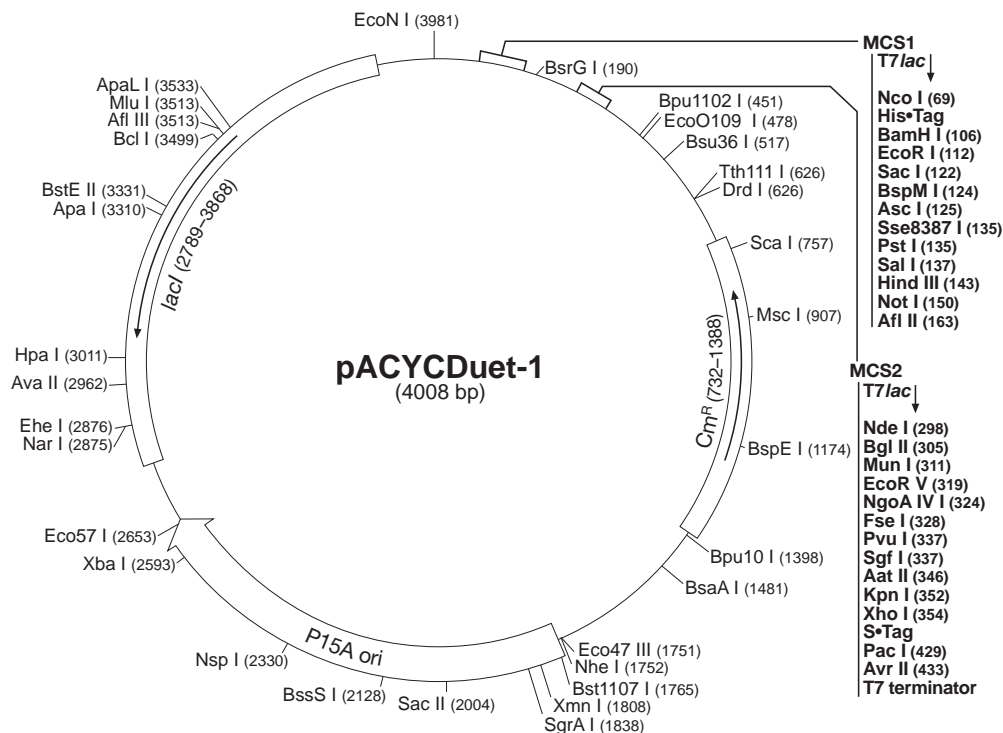


## pACYCDuet-1 Vector

TB336 10/02

	Cat. No.
pACYCDuet-1 DNA	71147-3
<b>pACYCDuet-1 sequence landmarks</b>	
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<sup>R</sup></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).



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          ACYCDuetUP1
          Primer #71178-3
GCCATACGCGAAAGGTTTTGCGCCATTGATGGTGTCCGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAATTAATACGACTCACTATA

          T7 promoter-1
          T7 transcription start-1
          lac operator
GGGGAATTGTGAGCGGATAACAATTCCTGTAGAAATAATTTGTTAACTTAATAAGGAGATATACCATGGGCAGCAGCCATCACCATCATCACCAC
MetGlySerSerHisHisHisHisHisHisHis

          BspM I
          Pst I
          BamH I EcoR I Eco136 I Asc I Sse8387 I Sal I Hind III Not I Afl II
AGCCAGGATCCGAATTCGAGCTCGGCGGCCCTGCAGGTCGACAAAGCTTGCGGCCGCATAATGCTTAAGTCGAACAGAAAGTAATCGTATTGTACACGGCC
SerGlnAspProAsnSerSerSerAlaArgLeuGlnValAspLysLeuAlaAlaAlaEnd

          DuetUP2 Primer #71180-3
          T7 promoter-2
          T7 transcription start-2
          lac operator
GCATAATCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCATCTAGTATATTAGTTAAGTATAAGAGGAGATATACAT
DuetDOWN1 Primer #71179-3

          NgoA IV Pvu I Xho I S•Tag
Nde I Bgl II Mun I EcoR V Fse I Sgf I Aat II Kpn I Ava I
ATGGCAGATCTCAATTGGATATCGGCGGCCACGCGATCGTGACGTCGGTACCCTCGAGTCTGGTAAGAAACCGCTGCTGCGAAATTTGAACGCCGAC
MetAlaAspLeuAsnTrpIleSerAlaGlyHisAlaIleAlaAspValGlyThrLeuGluSerGlyLysGluThrAlaAlaAlaLysPheGluArgGln

          S•Tag
          Pac I Avr II Bpu1102 I EcoO109 I T7 terminator
CACATGGACTCGTCTACTAGCGCAGCTTAATTAACCTAGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGCTTGG
HisMetAspSerSerThrSerAlaAlaEnd
          T7 Terminator Primer #69337-3

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### pACYCDuet-1 cloning/expression regions

