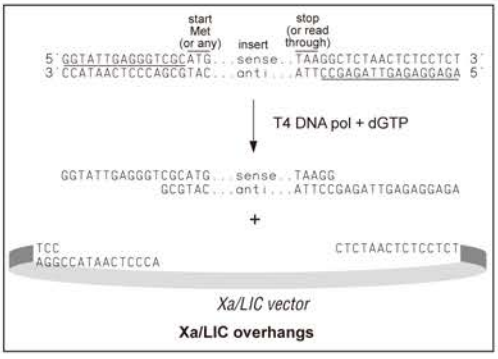
pET-35b(+) Vector

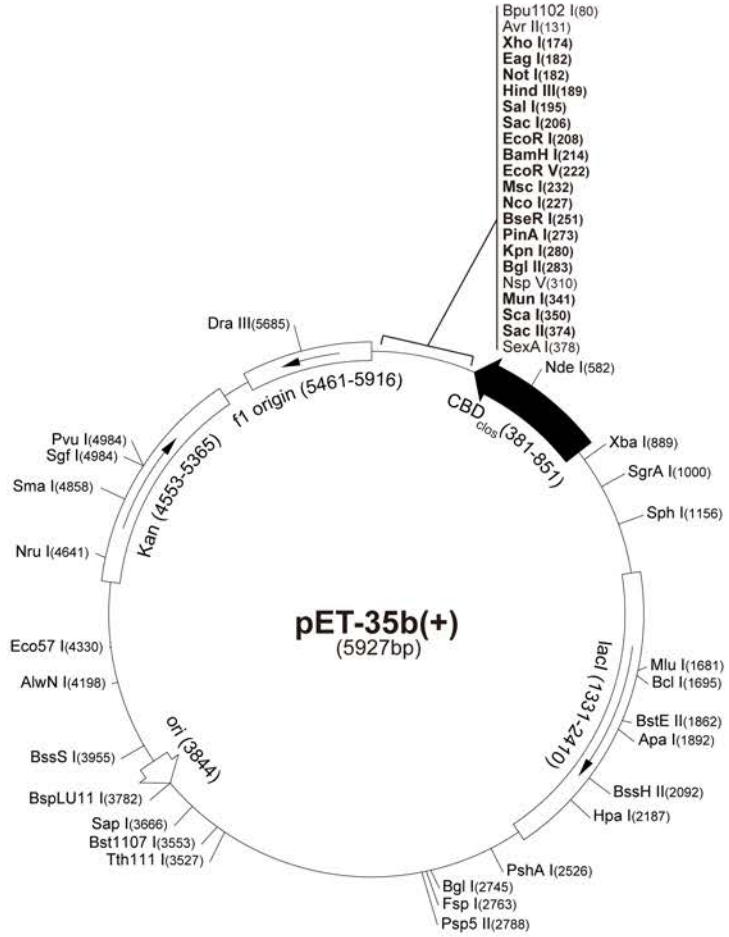
The pET-35b(+) (Cat. No. 70103-3) vector is designed for expression of CBD_{clos} fusion proteins. A variety of cloning sites and strategies are available. Rapid directional cloning of PCR-amplified DNA is available with the pET-35 Xa/LIC Vector Kit (Cat. No. 70115-3), which contains linearized pET-35b(+) ready for annealing with appropriately prepared inserts. When cloned with the Xa/LIC method, resulting CBD_{clos}•Tag™ fusion proteins can be cleaved precisely at the vector-encoded junction using Factor Xa. 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).

