

## pCOLADuet™-1 Vector

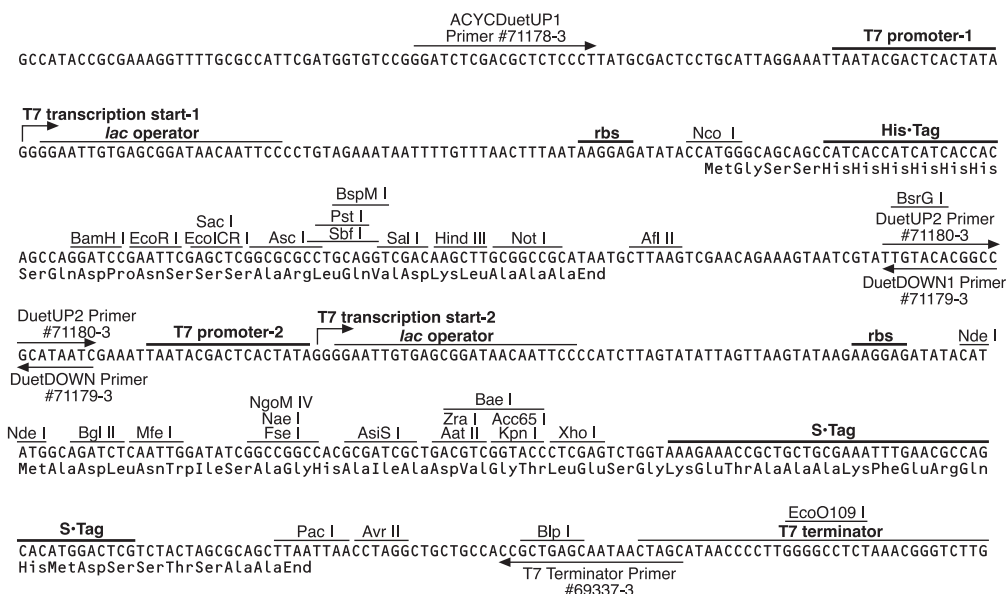
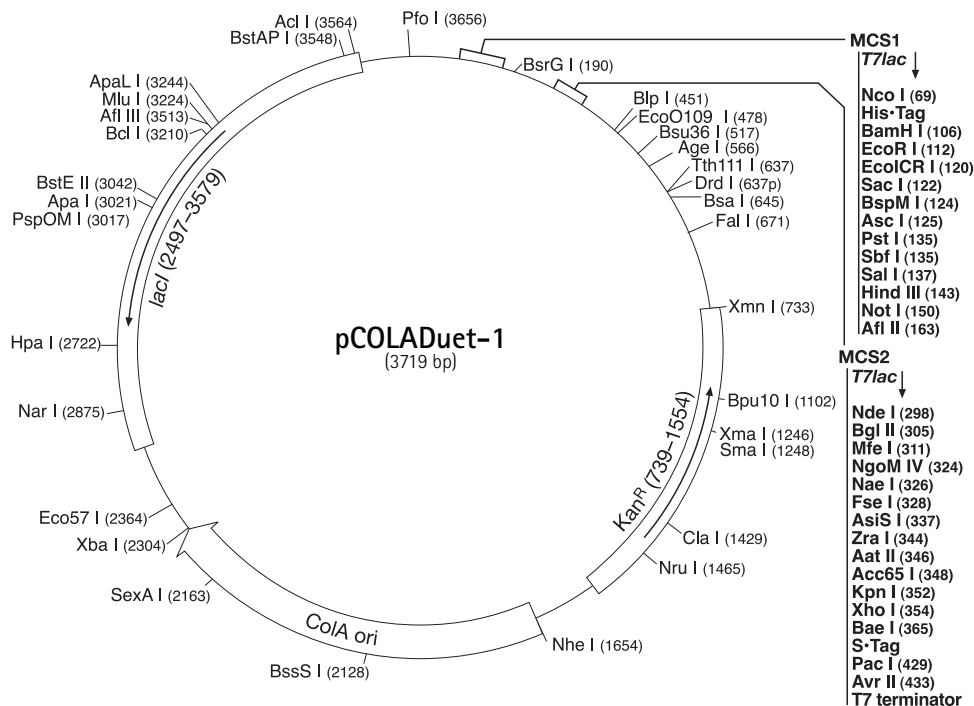
TB408 0304

