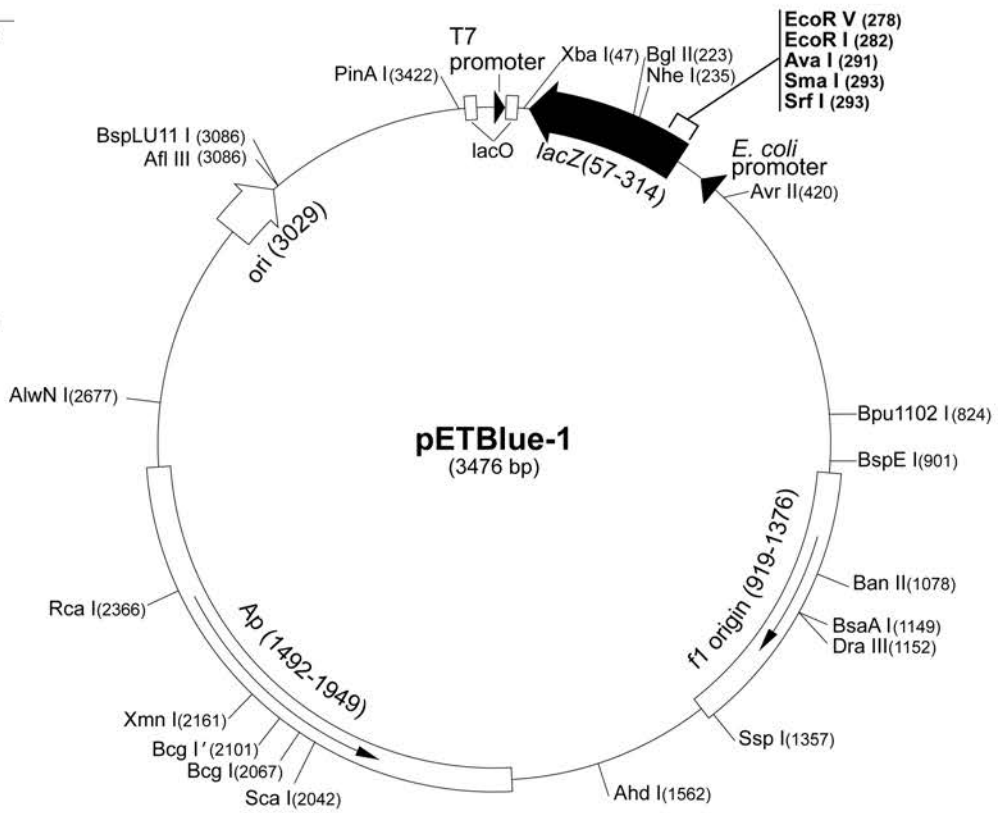
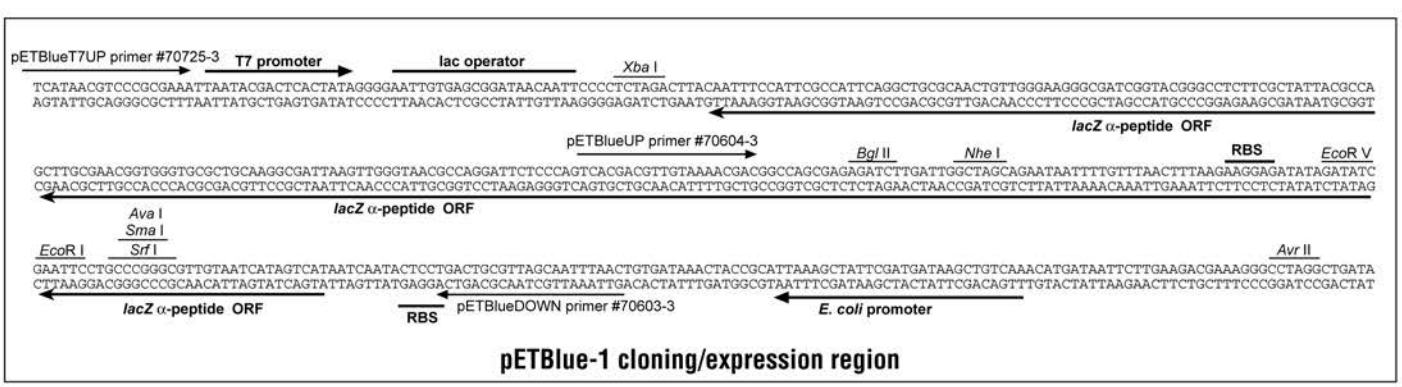
pETBlue-1 Vector

The pETBlue™ vectors are designed to identify recombinants by traditional blue/white screening while also allowing T7*lac* promoter based expression of target genes. Screening is independent of expression because the T7*lac* expression promoter is in an opposed orientation relative to the *E. coli* promoter that mediates blue/white screening. pETBlue-1 is a vector that facilitates the expression of native unfused proteins and allows convenient subcloning of target genes already fused to existing detection and purification tags. The *Eco*R V cloning site is appropriately spaced downstream of an *E. coli* ribosome binding site. Inserts must encode an ATG start codon at their 5' end if expression is desired. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The sequence is numbered from the first base of the T7 promoter sequence. Unique sites are shown on the circle map. The f1 origin in pETBlue-1 is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the T7 RNA polymerase coding strand. Therefore, single stranded sequencing should be performed using the pETBlueDOWN primer (Cat. No. 70603-3).

