pETDuet-1 Vector

	Cat. No.
pETDuet-1 DNA	71146-3

pETDuet-1 sequence landmarks

T7 promoter-1	5404–5420
T7 transcription start-1	1
His•Tag® coding sequence	83–100
Multiple cloning sites-1 (Nco I–Afl II)	69–168
T7 promoter-2	214–230
T7 transcription start-2	231
Multiple cloning sites-2 (Nde I–Avr II)	297–438
S•Tag™ coding sequence	366–410
T7 terminator	462–509
lacI coding sequence	3931–5013
pBR322 origin	2737
bla (Ap) coding sequence	1119–1976
f1 origin	547–994

pETDuet™-1 is designed for the coexpression of two target genes. The vector contains two multiple cloning sites (MCS), each of which is preceded by a T7 promoter/lac operator and a ribosome binding site (rbs). The vector also carries the pBR322-derived ColE1 replicon, lacI gene and ampicillin resistance gene. This vector can be used in combination with pACYCDuet™-1 (Cat. No. 71147-3) in an appropriate host strain for the coexpression of up to 4 target genes. Genes inserted into MCS1 can be sequenced using the pET Upstream Primer (Cat. No. 69214-3) and DuetDOWN1 Primer (Cat. No. 71179-3). Genes inserted into MCS2 can be sequenced using the DuetUP2 Primer (Cat. No. 71180-3) and T7 Terminator Primer (Cat. No. 69337-3).

pETDuet-1 cloning/expression regions
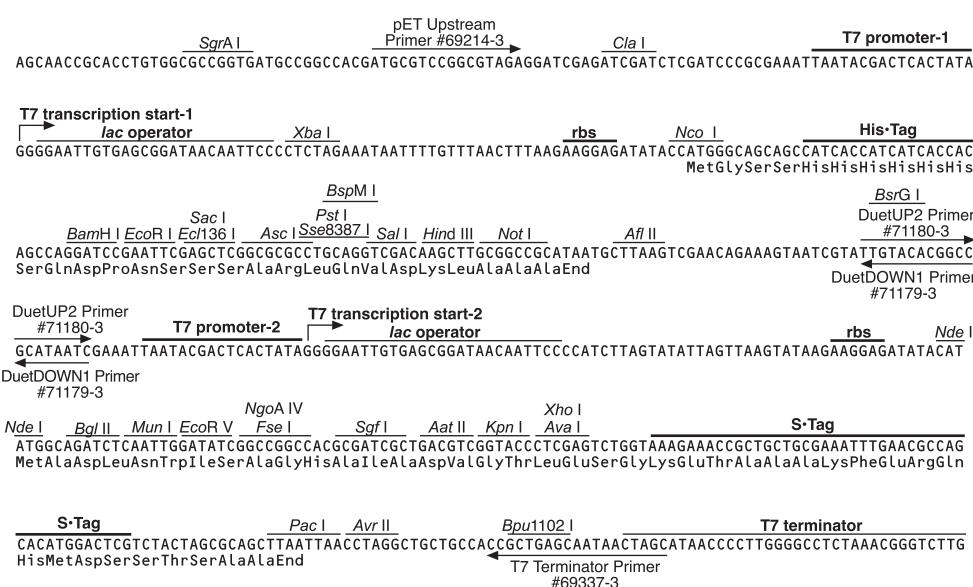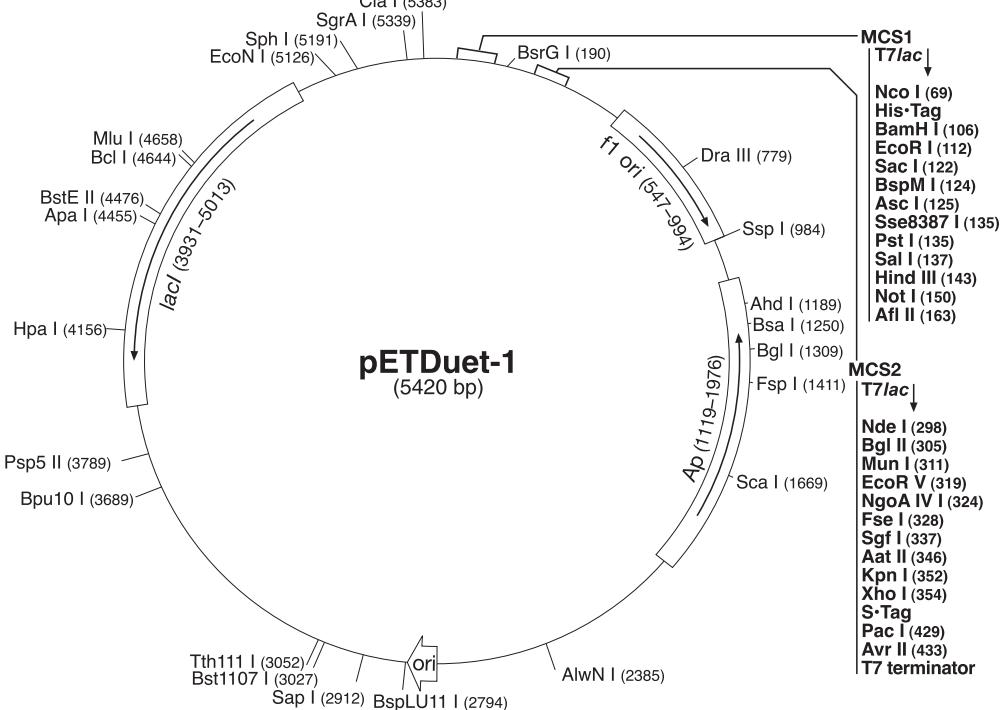
pETDuet-1 Restriction Sites