	Cat No.
pCOLADuet-1 DNA	71406-3
<b>pCOLADuet-1 sequence landmarks</b>	
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 <sup>R</sup>	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					Enzyme	# Sites	Locations				
AatII	1	346					EarI	4	1306	1562	2332	3607	
Acc65I	1	348					EciI	2	1730	3439			
AccI	3	138	411	1922			Ecl136II	1	120				
AcII	1	3564					Eco57I	1	2364				
AflII	1	163					Eco57MI	3	2364	2903	3392		
AflIII	1	3224					EcoICRI	1	120				
AgeI	1	566					EcoNI	3	1209	2107	3692		
Apal	1	3021					EcoO109I	1	478				
ApaLI	1	3244					EcoRI	1	112				
Ascl	1	125					EcoRV	2	319	2080			
Asel	6	213	732	921	2482	2541	FalI	1	671				
		3702					FseI	1	328				
AsiSI	1	337					HaeII	3	2589	2832	3613		
AvaI	2	354	1246				HincII	2	139	2722			
AvrII	1	433					HindIII	1	143				
BaeI	1	365					HpaI	1	2722				
BamHI	1	106					KasI	1	2585				
BanI	4	348	2455	2585	3304		KpnI	1	352				
BanII	3	122	1471	3021			MfeI	1	311				
BbeI	1	2589					MluI	1	3224				
BbsI	2	2739	3078				MsiI	3	2858	2888	3176		
BceAI	5	211	801	1708	2740	3367	NaeI	1	326				
BcgI	2	162	2904				NarI	1	2586				
BciVI	4	728	1604	1681	2772		NcoI	1	69				
BclI	1	3210					NdeI	1	298				
BfrBI	2	1008	1274				NgoMIV	1	324				
BglII	1	305					NheI	1	1654				
BlpI	1	451					NotI	1	150				
Bme1580I	2	3021	3248				NruI	1	1465				
BmgBI	1	1908					NsiI	2	1010	1276			
BmrI	3	2426	3066	3303			NspI	1	1654				
BmtI	1	1658					PacI	1	429				
Bpml	2	2903	3392				PfiMI	3	401	862	3649		
Bpu10I	1	1102					PfoI	1	3656				
BpuEI	4	515	2024	2252	2416		PinAI	1	566				
BsaHI	4	343	1870	2586	3269		PspOMI	1	3017				
Bsal	1	645					PstI	1	135				
BsaWI	6	551	566	983	1988	2402	PvuI	2	337	2255			
		2905					PvuII	2	2535	2628			
BsaXI	2	655	2556				SacI	1	122				
BseYI	3	1939	2690	2825			SalI	1	137				
BsgI	2	3179	3379				SbfI	1	135				
BsiEI	8	153	199	325	337	636	SexAI	1	2163				
		1124	2255	2445			SfcI	4	29	131	226	3715	
BsiHKAI	2	122	3248				SfoI	1	2587				
BsmAI	8	645	1102	1604	1695	2609	SmaI	1	1248				
		2996	3122	3527			SmlI	6	163	354	494	2003	
BsmBI	2	1102	2609						2431			2267	
BsmFI	1	1894					SphI	1	1654				
BsmI	2	1163	1240				Sse8387I	1	135				
Bsp1286I	4	122	1471	3021	3248		Sspl	2	1197	1571			
BspCNI	5	443	530	1094	2051	2645	StyI	3	69	433	473		
BspHI	2	725	1602				TaqII	2	870	2431			
BspMI	1	124					TatI	1	190				
BsrBI	4	13	243	723	1608		TspGWI	5	1303	1315	1863	1906	
BsrDI	2	2817	3183				Tth111I	1	637			2298	
BsrFI	4	324	566	1164	3538		XbaI	1	2304				
BsrGI	1	190					XcmI	3	2839	2857	3373		
BssHII	2	125	2813				XhoI	1	354				
BstAPI	1	3548					XmaI	1	1246				
BstEII	1	3042					XmnI	1	733				
BstXI	3	3178	3301	3430			ZraI	1	344				
BstYI	7	106	305	869	2263	2274							
		2448	3660				Enzymes that do not cut pCOLADuet-1:						
Bsu36I	1	517					AarI	AfeI	AhdI	AleI	Alol	AlwNI	
BtgI	1	69					BbvCI	BglI	BpII	BsaAI	BsaBI	BseRI	
BtrI	1	1908					BsiWI	BspEI	BspLU11I	BssSI	Bst1107I	BstBI	
BtsI	5	543	1176	1263	2497	2865	BstZ17I	Dral	DrallI	FspAI	FspI	MscI	
ClaI	1	1429					NspV	PciI	PmeI	PmlI	Ppil	PpuMI	
DrdI	1	637					PshAI	Psil	Psrl	RsrII	SacII	SanDI	
EaeI	5	150	196	322	326	2550	SapI	Scal	Sfil	SgrAI	SnaBI	SpeI	
EagI	3	150	196	322			SrfI	StuI	Swal				