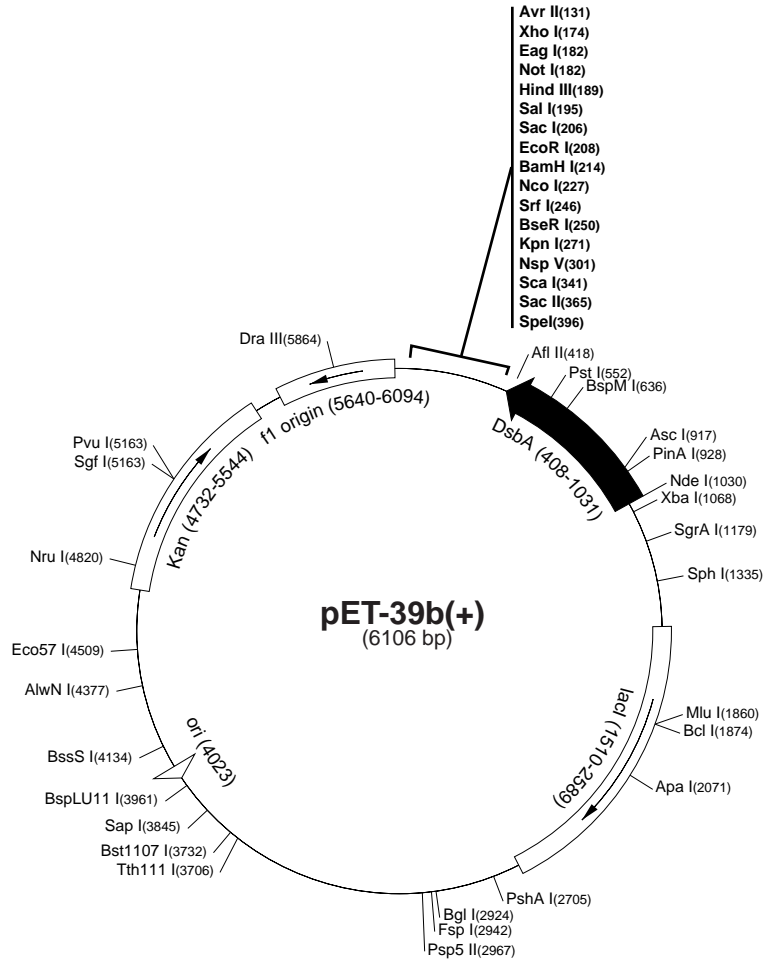


pET-39b(+)TM Vector

The pET-39b(+)TM vector (Cat. No. 70090-3) is designed for expression of DsbA 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-39b(+)TM sequence landmarks

T7 promoter	1103-1119
T7 transcription start	1102
DsbA•Tag TM coding seq.	408-1031
His•Tag [®] coding sequence	369-386
S•Tag TM 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	1510-2589
pBR322 origin	4023
Kan coding sequence	4732-5544
f1 origin	5640-6094



pET upstream primer #69214-3

ATGCGTCCGGCTAGAGGATCGAGATCGATCTCGATCCCGGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCTCTAGAAATAATTTTGTTAACTTTAAGAAGGAGATACAT

DsbA S-DsbA•Tag primer #70177-3

ATGAAAAAGATTGGCTGGCGCTGGCTGGTTAGTTTTAGCGTTTAGCGCATCGGCGCGG...522bp...CAGCAGTATGCTGATACAGTGAATACTTAAGCGAGAAAAAGGATCAACTAGTGGTTCTGGT
Met Lys Lys Ile Trp Leu Ala Leu Ala Gly Leu Val Pro Arg Gly Ser Thr Ala Ile Gly Met Lys Glu Thr Ala Lys Phe Glu Arg Gln His Met Asp Ser Pro Asp Leu Gly Thr

