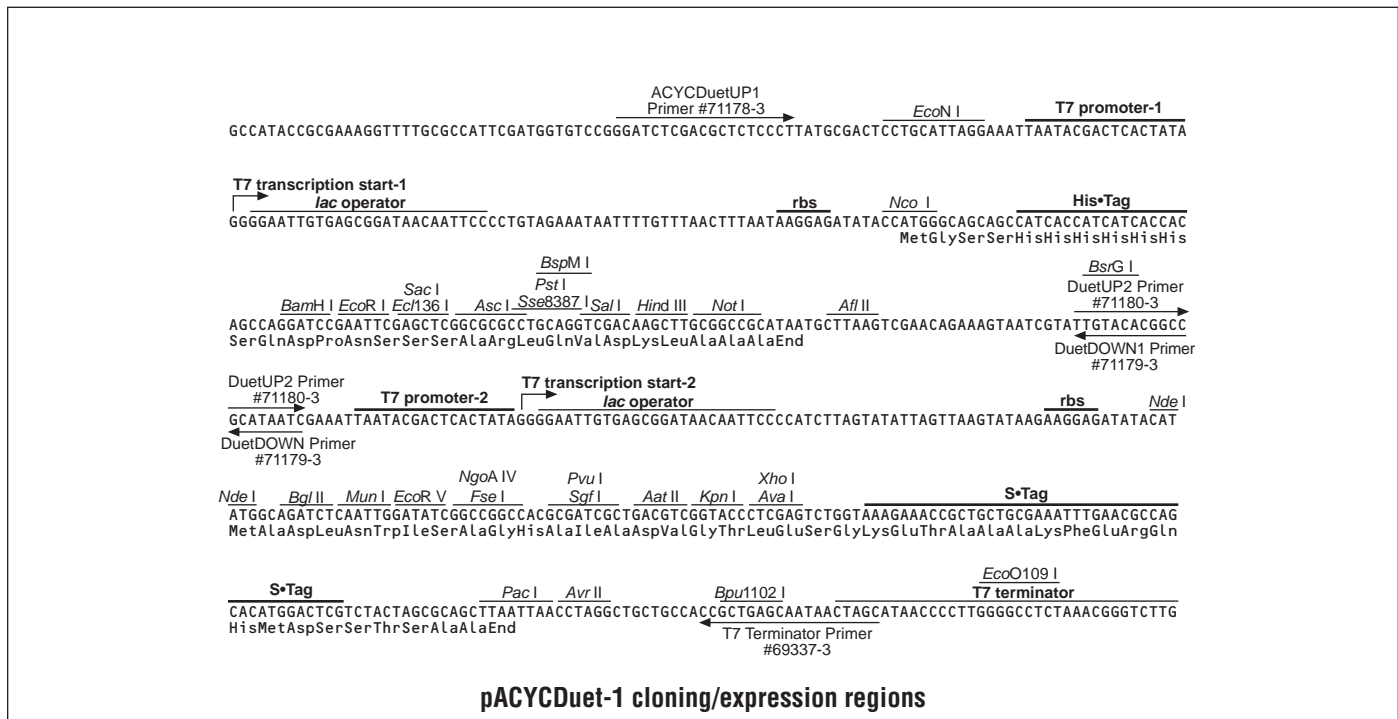
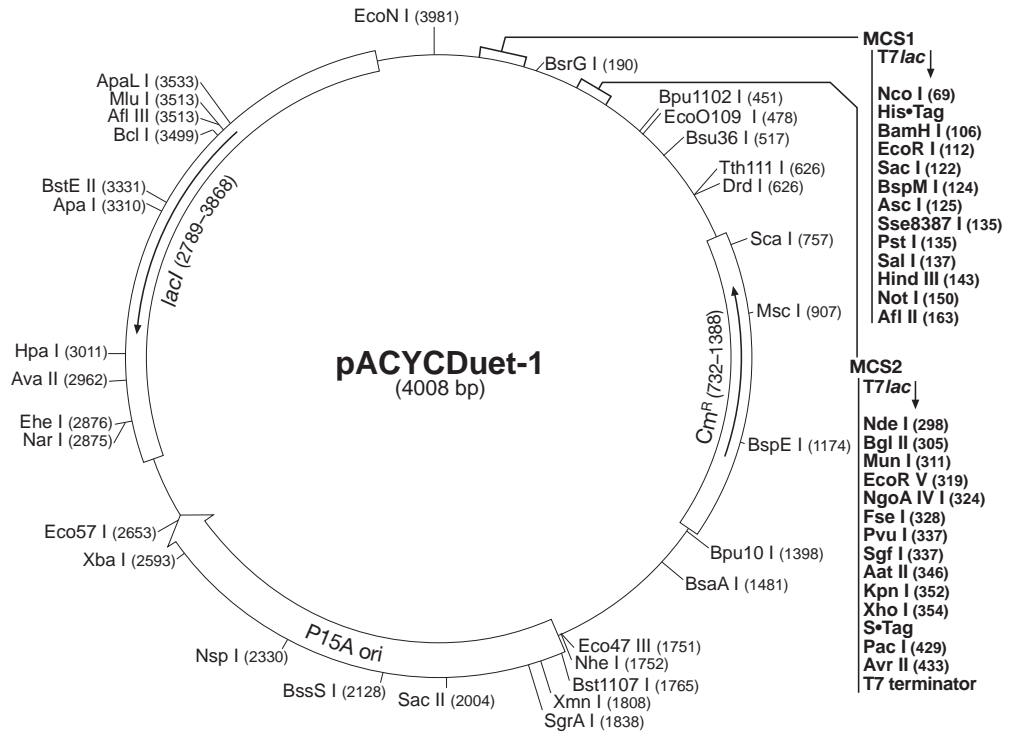


