pCOLADuet™-1 Vector

TB408 0304

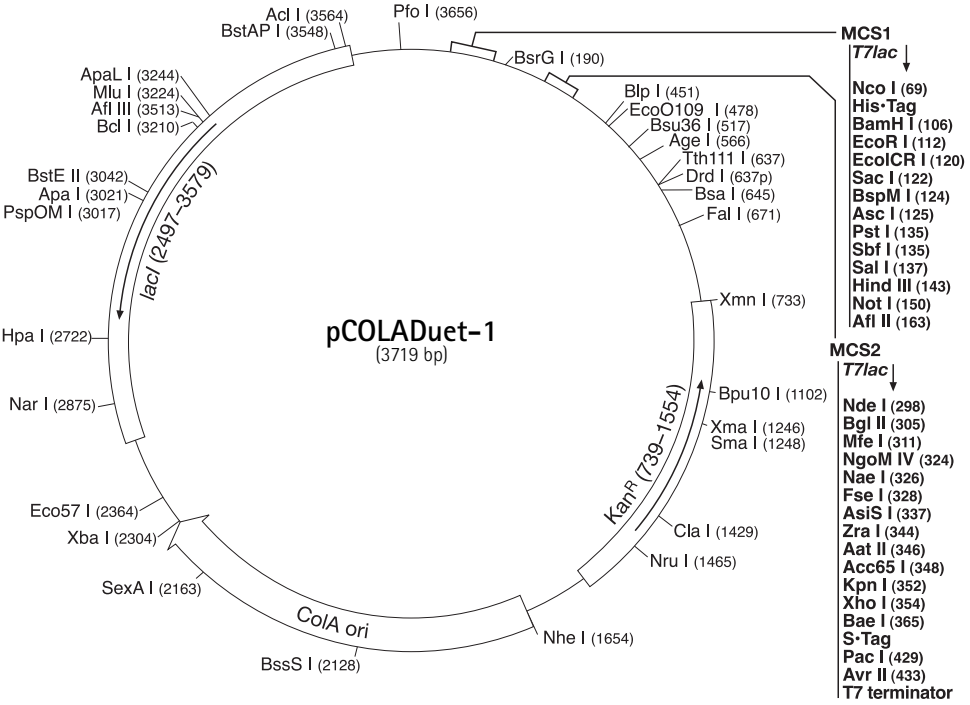
	Cat No.
pCOLADuet-1 DNA	71406-3

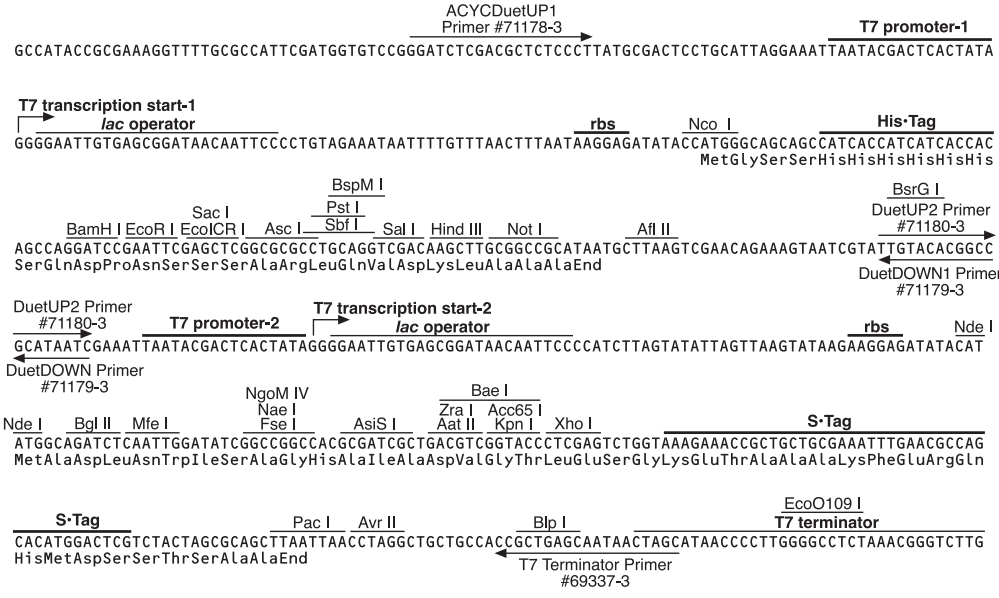
pCOLADuet-1 sequence landmarks	
T7 promoter-1	3703–3719
T7 transcription start-1	1
His•Tag® coding sequence	83–100
Multiple cloning sites-1 (*Nco* I–*Afl* II)	68–168
T7 promoter-2	214-230
T7 transcription start-2	231
Multiple cloning sites-2 (*Nde* I–*Avr* II)	297–438
S•Tag™ coding sequence	366-410
T7 terminator	462-509
Kan^R	739–1554
ColA ori	1664-2299
lacI coding sequence	2497-3579

pCOLADuet™-1 is designed for the coexpression of two target genes from a single plasmid. The vector encodes two multiple cloning sites (MCS) each of which is preceded by a T7 promoter, *lac* operator, and ribosome binding site (rbs). MCS-1 encodes the six-amino acid His•Tag® sequence for the creation of a N-terminal fusion and MCS2 encodes the 15 amino acid S•Tag™ peptide after the last restriction site for the creation of a C-terminal fusion if desired. Genes inserted into MCS-1 can be sequenced using the ACYCDuetUP1 Primer and DuetDOWN1 Primer. Genes inserted into MCS-2 can be sequenced using the DuetUP2 Primer and T7 Terminator Primer. The vector has the COLA replicon from ColA(1) and the kanamycin resistance gene. This vector can be transformed into the same cell with plasmids containing compatible origins of replication and drug resistance genes for coexpression of up to 8 target genes.

