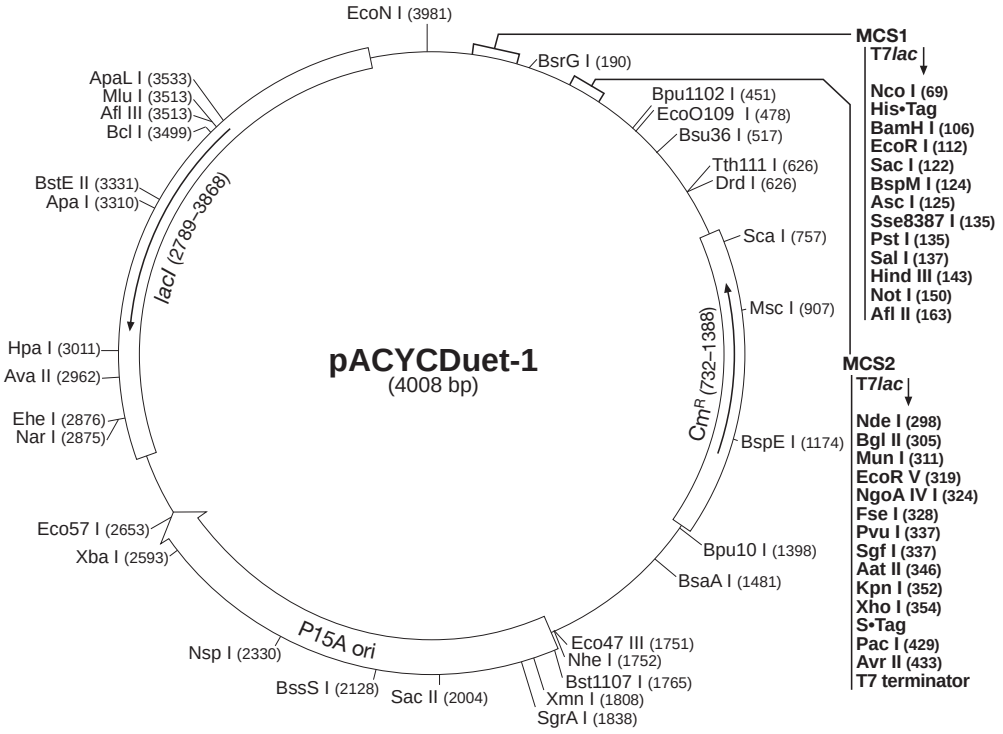
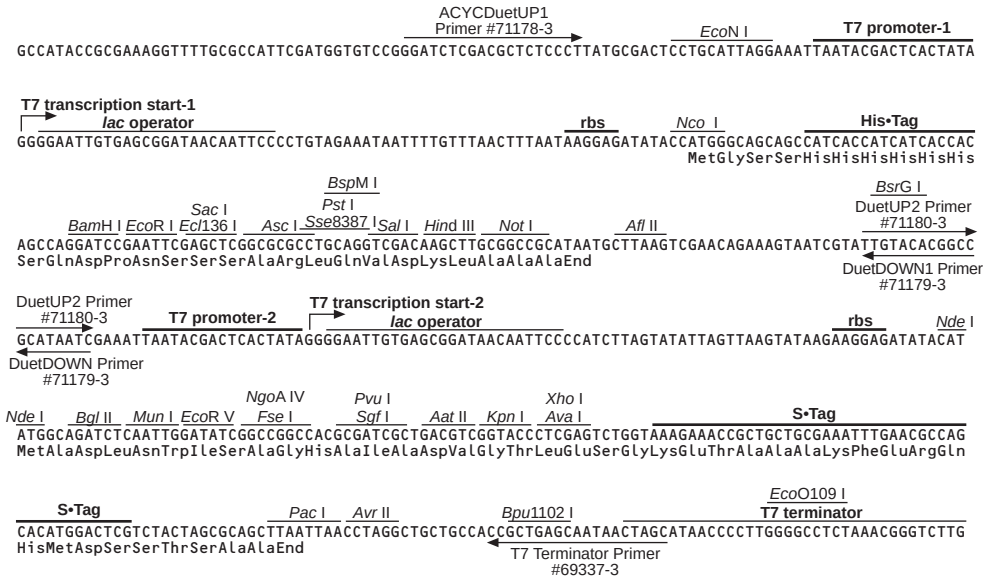
pACYCDuet-1 Vector

	Cat. No.
pACYCDuet-1 DNA	71147-3
pACYCDuet-1 sequence landmarks	
T7 promoter-1	3992–4008
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
P15A origin	1750–2662
cat (Cm^R) coding sequence	732–1388
lacI coding sequence	2789–3868

pACYCDuet™-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 ribosome binding site (rbs). The vector also carries the P15A replicon, *lacI* gene and chloramphenicol resistance gene. This vector can be used in combination with pETDuet™-1 (Cat. No. 71146-3) in an appropriate host strain for the coexpression of up to 4 target genes. Genes inserted into MCS1 can be sequenced using the ACYCDuetUP1 Primer (Cat. No. 71178-3) 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).