pETBlue-1 sequence landmarks

lac operator	3429–3448
T7 promoter	1–17
lac operator	22–42
T7 transcription start	18
multiple cloning region (*Eco*R V–*Srf* I)	276–297
lacZ start codon	314
lacZ α-peptide ORF	57–314
E. coli promoter	364–392
f1 origin	919–1374
bla coding sequence	1492–2349
pUC origin	3029





pETBlue-1 cloning/expression region

pETBlue-1 Restriction Sites

Enzyme	# Sites	Locations				
AciI	39					
AflIII	1	3086				
AhdI	1	1562				
AluI	13					
AlwI	8	1414	1415	1878	2195	2199
		2440	2442	2528		
Alw26I	2	532	1623			
AlwNI	1	2677				
ApaLI	2	2229	2772			
ApoI	4	282	437	1332	1343	
AvaI	1	291				
AvaII	5	464	1700	1922	3248	3336
AvrII	1	420				
BanI	3	1108	1510	3368		
BanII	1	1078				
BbsI	2	414	3404			
BbvI	12					
BcgI	1	2067				
BcgI'	1	2101				
BfaI	8	48	236	421	835	996
			1417	1752	2593	
BglI	2	75	1682			
BglII	1	223				
BpmI	2	1632	3308			
Bpu1102I	1	824				
BsaI	2	532	1623			
BsaAI	1	1149				
BsaHI	2	705	2099			
BsaJI	4	291	420	846	2926	
BsaWI	5	901	1853	2733	2880	3422
BsiEI	6	103	1932	2081	2752	3176
		3423				
BsiHKAI	3	2148	2233	2776		
BslI	8	692	860	930	1256	2608
		2887	3053	3071		
BsmFI	4	450	930	3261	3450	
Bsp1286I	4	1078	2148	2233	2776	
BspEI	1	901				
BspLU11I	1	3086				
BsrI	12					
BsrBI	4	30	658	1005	3157	
BsrDI	2	1623	1797			
BsrFI	5	1044	1642	3205	3365	3422
BssSI	3	2226	2913	3449		
BstYI	7	223	1407	1419	2187	2204
		2434	2445			
Cac8I	19					
CviJI	52					
DdeI	8	488	578	634	824	1519
		2059	2403	2812		
DpnI	16					
DraI	3	1428	1447	2139		
DraIII	1	1152				
DrdI	2	1196	2984			
EaeI	2	212	1950			
EarI	2	120	2357			
Eco47III	2	3361	3438			
Eco57I	2	2229	2544			
EcoO109I	2	417	851			
EcoRI	1	282				
EcoRII	7	177	443	553	721	2925
		2938	3059			
EcoRV	1	278				
FauI	6	689	716	941	1010	3238
		3475				
Fnu4HI	23					
FokI	5	733	769	1528	1709	1996
FspI	2	82	1784			
HaeII	6	511	994	1002	2846	3363
		3440				
HaeIII	19					
HgaI	5	713	927	2088	2396	2974

Enzyme	# Sites	Locations				
HhaI	22					
HinfI	9	7	182	1197	1219	1557
		2716	3112	3187	3283	
HphI	8	633	1149	1405	1632	2048
		2254	2289	3437		
MaeIII	14					
MboII	9	107	419	1010	1402	2157
		2235	2344	2429	3409	
MnlI	17					
MseI	21					
MslI	3	1814	1973	2332		
MspI	17					
MspA1I	4	823	2197	2503	2748	
MwoI	14					
NciI	7	292	293	653	686	1745
		2096	2709			
NgoAIV	3	1044	3205	3365		
NheI	1	235				
NlaIII	11					
NlaIV	13					
NspI	1	3090				
PinAI	1	3422				
PleI	6	1	1205	1213	1551	2710
		3195				
Psp1406I	5	668	1362	1788	2161	3235
PvuI	2	103	1932			
RcaI	1	2366				
RsaI	2	107	2042			
Sau3AI	16					
Sau96I	13					
ScaI	1	2042				
ScrFI	14					
SfaNI	6	567	735	1818	2009	2258
		2989				
SfcI	5	13	926	1803	2630	2821
SmaI	1	293				
SrfI	1	293				
SspI	1	1357				
StyI	2	420	846			
TaiI	12					
TaqI	6	280	375	583	1114	2213
		2988				
TfiI	3	182	3112	3283		
ThaI	11					
TseI	12					
Tsp45I	6	191	971	1818	2029	3253
		3451				
Tsp509I	17					
TspRI	9	1510	1615	1962	1989	2407
		2678	2691	3197	3258	
VspI	1	1734				
XbaI	1	47				
XmnI	1	2161				

Enzymes that do not cut pETBlue-1:

AatII	AccI	AflII	ApaI	AscI	BamHI
BclI	Bpu10I	BsaBI	BseRI	BsgI	BsmI
BsmBI	BspMI	BsrGI	BssHII	Bst1107I	BstEII
BstXI	Bsu36I	ClaI	DsaI	EagI	EcoNI
FseI	HincII	HindIII	HpaI	KpnI	MluI
MscI	MunI	NarI	NcoI	NdeI	NotI
NruI	NsiI	NspV	PacI	PflMI	PmeI
PmlI	PshAI	Psp5II	PstI	PvuII	RsrII
SacI	SacII	SalI	SanDI	SapI	SexAI
SfiI	SgfI	SgrAI	SnaBI	SpeI	SphI
Sse8387I	StuI	SunI	SwaI	Tth111I	UbaEI
XcmI	XhoI				