Reference

1. Zverev, V.V. and Khmel, I.A. (1985) *Plasmid* **14**, 192-199.





pCOLADuet-1 cloning/expression regions

pCOLADuet™-1 Restriction Sites

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Enzyme	# Sites	Locations				
AatII	1	346				
Acc65I	1	348				
AccI	3	138	411	1922		
AclI	1	3564				
AflII	1	163				
AflIII	1	3224				
AgeI	1	566				
ApaI	1	3021				
ApaLI	1	3244				
AscI	1	125				
AseI	6	213	732	921	2482	2541
		3702				
AsiSI	1	337				
AvaI	2	354	1246			
AvrII	1	433				
BaeI	1	365				
BamHI	1	106				
BanI	4	348	2455	2585	3304	
BanII	3	122	1471	3021		
BbeI	1	2589				
BbsI	2	2739	3078			
BceAI	5	211	801	1708	2740	3367
BcgI	2	162	2904			
BciVI	4	728	1604	1681	2772	
BclI	1	3210				
BfrBI	2	1008	1274			
BglII	1	305				
BlpI	1	451				
Bme1580I	2	3021	3248			
BmgBI	1	1908				
BmrI	3	2426	3066	3303		
BmtI	1	1658				
BpmI	2	2903	3392			
Bpu10I	1	1102				
BpuEI	4	515	2024	2252	2416	
BsaHI	4	343	1870	2586	3269	
BsaI	1	645				
BsaWI	6	551	566	983	1988	2402
		2905				
BsaXI	2	655	2556			
BseYI	3	1939	2690	2825		
BsgI	2	3179	3379			
BsiEI	8	153	199	325	337	636
		1124	2255	2445		
BsiHKAI	2	122	3248			
BsmAI	8	645	1102	1604	1695	2609
		2996	3122	3527		
BsmBI	2	1102	2609			
BsmFI	1	1894				
BsmI	2	1163	1240			
Bsp1286I	4	122	1471	3021	3248	
BspCNI	5	443	530	1094	2051	2645
BspHI	2	725	1602			
BspMI	1	124				
BsrBI	4	13	243	723	1608	
BsrDI	2	2817	3183			
BsrFI	4	324	566	1164	3538	
BsrGI	1	190				
BssHII	2	125	2813			
BstAPI	1	3548				
BstEII	1	3042				
BstXI	3	3178	3301	3430		
BstYI	7	106	305	869	2263	2274
		2448	3660			
Bsu36I	1	517				
BtgI	1	69				
BtrI	1	1908				
BtsI	5	543	1176	1263	2497	2865
ClaI	1	1429				
DrdI	1	637				
EaeI	5	150	196	322	326	2550
EagI	3	150	196	322		

Enzyme	# Sites	Locations				
EarI	4	1306	1562	2332	3607	
EciI	2	1730	3439			
Ecl136II	1	120				
Eco57I	1	2364				
Eco57MI	3	2364	2903	3392		
EcoICRI	1	120				
EcoNI	3	1209	2107	3692		
EcoO109I	1	478				
EcoRI	1	112				
EcoRV	2	319	2080			
FalI	1	671				
FseI	1	328				
HaeII	3	2589	2832	3613		
HinclI	2	139	2722			
HindIII	1	143				
HpaI	1	2722				
KasI	1	2585				
KpnI	1	352				
MfeI	1	311				
MluI	1	3224				
MslI	3	2858	2888	3176		
NaeI	1	326				
NarI	1	2586				
NcoI	1	69				
NdeI	1	298				
NgoMIV	1	324				
NheI	1	1654				
NotI	1	150				
NruI	1	1465				
NsiI	2	1010	1276			
NspI	1	1654				
PacI	1	429				
PflMI	3	401	862	3649		
PfoI	1	3656				
PinAI	1	566				
PspOMI	1	3017				
PstI	1	135				
PvuI	2	337	2255			
PvuII	2	2535	2628			
SacI	1	122				
SalI	1	137				
SbfI	1	135				
SexAI	1	2163				
SfcI	4	29	131	226	3715	
SfoI	1	2587				
SmaI	1	1248				
SmlI	6	163	354	494	2003	2267
		2431				
SphI	1	1654				
Sse8387I	1	135				
SspI	2	1197	1571			
StyI	3	69	433	473		
TaqII	2	870	2431			
TatI	1	190				
TspGWI	5	1303	1315	1863	1906	2298
Tth111I	1	637				
XbaI	1	2304				
XcmI	3	2839	2857	3373		
XhoI	1	354				
XmaI	1	1246				
XmnI	1	733				
ZraI	1	344				

Enzymes that do not cut pCOLADuet-1:

AarI	AfeI	AhdI	AleI	AloI	AlwNI
BbvCI	BglI	BplI	BsaAI	BsaBI	BseRI
BsiWI	BspEI	BspLU11I	BssSI	Bst1107I	BstBI
BstZ17I	DraI	DraIII	FspAI	FspI	MscI
NspV	PciI	PmeI	PmlI	PpiI	PpuMI
PshAI	PsiI	PsrI	RsrII	SacII	SanDI
SapI	ScaI	SfiI	SgrAI	SnaBI	SpeI
SrfI	StuI	SwaI			