pACYCDuet-1 Vector

TB336 10/02

	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 (<i>Nco</i> I- <i>Afl</i> II)	69-168
T7 promoter-2	214-230
T7 transcription start-2	231
Multiple cloning sites-2 (<i>Nde</i> I- <i>Avr</i> II)	297-438
S•Tag™ coding sequence	366-410
T7 terminator	462-509
P15A origin	1750-2662
<i>cat</i> (<i>Cm^r</i>) coding sequence	732-1388
<i>lacI</i> 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

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			
Avall	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				
Bcgl	2	162	3193		
BclI	1	3499			
BfaI	5	415	434	462	1753 2594
BglIII	1	305			
Bpml	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	
BsII	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			
CacBI	22				
CviJI	61				
Ddel	8	262	451	517	950 1398
		2213	2476	2942	
Dpnl	13				
DraI	2	915	1254		
DrdI	1	626			
Dsal	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				
Hinfl	11					
HpaI	1	3011				
HphI	16					
KpnI	1	352				
MaellI	10	539	1007	1112	1578 1721	
		2294	2427	2449	3331 3854	
MbolI	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			
Pacl	1	429				
PfIMI	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				
Sall	1	137				
Sau3AI	13					
Sau96I	8	478	1515	1998	2938 2962	
		3306	3307	3652		
Scal	1	757				
ScrFI	23					
SfaNI	10	842	1327	1605	1955 2053	
		2736	3148	3151	3339 3480	
Sfcl	4	29	131	226	4004	
Sgfl	1	337				
SgrAI	1	1838				

Enzyme	# Sites	Locations				
Sse8387I	1	135				
SspI	2	862	2589			
StyI	3	69	433	473		
TaqI	7	346	913	1088	1483 1495	
		3783	3856			
TaqI	15					
Tfil	2	821	2835			
Thal	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	Bsal	BspLU11I	Clal
DraIII	FspI	NruI	Nsil	Pmel	PmlI
PshAI	Psp5II	RcaI	RsrII	SanDI	SapI
SexAI	SfiI	Smal	SnaBI	SpeI	SphI
SrfI	StuI	SunI	Swal		