# pACYCDuet-1 Restriction Sites

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Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
AatII	1	346	Eco47III	1	1751	Sse8387I	1	135
AccI	3	138 411 1764	Eco57I	1	2653	SspI	2	862 2589
AcII	49		EcoNI	1	3981	StyI	3	69 433 473
AflII	1	163	EcoO109I	1	478	TaiI	7	346 913 1088 1483 1495
AflIII	1	3513	EcoRI	1	112			3783 3856
AluI	18		EcoRII	14		TaqI	15	
Alw26I	7	946 1499 2198 2898 3285	EcoRV	1	319	TfiI	2	821 2835
		3411 3816	EheI	1	2876	ThaI	22	
AlwI	4	101 114 2732 3957	FauI	10	743 1187 2690 2800 2842	TseI	16	
AlwNI	2	1706 2354			3009 3317 3704 3771 3796	Tsp45I	4	539 1578 2427 3331
ApaI	1	3310	Fnu4HI	30		Tsp509I	23	
ApaLI	1	3533	FokI	4	644 1190 3458 3467	TspRI	14	
ApoI	5	112 384 632 644 3238	FseI	1	328	Tth111I	1	626
AscI	1	125	HaeII	4	1753 2878 3121 3902	VspI	5	213 2575 2771 2830 3991
AvaI	1	354	HaeIII	17		XbaI	1	2593
Avall	1	2962	HgaI	8	1595 1833 2067 3286 3292	XcmI	3	3128 3146 3662
AvrII	1	433			3521 3566 3965	XhoI	1	354
BamHI	1	106	HhaI	26		XmnI	1	1808
BanI	5	348 704 2744 2874 3593	HincII	2	139 3011			
BanII	2	122 3310	HindIII	1	143			
BbsI	2	3028 3367	Hinfl	11				
BbvI	16		HpaI	1	3011			
BcgI	2	162 3193	HphI	16				
BclI	1	3499	KpnI	1	352			
BfaI	5	415 434 462 1753 2594	MaeIII	10	539 1007 1112 1578 1721			
BglII	1	305			2294 2427 2449 3331 3854			
BpmI	4	1054 1647 3192 3681	MbolI	10	900 1565 1974 1985 2574			
Bpu10I	1	1398			2608 3028 3367 3538 3883			
Bpu1102I	1	451	MluI	1	3513			
BsaAI	1	1481	MnlI	18				
BsaHI	3	343 2875 3558	MscI	1	907			
BsaJI	11		MseI	23				
BsaWI	8	551 566 1174 1838 2161	MslI	4	1458 3147 3177 3465			
		2291 2691 3194	MspA1I	11				
BseRI	2	2385 2428	MspI	24				
BsgI	3	1819 3468 3668	MunI	1	311			
BsiEI	8	153 199 325 337 625	MwoI	24				
		1909 2278 2734	NarI	1	2875			
BsiHKA1	3	122 1663 3537	NciI	9	626 1436 1528 2221 2318			
BsiI	11				2742 3087 3896 3947			
BsmBI	3	946 1499 2898	NcoI	1	69			
BsmFI	2	1554 1674	NdeI	1	298			
BsmI	2	776 1183	NgoAI	1	324			
Bsp1286I	5	122 707 1663 3310 3537	NheI	1	1752			
BspEI	1	1174	NlaI	15				
BspMI	1	124	NlaIV	11				
BsrBI	3	13 243 1926	NotI	1	150			
BsrDI	3	1157 3106 3472	NspI	1	2330			
BsrFI	6	324 566 1838 2161 2394	NspV	2	642 2488			
		3827	PacI	1	429			
BsrGI	1	190	PfIMI	4	401 945 1512 3938			
BsrI	16		PinAI	3	566 1838 2161			
BssHII	2	125 3102	PleI	9	214 365 399 1887 2317			
BssSI	1	2128			3084 3880 3967 3992			
Bst1107I	1	1765	Psp1406I	2	1085 3853			
BstEII	1	3331	PstI	1	135			
BstXI	3	3467 3590 3719	PvuI	1	337			
BstYI	4	106 305 2737 3949	PvuII	4	1274 1686 2824 2917			
Bsu36I	1	517	RsaI	5	192 350 757 1295 3370			
CacBI	22		SacI	1	122			
CviJI	61		SacII	1	2004			
DdeI	8	262 451 517 950 1398	Sall	1	137			
		2213 2476 2942	Sau3AI	13				
DpnI	13		Sau96I	8	478 1515 1998 2938 2962			
DraI	2	915 1254			3306 3307 3652			
DrdI	1	626	Scal	1	757			
Dsal	2	69 2001	ScrFI	23				
EaeI	7	150 196 322 326 905	SfaNI	10	842 1327 1605 1955 2053			
		2182 2839			2736 3148 3151 3339 3480			
EagI	3	150 196 322	Sfcl	4	29 131 226 4004			
EarI	2	2621 3896	Sgfl	1	337			
Ecl136II	1	120	SgrAI	1	1838			

Enzymes that do not cut pACYCDuet-1:

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