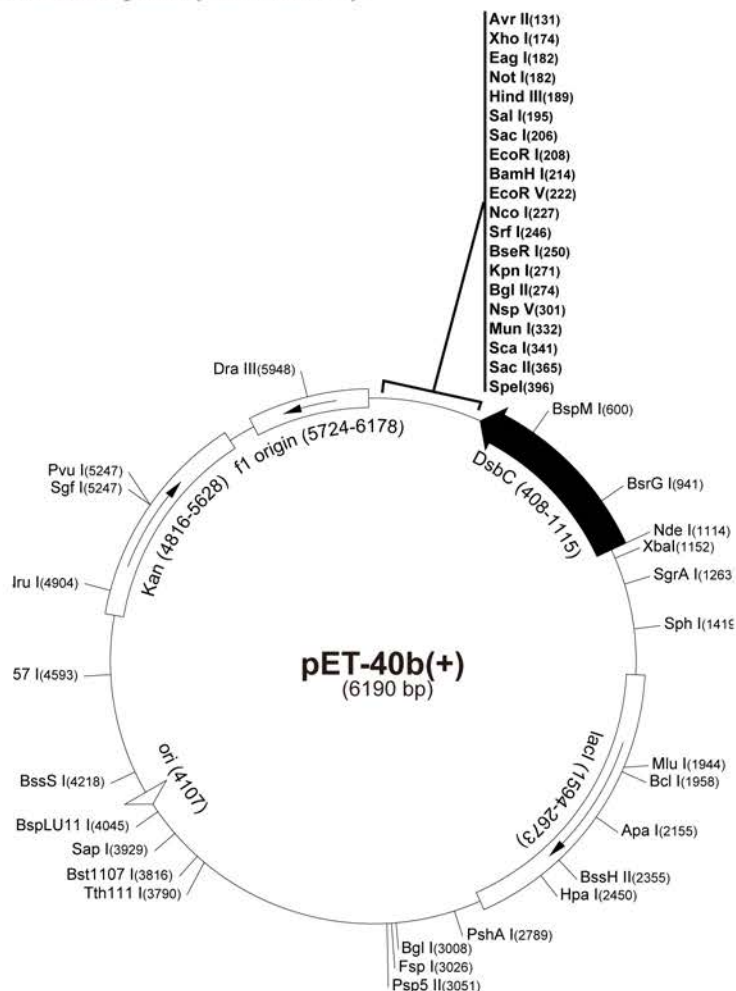


pET-40b(+) Vector

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	
(Srf I - Xho I)	174-250
His•Tag coding sequence	150-173
T7 terminator	26-72
lacI coding sequence	1594-2673
pBR322 origin	4107
Kan coding sequence	4816-5628
f1 origin	5724-6178



pET upstream primer #69214-3

ATGCGTCCGGCTAGAGGATCGAGATCGATCGATCCGCGAAATTAACGACTCACTATAGGGAAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTGTTAACTTTAAGAAGGAGATATACAT

DsbC S-DsbC•Tag primer #70178-3

ATGAAGAAAGGTTTTATGTTGTTACTTTGTTAGCGGCTTTTCAGGCTTTGCTCAGGCTGAT...606bp...GAATTTCTCGACGAACACCAAAAAATGACACCGGTAAGGATCACTAGTGGTTCTGGT
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

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

thrombin

GATGACGACGACAAGAGCCCGGCTTCTCCTAACCATGGCGATATCGGATCCGAATTCGAGCTCCGTCGACAAAGCTTGGCGCCGCACTCGAGCACCACCACCACCACCACCACCACTAA
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 His End

