pETBlue-1 Vector

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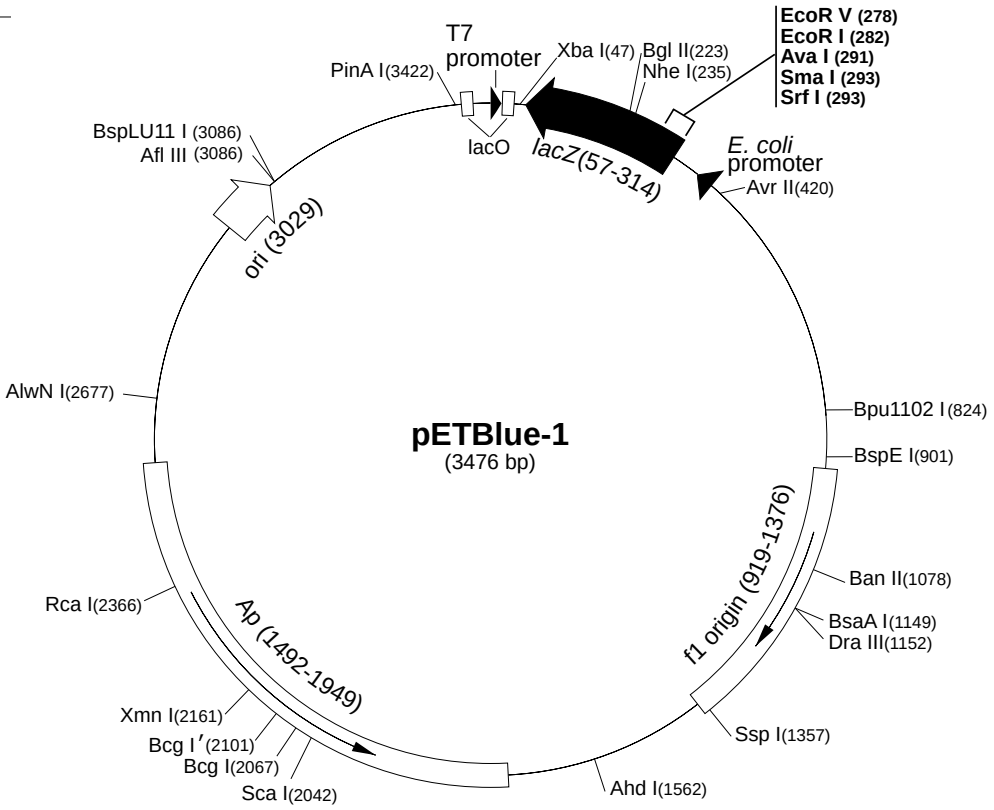
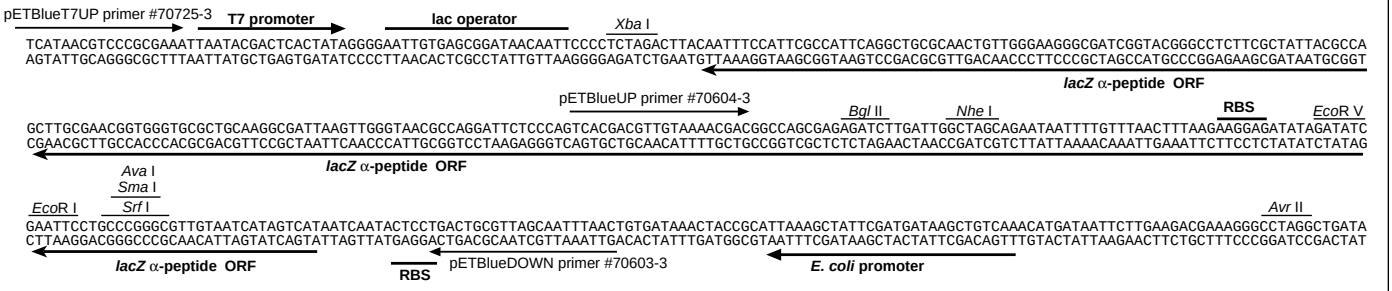
The pETBlue™ vectors are designed to identify recombinants by traditional blue/white screening while also allowing T7*lac* promoter based expression of target genes. Screening is independent of expression because the T7*lac* expression promoter is in an opposed orientation relative to the *E. coli* promoter 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

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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			
BsiI	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				