

pET-34b(+)⁺ Vector

Developed through collaboration between
Novagen and CBD Technologies, Inc.

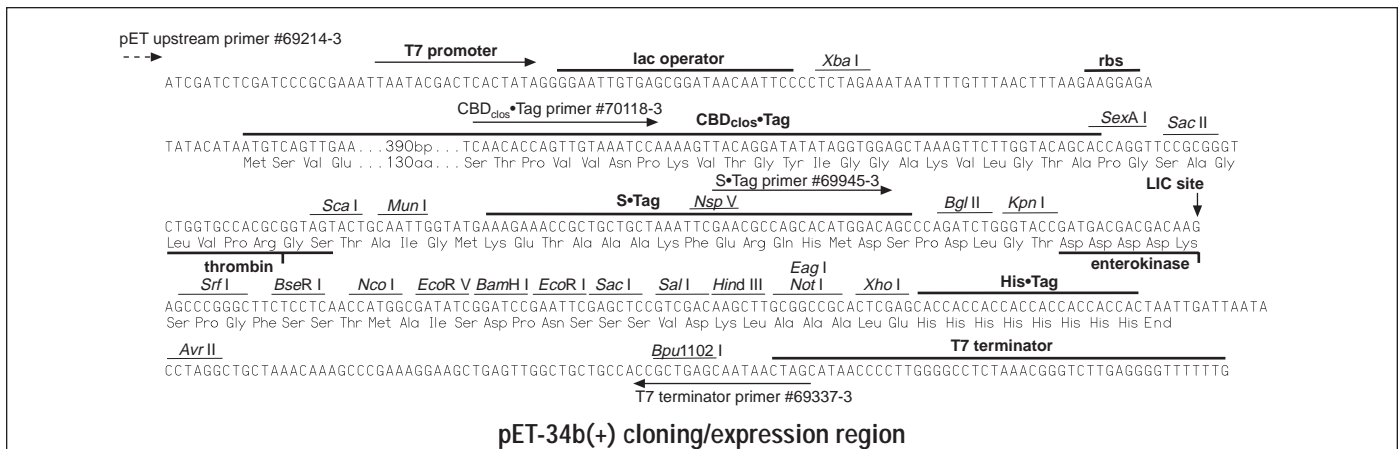
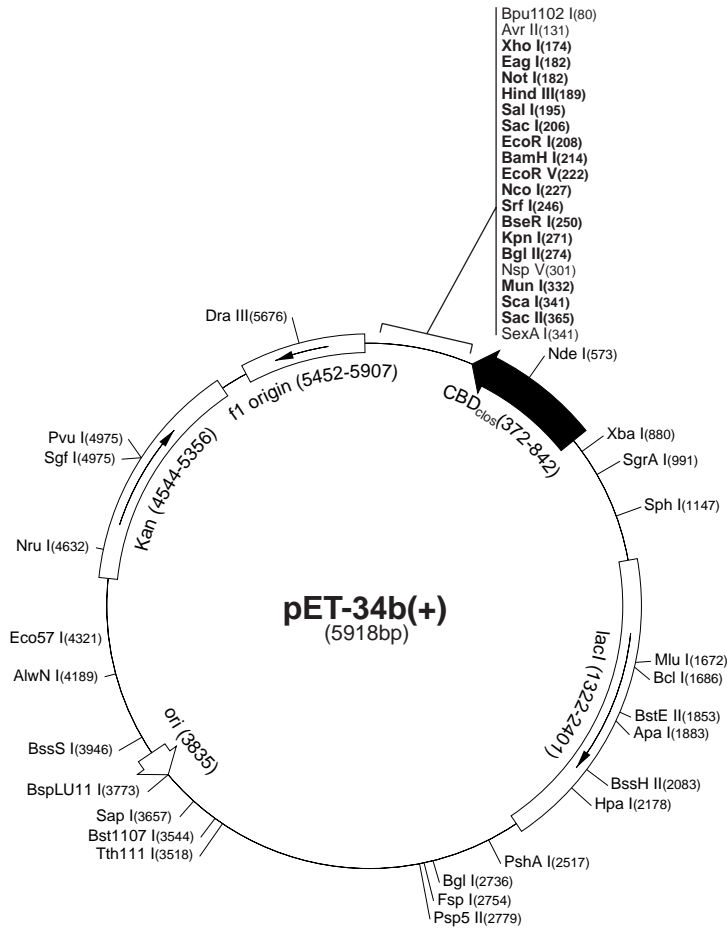
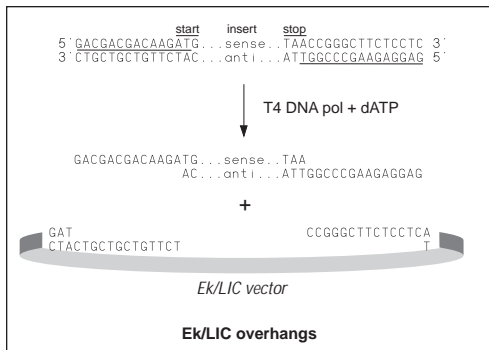