enterokinase

TTGATTAATACCTAGGCTGCTAAACAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCTCTAAACGGGCTTTGAGGGGTTTTTTTG

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	Eco47III	3	1349	2850	3299	SphI	1	1419						
		5263					Eco57I	1	4593			SrfI	1	246						
AlwNI	2	677	4461				EcoNI	2	1479	5159		Sspl	2	5172	5740					
ApaI	1	2155					EcoO109I	3	53	1377	3051	StyI	3	57	131	227				
ApaLI	3	1924	3859	4359			EcoRI	1	208			TalI	18							
ApoI	8	208	303	441	2219	4860	EcoRII	10	676	1667	1982	2522	2579	TaqI	18					
		5044	5750	5761					4071	4192	4205	5135	5492	TfiI	9	2623	2925	3095	3599	4020
AvaI	3	174	244	5119			EcoRV	1	222						5158	5214	5386	5477		
Avall	5	2496	2872	2960	3051	3330	FauI	20						ThaI	41					
AvrII	1	131					Fnu4HI	49						TseI	26					
BamHI	1	214					FokI	9	1990	1999	3264	3326	3404	Tsp45I	8	867	2125	2953	3484	3697
BanI	10	267	352	1266	1287	1401			3590	3731	4885	5491			3792	5394	6121			
		1864	2583	2713	2839	5985	FspI	1	3026					Tsp509I	25					
BanII	7	206	251	1328	1342	2155	Haell	15						TspRI	12					
		4902	6023				HaellI	24						Tth111I	1	3790				
BbsI	5	963	2090	2429	2803	3163	HgaI	14						VspI	6	139	1201	2629	2688	5446
BbvI	26						HhaI	51								5635				
BcgI	4	210	2236	2804	3656		HincII	2	197	2450				XbaI	1	1152				
BcgI'	4	176	2270	2770	3622		HindIII	1	189					XcmI	5	542	567	1800	2316	2334
BclI	1	1958					HinfI	18						XhoI	1	174				
Bfal	8	70	132	397	1153	3059	HpaI	1	2450					XmnI	2	3603	5636			
		4540	4847	6099			HphI	22						Enzymes that do not cut pET-40b(+):						
BglI	1	3008					KpnI	1	271					AatII	AflII	AhdI	AscI		BsaI	
BglII	1	274					MaeIII	19						Bsu36I	DraI	FseI	MscI		NheI	
BpmI	4	1782	2271	2905	3572		MbolI	14						PacI	PinAI	PmeI	PmlI		PstI	
Bpu10I	3	1059	3151	5264			MluI	1	1944					RsrII	SanDI	SexAI	SfiI		SnaBI	
Bpu1102I	2	80	493				MnlI	26						Sse8387I	StuI	SunI	Swal		UbaEI	
BsaAI	2	3797	5948				MseI	27												
BsaBI	3	1217	1227	3242			MslI	7	784	1996	2284	2314	3032							
BsaHI	6	532	1267	1288	1402	1901			3227	3618										
		2584					MspI	32												
BsaJI	14						MspA1I	12												
BsaWI	8	2	877	2263	2766	3234	MunI	1	332											
		4251	4398	5382			MwoI	43												
BseRI	1	250					NarI	4	1267	1288	1402	2584								
BsgI	3	1795	1995	3205			NciI	15												
BsiEI	5	185	2729	3961	4385	5247	NcoI	1	227											
BsiHKA1	7	175	206	1444	1928	3039	NdeI	1	1114											
		3863	4363				NgoAIV	4	1254	2842	3002	6049								
BsiI	30						NlaIII	28												
BsmI	3	838	5131	5208			NlaIV	25												
BsmBI	3	2559	3686	5263			NotI	1	182											
BsmFI	4	1405	2946	3316	6163		NruI	1	4904											
Bsp1286I	13						NsiI	2	5097	5363										
BspEI	2	2	3234				NspI	4	1419	3390	3682	4049								
BspLU11I	1	4045					NspV	1	301											
BspMI	1	600					PilMI	4	293	677	1526	5510								
BsrI	22						PleI	9	1201	1493	1580	2376	3939							
BsrBI	4	1173	3978	5646	6092				4424	5479	5883	5891								
BsrDI	3	487	1991	2357			PshAI	1	2789											
BsrFI	7	1254	1263	1630	2842	3002	Psp1406I	5	1031	1606	2974	3370	5733							
		5201	6049				Psp5II	1	3051											
BsrGI	1	941					PvuI	1	5247											
BssHII	1	2355					PvuII	3	2544	2637	3636									
BssSI	1	4218					RcaI	3	1342	4765	5640									
Bst1107I	1	3816					RsaI	7	269	341	512	943	2091							
BstEII	2	467	2125						3851	5082										
BstXI	3	1746	1875	1998			SacI	1	206											
BstYI	8	214	274	1508	2720	3237	SacII	1	365											
		4686	4697	5496			Sall	1	195											
Cac8I	42						SapI	1	3929											
Clal	2	1221	4938				Sau3AI	25												
CviJI	97						Sau96I	15												
DdeI	12						Scal	1	341											
DpnI	25						ScrFI	25												
DrallI	1	5948					SfaNI	26												