
BsgI	3	1795 1995 3205	MspA1I	12				
BsiEI	5	185 2729 3961 4385 5247	MunI	1	332			
BsiHKAI	7	175 206 1444 1928 3039 3863 4363	MwoI	43				
BsII	30		NarI	4	1267 1288 1402 2584			
BsmI	3	838 5131 5208	NciI	15				
BsmBI	3	2559 3686 5263	NcoI	1	227			
BsmFI	4	1405 2946 3316 6163	NdeI	1	1114			
Bsp1286I	13		NgoAIV	4	1254 2842 3002 6049			
BspEI	2	2 3234	NlaIII	28				
BspLU11I	1	4045	NlaIV	25				
BspMI	1	600	NottI	1	182			
BsrI	22		NruI	1	4904			
BsrBI	4	1173 3978 5646 6092	Nsil	2	5097 5363			
BsrDI	3	487 1991 2357	Nspl	4	1419 3390 3682 4049			
BsrFI	7	1254 1263 1630 2842 3002 5201 6049	NspV	1	301			
BsrGI	1	941	PfIMI	4	293 677 1526 5510			
BssHII	1	2355	PleI	9	1201 1493 1580 2376 3939 4424 5479 5883 5891			
BssSI	1	4218	PshAI	1	2789			
Bst1107I	1	3816	Psp1406I	5	1031 1606 2974 3370 5733			
BstEII	2	467 2125	Psp5II	1	3051			
BstXI	3	1746 1875 1998	PvuI	1	5247			
BstYI	8	214 274 1508 2720 3237 4686 4697 5496	PvuII	3	2544 2637 3636			
Cac8I	42		RcaI	3	1342 4765 5640			
Clal	2	1221 4938	RsaI	7	269 341 512 943 2091 3851 5082			
CviJI	97		SacI	1	206			
Ddel	12		SacII	1	365			
DpnI	25		Sall	1	195			
DrallI	1	5948	SapI	1	3929			
			Sau3AI	25				
			Sau96I	15				
			Scal	1	341			
			ScrFI	25				
			SfaNI	26				

Enzymes that do not cut pET-40b(+):

AatII	AflII	AhdI	AscI	BsaI
Bsu36I	DraI	FseI	MscI	NheI
PacI	PinAI	PmeI	PmlI	PstI
RsrII	SanDI	SexAI	SfiI	SnaBI
Sse8387I	StuI	SunI	Swal	UbaEI