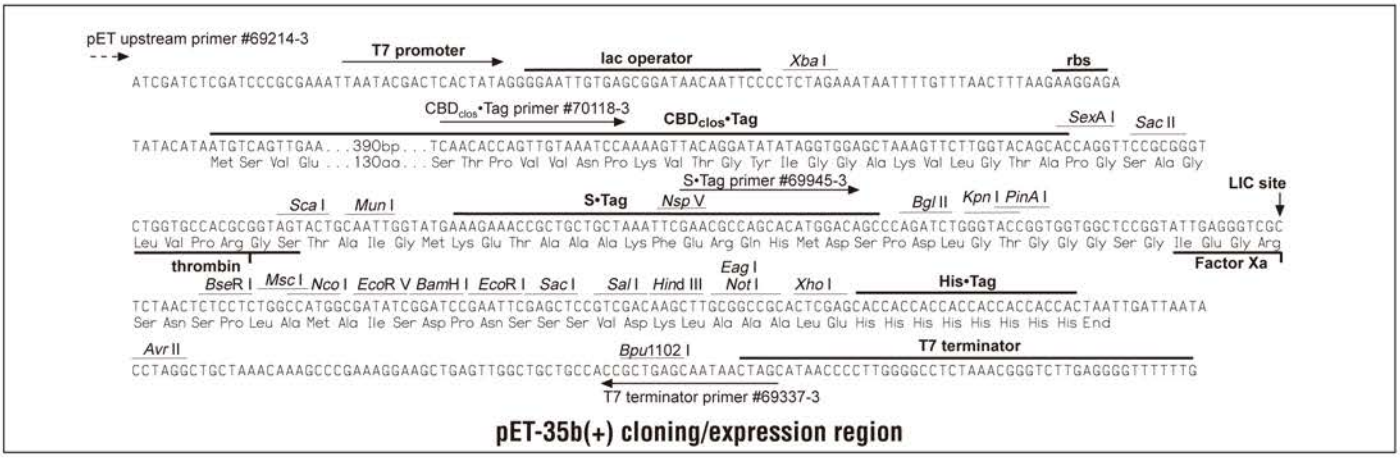
pET-35b(+) sequence landmarks

T7 promoter	924-940
T7 transcription start	923
CBD_{clos}•Tag coding seq.	381-851
S•Tag coding sequence	291-335
Multiple cloning sites	
(*Bse*R I - *Xho* I)	174-241
His•Tag coding sequence	150-173
T7 terminator	26-72
lacI coding sequence	1331-2410
pBR322 origin	3844
Kan coding sequence	4553-5365
f1 origin	5461-5916

Note: Primer sequence extensions required for Xa/LIC compatibility are underlined in the diagram below.







pET-35b(+) cloning/expression region

pET-35b(+) Restriction Sites

Enzyme	# Sites	Locations				
AccI	2	196	3552			
AciI	78					
AflIII	2	1681	3782			
AluI	25					
AlwI	12					
Alw26I	6	1378	1783	1909	2296	3423
		5000				
AlwNI	1	4198				
ApaI	1	1892				
ApaLI	3	1661	3596	4096		
ApoI	10	208	312	573	754	837
		1956	4597	4781	5487	5498
AvaI	2	174	4856			
AvaII	5	2233	2609	2697	2788	3067
AvrII	1	131				
BamHI	1	214				
BanI	10	276	361	1003	1024	1138
		1601	2320	2450	2576	5722
BanII	6	206	1065	1079	1892	4639
		5760				
BbsI	4	1827	2166	2540	2900	
BbvI	26					
BcgI	4	210	1973	2541	3393	
BcgI'	4	176	2007	2507	3359	
BclI	1	1695				
BfaI	8	70	132	643	890	2796
		4277	4584	5836		
BglI	1	2745				
BglII	1	283				
BpmI	4	1519	2008	2642	3309	
Bpu10I	2	2888	5001			
Bpu1102I	1	80				
BsaAI	2	3534	5685			
BsaBI	3	954	964	2979		
BsaHI	5	1004	1025	1139	1638	2321
BsaJI	12					
BsaWI	9	2	261	273	2000	2503
		2971	3988	4135	5119	
BseRI	1	251				
BsgI	3	1532	1732	2942		
BsiEI	5	185	2466	3698	4122	4984
BsiHKAI	7	175	206	1181	1665	2776
		3600	4100			
BsiI	26					
BsmI	2	4868	4945			
BsmBI	3	2296	3423	5000		
BsmFI	4	1142	2683	3053	5900	
Bsp1286I	12					
BspEI	2	2	2971			
BspLU11I	1	3782				
BsrI	23					
BsrBI	4	910	3715	5383	5829	
BsrDI	2	1728	2094			
BsrFI	8	273	991	1000	1367	2579
		2739	4938	5786		
BssHII	1	2092				
BssSI	1	3955				
Bst1107I	1	3553				
BstEII	1	1862				
BstXI	3	1483	1612	1735		
BstYI	8	214	283	1245	2457	2974
		4423	4434	5233		
Cac8I	42					
ClaI	2	958	4675			
CviJI	89					
DdeI	10	80	101	2253	2888	3050
		3590	4057	4466	5001	5365
DpnI	22					
DraI	2	753	759			
DraIII	1	5685				
DrdI	3	3475	3890	5640		
DsaI	4	227	371	1118	2754	