signal peptidase

S•Tag primer #69945-3

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

thrombin

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

enterokinase

Avr II

Bpu1102 I

T7 terminator

TTGATTAACTAGGCTGCTAAACAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCTCTAAACGGGCTTGAGGGGTTTTTGT

T7 terminator primer #69337-3

pET-39b(+)TM cloning/expression regions

pET-39b(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations			
AccI	2	196 3731	DsaI	4	227 362 1297 2933	SgfI	1	5163			
AcII	84		EaeI	4	182 1168 1300 2534	SgrAI	1	1179			
AflII	1	418	EagI	1	182	Smal	2	246 5037			
AflIII	2	1860 3961	EarI	4	618 1478 3845 4976	SpeI	1	396			
AluI	27		Eco47III	3	1265 2766 3215	SphI	1	1335			
AlwI	13		Eco57I	1	4509	SrfI	1	246			
Alw26I	6	1557 1962 2088 2475 3602	EcoNI	2	1395 5075	Sspl	2	5088 5656			
		5179	EcoO109I	3	53 1293 2967	StyI	3	57 131 227			
AlwNI	1	4377	EcoRI	1	208	TalI	16				
ApaI	1	2071	EcoRII	11		TaqI	17				
ApaLI	3	1840 3775 4275	EcoRV	2	222 653	TfiI	10	488 2539 2841 3011 3515			
ApoI	7	208 303 2135 4776 4960	FauI	19				3936 5074 5130 5302 5393			
		5666 5677	Fnu4HI	48		ThaI	40				
AscI	1	917	FokI	10	444 1906 1915 3180 3242	TseI	27				
AvaI	3	174 244 5035			3320 3506 3647 4801 5407	Tsp45I	9	708 773 2041 2869 3400			
Avall	5	2412 2788 2876 2967 3246	FspI	1	2942			3613 3708 5310 6037			
AvrII	1	131	HaeII	16		Tsp509I	24				
BamHI	1	214	HaeIII	23		TspRI	14				
BanI	10	267 352 1182 1203 1317	HgaI	12		Tth111I	1	3706			
		1780 2499 2629 2755 5901	HhaI	52		VspI	6	139 1117 2545 2604 5362			
BanII	7	206 251 1244 1258 2071	HincII	4	197 509 791 2366			5551			
		4818 5939	HindIII	1	189	XbaI	1	1068			
BbsI	5	710 2006 2345 2719 3079	HinfI	20		XcmI	3	1716 2232 2250			
BbvI	27		HpaI	2	509 2366	XhoI	1	174			
BcgI	4	210 2152 2720 3572	HphI	21		XmnI	4	592 861 3519 5552			
BcgI'	4	176 2186 2686 3538	KpnI	1	271						
BclI	1	1874	MaeIII	18		Enzymes that do not cut pET-39b(+):					
Bfal	8	70 132 397 1069 2975	MbolI	19		AatII	AhdI	BsaI	BsrGI	Bsu36I	
		4456 4763 6015	MluI	1	1860	DraI	FseI	MscI	NheI	Pacl	PmeI
BglI	1	2924	MnlI	26		PmlI	RsrII	SanDI	SexAI	SfiI	SnaBI
BglII	2	274 760	MseI	29		Sse8387I	StuI	SunI	Swal	UbaEI	
Bpml	5	888 1698 2187 2821 3488	MslI	6	1912 2200 2230 2948 3143						
Bpu10I	2	3067 5180			3534						
Bpu1102I	2	80 564	MspI	32							
BsaAI	2	3713 5864	MspA1I	13							
BsaBI	3	1133 1143 3158	MunI	2	332 534						
BsaHI	5	1183 1204 1318 1817 2500	MwoI	43							
BsaJI	15		NarI	4	1183 1204 1318 2500						
BsaWI	8	2 928 2179 2682 3150	NciI	14							
		4167 4314 5298	NcoI	1	227						
BseRI	1	250	NdeI	1	1030						
BsgI	3	1711 1911 3121	NgoAIV	4	1170 2758 2918 5965						
BsiEI	5	185 2645 3877 4301 5163	NlaIII	28							
BsiHKAI	7	175 206 1360 1844 2955	NlaIV	23							
		3779 4279	NottI	1	182						
BsII	27		NruI	1	4820						
BsmI	2	5047 5124	Nsil	2	5013 5279						
BsmBI	3	2475 3602 5179	NspI	4	1335 3306 3598 3965						
BsmFI	4	1321 2862 3232 6079	NspV	1	301						
Bsp1286I	13		PfiMI	3	293 1442 5426						
BspEI	2	2 3150	PinAI	1	928						
BspLU11I	1	3961	PleI	10	763 1117 1409 1496 2292						
BspMI	1	636			3855 4340 5395 5799 5807						
BsrI	21		PshAI	1	2705						
BsrBI	4	1089 3894 5562 6008	Psp1406I	4	1522 2890 3286 5649						
BsrDI	2	1907 2273	Psp5II	1	2967						
BsrFI	8	928 1170 1179 1546 2758	PstI	1	552						
		2918 5117 5965	PvuI	1	5163						
BssHII	2	917 2271	PvuII	5	494 547 2460 2553 3552						
BssSI	1	4134	RcaI	3	1258 4681 5556						
Bst1107I	1	3732	RsaI	8	269 341 610 687 949						
BstEII	2	773 2041			2007 3767 4998						
BstXI	4	468 1662 1791 1914	SacI	1	206						
BstYI	9	214 274 760 1424 2636	SacII	1	365						
		3153 4602 4613 5412	Sall	1	195						
Cac8I	45		SapI	1	3845						
Clal	2	1137 4854	Sau3AI	24							
CviJI	93		Sau96I	14							
Ddel	12		Scal	1	341						
DpnI	24		ScrFI	25							
DrallI	1	5864	SfaNI	24							
DrdI	3	3654 4069 5819	Sfcl	5	548 1102 4226 4417 6083						