The pET-34b(+)⁺ vector (Cat. No. 70102-3) is designed for expression of CBD_{clos} fusion proteins. A variety of cloning sites and strategies are available. Rapid directional cloning of PCR-amplified DNA is available with the pET-34 Ek/LIC Vector Kit (Cat. No. 70114-3), which contains linearized pET-34b(+)⁺ ready for annealing with appropriately prepared inserts. When cloned with the LIC method, resulting CBD_{clos}•TagTM fusion proteins can be cleaved precisely at the vector-encoded junction using enterokinase. 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-34b(+)⁺ sequence landmarks

T7 promoter	915-931
T7 transcription start	914
CBD _{clos} •Tag coding seq.	372-842
S•Tag coding sequence	284-326
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	1332-2401
pBR322 origin	3835
Kan coding sequence	4544-5356
f1 origin	5452-5907

Notes: the *Srf*I site is destroyed during Ligation Independent Cloning. Primer sequence extensions required for LIC compatibility are underlined in the diagram below.



pET-34b(+) Restriction Sites

Enzyme	# Sites	Locations				
AccI	2	196	3543			
AcII	78					
AflIII	2	1672	3773			
AluI	25					
AlwI	12					
Alw26I	6	1369	1774	1900	2287	3414
		4991				
AlwNI	1	4189				
ApaI	1	1883				
ApaLI	3	1652	3587	4087		
ApoI	10	208	303	564	745	828
		1947	4588	4772	5478	5489
AvaI	3	174	244	4847		
Avall	5	2224	2600	2688	2779	3058
AvrII	1	131				
BamHI	1	214				
BanI	10	267	352	994	1015	1129
		1592	2311	2441	2567	5713
BanII	7	206	251	1056	1070	1883
		4630	5751			
BbsI	4	1818	2157	2531	2891	
BbvI	26					
Bcgl	4	210	1964	2532	3384	
Bcgl'	4	176	1998	2498	3350	
BclI	1	1686				
Bfal	8	70	132	634	881	2787
		4268	4575	5827		
BglI	1	2736				
BglII	1	274				
BpmI	4	1510	1999	2633	3300	
Bpu10I	2	2879	4992			
Bpu1102I	1	80				
BsaAI	2	3525	5676			
BsaBI	3	945	955	2970		
BsaHI	5	995	1016	1130	1629	2312
BsaJI	13					
BsaWI	7	2	1991	2494	2962	3979
		4126	5110			
BseRI	1	250				
BsgI	3	1523	1723	2933		
BsiEI	5	185	2457	3689	4113	4975
BsiHKAI	7	175	206	1172	1656	2767
		3591	4091			
BsII	26					
BsmI	2	4859	4936			
BsmBI	3	2287	3414	4991		
BsmFI	4	1133	2674	3044	5891	
Bsp1286I	13					
BspEI	2	2	2962			
BspLU111	1	3773				
BsrI	23					
BsrBI	4	901	3706	5374	5820	
BsrDI	2	1719	2085			
BsrFI	7	982	991	1358	2570	2730
		4929	5777			
BssHII	1	2083				
BssSI	1	3946				
Bst1107I	1	3544				
