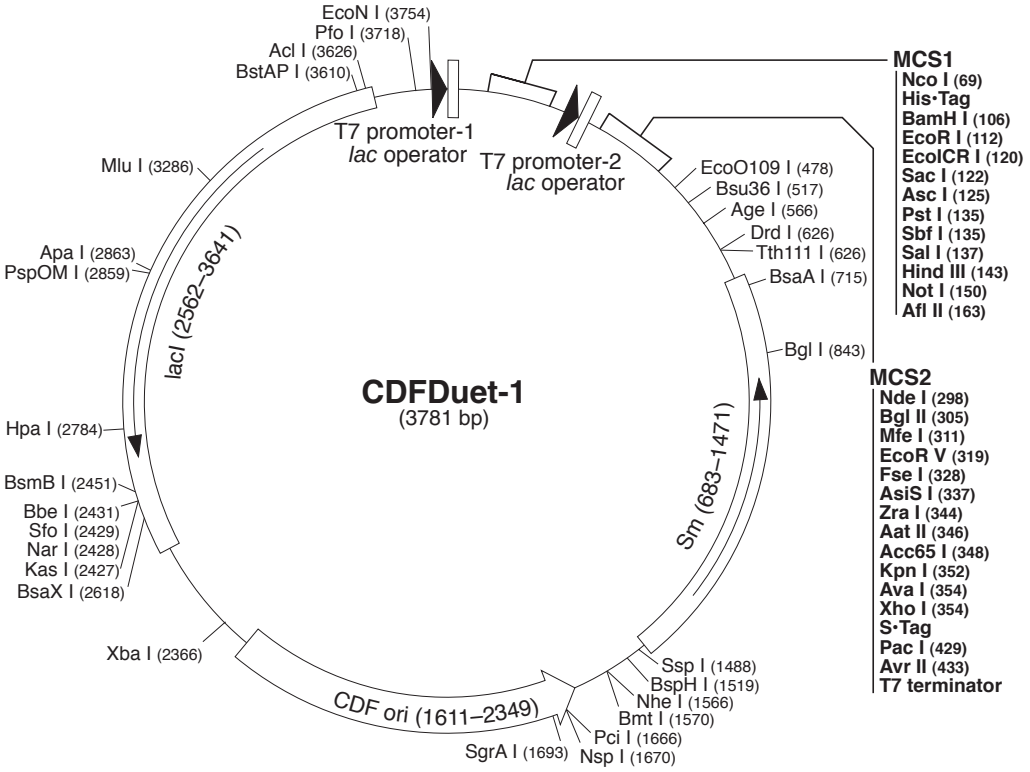
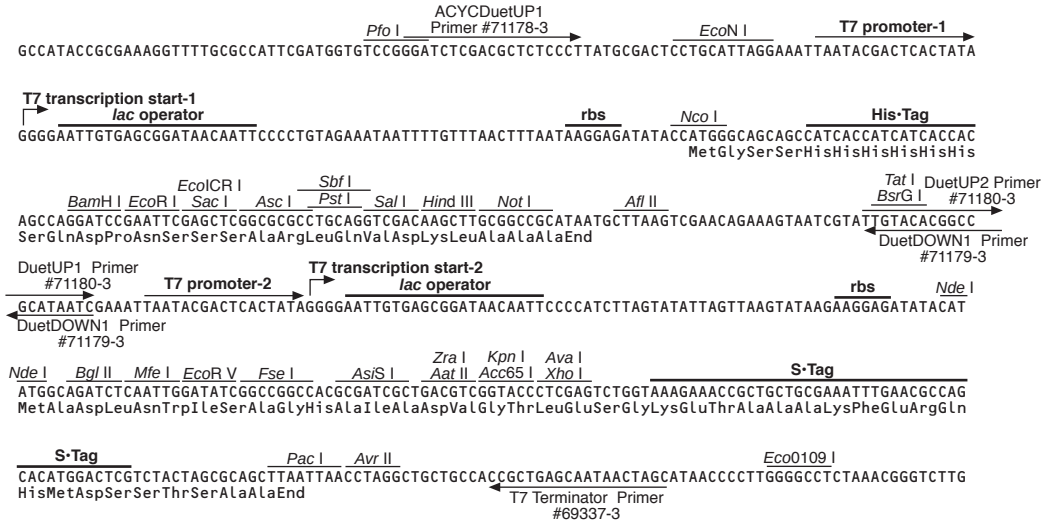
pCDFDuet-1 Vector

	Cat. No.
pCDFDuet-1 DNA	71340-3
pCDFDuet-1 sequence landmarks	
T7 promoter-1	3765–3781
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
aadA (SmR) coding sequence	683–1471
CDF origin	1611–2349
lacI coding sequence	2562–3641

pCDFDuet™-1 is designed for the coexpression of two target ORFs. The vector contains two multiple cloning sites (MCS), each of which is preceded by a T7*lac* promoter and ribosome binding site (rbs). The vector also carries the CloDF13-derived CDF replicon, *lacI* gene and streptomycin/spectinomycin resistance gene (Sm^R). This vector can be used in combination with pACYCDuet™-1 (Cat. No. 71147-3), pRSFDuet™-1 (Cat. No. 71341-3), and pETDuet™-1 (Cat. No. 71146-3) in an appropriate host strain for the coexpression of 4 to 8 target proteins. ORFs inserted into MCS1 can be sequenced using the ACYCDuetUP1 Primer (Cat. No. 71178-3) and DuetDOWN1 Primer (Cat. No. 71179-3). ORFs inserted into MCS2 can be sequenced using the DuetUP2 Primer (Cat. No. 71180-3) and T7 Terminator Primer (Cat. No. 69337-3). Unique sites are shown on the circle map.