Enzyme	# Sites	Locations				
AatII	1	346				
AccI	3	138	411	3026		
AciI	78					
AflIII	1	163				
AflIII	2	2794	4658			
AhdI	1	1189				
AluI	25					
Alw26I	7	1250	2027	3153	4043	4430
		4556	4961			
AlwI	15					
AlwNI	1	2385				
ApaI	1	4455				
ApaLI	4	1856	2480	2980	4678	
ApoI	5	112	384	959	970	4383
AscI	1	125				
AvaI	1	354				
AvaII	5	1327	1549	3510	3789	4107
AvrII	1	433				
BamHI	1	106				
BanI	9	348	735	1137	3889	4019
		4738	5201	5315	5336	
BanII	5	122	705	4455	5268	5282
BbsI	3	3676	4173	4512		
BbvI	28					
BcgI	4	162	1728	3223	4338	
BclI	1	4644				
BfaI	10	31	415	434	462	623
		1044	1379	2301	3782	3817
BglI	1	1309				
BglII	1	305				
BpmI	4	1259	3273	4337	4826	
Bpu10I	1	3689				
Bpu1102I	1	451				
BsaAI	2	776	3046			
BsaBI	3	3601	5379	5389		
BsaHI	7	343	1726	4020	4703	5202
		5316	5337			
BsaI	1	1250				
BsaJI	7	69	433	473	2634	4023
		5215	5221			
BsaWI	7	528	1480	2441	2588	3605
		3836	4339			
BsgI	3	3640	4613	4813		
BsiEI	9	153	199	325	337	1559
		1708	2460	2884	3879	
BsiHKAI	8	122	1775	1860	2484	2984
		3808	4682	5166		
BslI	22					
BsmBI	2	3153	4043			
BsmFI	3	557	3523	5197		
Bsp1286I	12					
BspEI	2	528	3605			
BspLU11I	1	2794				
BspMI	1	124				
BsrBI	5	13	243	632	2031	2865
BsrDI	4	1250	1424	4251	4617	
BsrFI	6	324	671	1269	4972	5339
		5348				
BsrGI	1	190				
BsrI	24					
BssHII	2	125	4247			
BssSI	2	1853	2621			
Bst1107I	1	3027				
BstEII	1	4476				
BstXI	3	4612	4735	4864		
BstYI	11					
Cac8I	36					
ClaI	1	5383				
CviJI	84					
DdeI	10	262	451	1146	1686	2111
		2520	2987	3527	3689	4087
DpnI	28					

Enzyme	# Sites	Locations				
DraI	3	1055	1074	1766		
DraIII	1	779				
DrdI	3	823	2692	3107		
DsaI	2	69	5221			
EaeI	8	150	196	322	326	1577
		3984	5218	5350		
EagI	3	150	196	322		
EarI	3	1984	2911	5041		
Ecl136II	1	120				
Eco47III	2	3544	5257			
Eco57I	2	1856	2252			
EcoNI	1	5126				
EcoO109I	3	478	3789	5226		
EcoRI	1	112				
EcoRII	8	102	2633	2646	2767	4022
		4079	4619	4934		
EcoRV	1	319				
EheI	4	4021	5203	5317	5338	
FauI	16					
Fnu4HI	48					
FokI	10	1155	1336	1623	3108	3249
		3435	3513	3575	4603	4612
FseI	1	328				
FspI	1	1411				
HaeII	13					
HaeIII	24					
HgaI	12					
HhaI	44					
HincII	2	139	4156			
HindIII	1	143				
HinfI	16					
HpaI	1	4156				
HphI	18					
KpnI	1	352				
MaeIII	17					
MboII	13					
MluI	1	4658				
MnlI	25					
MseI	33					
MslI	9	1441	1600	1959	3225	3616
		3811	4292	4322	4610	
MspA1I	10	375	450	1824	2211	2456
		3088	3207	3969	4062	4632
MspI	29					
MunI	1	311				
MwoI	34					
NarI	4	4020	5202	5316	5337	
NciI	12					
NcoI	1	69				
NdeI	1	298				
NgoAIV	3	324	671	5348		
NlaIII	26					
NlaIV	22					
NotI	1	150				
NspI	4	2798	3165	3457	5191	
PacI	1	429				
PflMI	2	401	5083			
PleI	12					
Psp1406I	5	989	1415	1788	3471	4998
Psp5II	1	3789				
PstI	1	135				
PvuI	2	337	1559			
PvuII	3	3207	3969	4062		
RcaI	4	1993	2025	2074	5260	
RsaI	5	192	350	1669	2992	4515
SacI	1	122				
SalI	1	137				
SapI	1	2911				
Sau3AI	28					
Sau96I	15					
ScaI	1	1669				
ScrFI	20					

Enzyme	# Sites	Locations				
SfaNI	20					
SfcI	7	131	226	553	1430	2338
		2529	5416			
SgfI	1	337				
SgrAI	1	5339				
SphI	1	5191				
Sse8387I	1	135				
SspI	1	984				
StyI	3	69	433	473		
TaiI	15					
TaqI	16					
TfiI	4	2820	3241	3745	3980	
ThaI	34					
TseI	28					
Tsp45I	7	598	1445	1656	3046	3141
		3354	4476			
Tsp509I	22					
TspRI	13					
Tth111I	1	3052				
VspI	5	213	1361	3916	3975	5403
XbaI	1	30				
XcmI	3	4273	4291	4807		
XhoI	1	354				
XmnI	2	1788	3240			

Enzymes that do not cut pETDuet-1:

BseRI	BsmI	Bsu36I	MscI	NheI	NruI
NsiI	NspV	PinAI	PmeI	PmlI	PshAI
RsrII	SacII	SanDI	SexAI	SfiI	SmaI
SnaBI	SpeI	SrfI	StuI	SunI	SwaI