pACYCDuet-1 cloning/expression regions

pACYCDuet-1 Restriction Sites

TB336 10/02

Enzyme	# Sites	Locations				
AatII	1	346				
AccI	3	138	411	1764		
AciI	49					
AflII	1	163				
AflIII	1	3513				
AluI	18					
Alw26I	7	946	1499	2198	2898	3285
		3411	3816			
AlwI	4	101	114	2732	3957	
AlwNI	2	1706	2354			
ApaI	1	3310				
ApaLI	1	3533				
ApoI	5	112	384	632	644	3238
AscI	1	125				
AvaI	1	354				
AvaII	1	2962				
AvrII	1	433				
BamHI	1	106				
BanI	5	348	704	2744	2874	3593
BanII	2	122	3310			
BbsI	2	3028	3367			
BbvI	16					
BcgI	2	162	3193			
BclI	1	3499				
BfaI	5	415	434	462	1753	2594
BglII	1	305				
BpmI	4	1054	1647	3192	3681	
Bpu10I	1	1398				
Bpu1102I	1	451				
BsaAI	1	1481				
BsaHI	3	343	2875	3558		
BsaJI	11					
BsaWI	8	551	566	1174	1838	2161
		2291	2691	3194		
BseRI	2	2385	2428			
BsgI	3	1819	3468	3668		
BsiEI	8	153	199	325	337	625
		1909	2278	2734		
BsiHKAI	3	122	1663	3537		
BslI	11					
BsmBI	3	946	1499	2898		
BsmFI	2	1554	1674			
BsmI	2	776	1183			
Bsp1286I	5	122	707	1663	3310	3537
BspEI	1	1174				
BspMI	1	124				
BsrBI	3	13	243	1926		
BsrDI	3	1157	3106	3472		
BsrFI	6	324	566	1838	2161	2394
		3827				
BsrGI	1	190				
BsrI	16					
BssHII	2	125	3102			
BssSI	1	2128				
Bst1107I	1	1765				
BstEII	1	3331				
BstXI	3	3467	3590	3719		
BstYI	4	106	305	2737	3949	
Bsu36I	1	517				
Cac8I	22					
CviJI	61					
DdeI	8	262	451	517	950	1398
		2213	2476	2942		
DpnI	13					
DraI	2	915	1254			
DrdI	1	626				
DsaI	2	69	2001			
EaeI	7	150	196	322	326	905
		2182	2839			
EagI	3	150	196	322		
EarI	2	2621	3896			
Ecl136II	1	120				

Enzyme	# Sites	Locations				
Eco47III	1	1751				
Eco57I	1	2653				
EcoNI	1	3981				
EcoO109I	1	478				
EcoRI	1	112				
EcoRII	14					
EcoRV	1	319				
EheI	1	2876				
FauI	10	743	1187	2690	2800	2842
		3009	3317	3704	3771	3796
Fnu4HI	30					
FokI	4	644	1190	3458	3467	
FseI	1	328				
HaeII	4	1753	2878	3121	3902	
HaeIII	17					
HgaI	8	1595	1833	2067	3286	3292
		3521	3566	3965		
HhaI	26					
HincII	2	139	3011			
HindIII	1	143				
HinfI	11					
HpaI	1	3011				
HphI	16					
KpnI	1	352				
MaeIII	10	539	1007	1112	1578	1721
		2294	2427	2449	3331	3854
MboII	10	900	1565	1974	1985	2574
		2608	3028	3367	3538	3883
MluI	1	3513				
MnlI	18					
MscI	1	907				
MseI	23					
MslI	4	1458	3147	3177	3465	
MspA1I	11					
MspI	24					
MunI	1	311				
MwoI	24					
NarI	1	2875				
NciI	9	626	1436	1528	2221	2318
		2742	3087	3896	3947	
NcoI	1	69				
NdeI	1	298				
NgoAIV	1	324				
NheI	1	1752				
NlaIII	15					
NlaIV	11					
NotI	1	150				
NspI	1	2330				
NspV	2	642	2488			
PacI	1	429				
PflMI	4	401	945	1512	3938	
PinAI	3	566	1838	2161		
PleI	9	214	365	399	1887	2317
		3084	3880	3967	3992	
Psp1406I	2	1085	3853			
PstI	1	135				
PvuI	1	337				
PvuII	4	1274	1686	2824	2917	
RsaI	5	192	350	757	1295	3370
SacI	1	122				
SacII	1	2004				
SalI	1	137				
Sau3AI	13					
Sau96I	8	478	1515	1998	2938	2962
		3306	3307	3652		
ScaI	1	757				
ScrFI	23					
SfaNI	10	842	1327	1605	1955	2053
		2736	3148	3151	3339	3480
SfcI	4	29	131	226	4004	
SgfI	1	337				
SgrAI	1	1838				

Enzyme	# Sites	Locations				
Sse8387I	1	135				
SspI	2	862	2589			
StyI	3	69	433	473		
TaiI	7	346	913	1088	1483	1495
		3783	3856			
TaqI	15					
TfiI	2	821	2835			
ThaI	22					
TseI	16					
Tsp45I	4	539	1578	2427	3331	
Tsp509I	23					
TspRI	14					
Tth111I	1	626				
VspI	5	213	2575	2771	2830	3991
XbaI	1	2593				
XcmI	3	3128	3146	3662		
XhoI	1	354				
XmnI	1	1808				

Enzymes that do not cut pACYCDuet-1:

AhdI	BglI	BsaBI	BsaI	BspLU11I	ClaI
DraIII	FspI	NruI	NsiI	PmeI	PmlI
PshAI	Psp5II	RcaI	RsrII	SanDI	SapI
SexAI	SfiI	SmaI	SnaBI	SpeI	SphI
SrfI	StuI	SunI	SwaI		