pCDFDuet-1 cloning/expression regions

pCDFDuet-1 Restriction Sites

TB390 0903

Enzyme	# Sites	Locations				
AatII	1	346				
Acc65I	1	348				
AccI	2	138	411			
AclI	1	3626				
AflII	1	163				
AflIII	2	1666	3286			
AgeI	1	566				
ApaI	1	3083				
ApaLI	2	1193	3306			
AscI	1	125				
AseI	4	213	2544	2603	3764	
AsiSI	1	337				
AvaI	1	354				
AvrII	1	433				
BaeI	2	365	1972			
BamHI	1	106				
BanI	4	348	2517	2647	3366	
BanII	2	122	3083			
BbeI	1	2651				
BbsI	2	2801	3140			
BceAI	7	211	1352	1396	1757	1868
		2802	3429			
BcgI	2	162	2966			
BciVI	3	1521	1887	2834		
BclI	2	1270	3272			
BglI	1	843				
BglII	1	305				
BlpI	2	451	1160			
Bme1580I	3	1197	3083	3310		
BmrI	7	824	833	929	2118	2488
		3128	3365			
BmtI	1	1570				
BpmI	3	1222	2965	3454		
BpuEI	4	515	2074	2315	2478	
BsaAI	1	715				
BsaHI	4	343	1416	2648	3331	
BsaWI	8	551	566	1002	1754	1890
		2038	2464	2967		
BsaXI	1	2618				
BseYI	3	1989	2752	2887		
BsgI	2	3241	3441			
BsiEI	8	153	199	325	337	625
		1093	1580	2507		
BsiHKAI	4	122	1197	2209	3310	
BsmAI	6	1521	1592	2671	3058	3184
		3589				
BsmBI	1	2671				
BsmFI	2	912	1673			
Bsp1286I	5	122	1197	2209	3083	3310
BspCNI	3	443	530	2707		
BspHI	1	1519				
BspMI	2	124	1121			
BsrBI	4	13	243	1525	2364	
BsrDI	4	832	1125	2879	3245	
BsrFI	6	324	566	819	872	1693
		3600				
BsrGI	1	190				
BssHII	3	125	744	2875		
BstAPI	1	3610				
BstEII	2	1307	3104			
BstXI	3	3240	3363	3492		
BstYI	9	106	305	641	987	1827
		2326	2337	2510	3722	
Bsu36I	1	517				
BtgI	4	69	1175	1645	2226	
BtsI	4	543	1357	2559	2927	
DraIII	2	718	1338			
DrdI	1	626				
EaeI	7	150	196	322	326	636
		1381	2612			
EagI	3	150	196	322		
EarI	3	1479	2394	3669		

Enzyme	# Sites	Locations				
EciI	4	1010	1706	1893	3501	
Eco57I	2	1328	2426			
Eco57MI	5	1222	1328	2426	2965	3454
EcoICRI	1	120				
EcoNI	1	3754				
EcoO109I	1	478				
EcoRI	1	112				
EcoRV	1	319				
FseI	1	328				
HaeII	8	830	979	1409	1614	1934
		2651	2894	3675		
HincII	2	139	2784			
HindIII	1	143				
HpaI	1	2784				
KasI	1	2647				
KpnI	1	352				
MfeI	1	311				
MluI	1	3286				
MslI	4	1200	2920	2950	3238	
NaeI	2	326	821			
NarI	1	2648				
NcoI	1	69				
NdeI	1	298				
NgoMIV	2	324	819			
NheI	1	1566				
NotI	1	150				
NspI	1	1670				
PacI	1	429				
PciI	1	1666				
PflMI	2	401	3711			
PfoI	1	3718				
PspOMI	1	3079				
PstI	1	135				
PvuI	2	337	1093			
PvuII	2	2597	2690			
SacI	1	122				
SalI	1	137				
SbfI	1	135				
SfcI	5	29	131	226	1213	3777
SfoI	1	2649				
SgrAI	1	1693				
SmlI	6	163	354	494	2053	2330
		2493				
SspI	1	1488				
StyI	5	69	433	473	694	1036
TaqII	2	1566	2493			
TatI	1	190				
TspGWI	2	1192	1634			
Tth111I	1	626				
XbaI	1	2366				
XcmI	4	1643	2901	2919	3435	
XhoI	1	354				
ZraI	1	344				

Enzymes that do not cut pCDFDuet-1:

AarI	AfeI	AhdI	AleI	AloI
AlwNI	BbvCI	BfrBI	BmgBI	BpiI
Bpu10I	BsaBI	BsaI	BseRI	BsiWI
BsmI	BspEI	BssSI	BstBI	BstZ17I
ClaI	DraI	FalI	FspAI	FspI
MscI	NruI	NsiI	PmeI	PmlI
PpiI	PpuMI	PshAI	PsiI	PsrI
RsrII	SacII	SanDI	SapI	ScaI
SexAI	SfiI	SmaI	SnaBI	SpeI
SphI	SrfI	StuI	SwaI	XmaI
XmnI				