BstEII	1	1853				
BstXI	3	1474	1603	1726		
BstYI	8	214	274	1236	2448	2965
		4414	4425	5224		
Cac8I	42					
Clal	2	949	4666			
CviJI	89					
Ddel	10	80	101	2244	2879	3041
		3581	4048	4457	4992	5356
Dpnl	22					
Dral	2	744	750			
DrallI	1	5676				
DrdI	3	3466	3881	5631		
Dsal	4	227	362	1109	2745	
EaeI	4	182	980	1112	2346	
EagI	1	182				
EarI	3	1290	3657	4788		
Eco47III	3	1077	2578	3027		
Eco57I	1	4321				
EcoNI	2	1207	4887			
EcoO109I	3	53	1105	2779		
EcoRI	1	208				
EcoRII	10	369	1395	1710	2250	2307
		3799	3920	3933	4863	5220
EcoRV	1	222				
FauI	18					
Fnu4HI	44					
FokI	9	1718	1727	2992	3054	3132
		3318	3459	4613	5219	
FspI	1	2754				
HaeII	14					
HaeIII	23					
Hgal	11					
HhaI	46					
HincII	2	197	2178			
HindIII	1	189				
HinfI	18					
HpaI	1	2178				
HphI	16					
KpnI	1	271				
MaeIII	19					
MbolI	13					
MluI	1	1672				
MnlI	26					
MseI	30					
MsiI	7	672	1724	2012	2042	2760
		2955	3346			
MspI	29					
MspA1I	10	84	316	364	1702	2272
		2365	3364	3483	4115	4360
MunI	1	332				
MwoI	39					
NarI	4	995	1016	1130	2312	
NciI	14					
NcoI	1	227				
NdeI	1	573				
NgoAIV	4	982	2570	2730	5777	
NlaIII	27					
NlaIV	24					
NottI	1	182				
NruI	1	4632				
NsiI	2	4825	5091			
Nspl	4	1147	3118	3410	3777	
NspV	1	301				
PfiMI	3	293	1254	5238		
PleI	9	929	1221	1308	2104	3667
		4152	5207	5611	5619	
PshAI	1	2517				
Psp1406I	4	1334	2702	3098	5461	
Psp5II	1	2779				
PvuI	1	4975				
PvuII	3	2272	2365	3364		
RcaI	3	1070	4493	5368		
RsaI	7	269	341	380	704	1819
		3579	4810			
SacI	1	206				
SacII	1	365				
SalI	1	195				
SapI	1	3657				
Sau3AI	22					
Sau96I	14					
Scal	1	341				
ScrFI	24					
SexAI	1	369				
SfaNI	24					
Sfcl	4	914	4038	4229	5895	
SgfI	1	4975				
SgrAI	1	991				
SmaI	2	246	4849			
SphI	1	1147				
SrfI	1	246				
Sspl	2	4900	5468			
SlyI	3	57	131	227		
Swal	2	744	750			
Tail	15					
TaqI	17					
TfiI	9	2351	2653	2823	3327	3748
		4886	4942	5114	5205	
Thal	37					
Tsel	26					
Tsp45I	9	624	677	1853	2681	3212
		3425	3520	5122	5849	
Tsp509I	29					
TspRI	13					
Tth111I	1	3518				
VspI	6	139	929	2357	2416	5174
		5363				
XbaI	1	880				
XcmI	3	1528	2044	2062		
XhoI	1	174				
XmnI	2	3331	5364			

Enzymes that do not cut pET-34b(+):					
AatII	AflII	AhdI	AscI	BsaI	
BspMI	BsrGI	Bsu36I	FseI	MscI	
NheI	PacI	PinAI	PmeI	PmlI	
PstI	RsrII	SanDI	SfiI	SnaBI	
SpeI	Sse8387I	StuI	SunI	UbaEI	