Enzyme	# Sites	Locations				
EaeI	5	182	230	989	1121	2355
EagI	1	182				
EarI	3	1299	3666	4797		
Eco47III	3	1086	2587	3036		
Eco57I	1	4330				
EcoNI	2	1216	4896			
EcoO109I	3	53	1114	2788		
EcoRI	1	208				
EcoRII	10	378	1404	1719	2259	2316
		3808	3929	3942	4872	5229
EcoRV	1	222				
FauI	18					
Fnu4HI	44					
FokI	9	1727	1736	3001	3063	3141
		3327	3468	4622	5228	
FspI	1	2763				
HaeII	14					
HaeIII	24					
HgaI	11					
HhaI	46					
HincII	2	197	2187			
HindIII	1	189				
HinfI	18					
HpaI	1	2187				
HphI	16					
KpnI	1	280				
MaeIII	19					
MboII	13					
MluI	1	1681				
MnlI	27					
MscI	1	232				
MseI	30					
MslI	7	681	1733	2021	2051	2769
		2964	3355			
MspI	30					
MspA1I	10	84	325	373	1711	2281
		2374	3373	3492	4124	4369
MunI	1	341				
MwoI	39					
NarI	4	1004	1025	1139	2321	
NciI	12					
NcoI	1	227				
NdeI	1	582				
NgoAIV	4	991	2579	2739	5786	
NlaIII	27					
NlaIV	25					
NotI	1	182				
NruI	1	4641				
NsiI	2	4834	5100			
NspI	4	1156	3127	3419	3786	
NspV	1	310				
PflMI	3	302	1263	5247		
PinAI	1	273				
PleI	9	938	1230	1317	2113	3676
		4161	5216	5620	5628	
PshAI	1	2526				
Psp1406I	4	1343	2711	3107	5470	
Psp5II	1	2788				
PvuI	1	4984				
PvuII	3	2281	2374	3373		
RcaI	3	1079	4502	5377		
RsaI	7	278	350	389	713	1828
		3588	4819			
SacI	1	206				
SacII	1	374				
SalI	1	195				
SapI	1	3666				
Sau3AI	22					
Sau96I	14					
ScaI	1	350				
ScrFI	22					
SexAI	1	378				

Enzyme	# Sites	Locations				
SfaNI	24					
SfcI	4	923	4047	4238	5904	
SgfI	1	4984				
SgrAI	1	1000				
SmaI	1	4858				
SphI	1	1156				
SspI	2	4909	5477			
StyI	3	57	131	227		
SwaI	2	753	759			
TaiI	15					
TaqI	17					
TfiI	9	2360	2662	2832	3336	3757
		4895	4951	5123	5214	
ThaI	37					
TseI	26					
Tsp45I	9	633	686	1862	2690	3221
		3434	3529	5131	5858	
Tsp509I	29					
TspRI	13					
Tth111I	1	3527				
VspI	6	139	938	2366	2425	5183
		5372				
XbaI	1	889				
XcmI	3	1537	2053	2071		
XhoI	1	174				
XmnI	2	3340	5373			

Enzymes that do not cut pET-35b(+):

AatII	AflII	AhdI	AscI	BsaI
BspMI	BsrGI	Bsu36I	FseI	NheI
PacI	PmeI	PmlI	PstI	RsrII
SanDI	SfiI	SnaBI	SpeI	SrfI
Sse8387I	StuI	SunI	UbaEI	