pBAC-7 Transfer Plasmid

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Locus	polh
Promoter	polh
N-terminal fusion	CBD_{clos}•Tag, S•Tag
C-terminal fusion option	His•Tag
Cloning options	LIC, polylinker

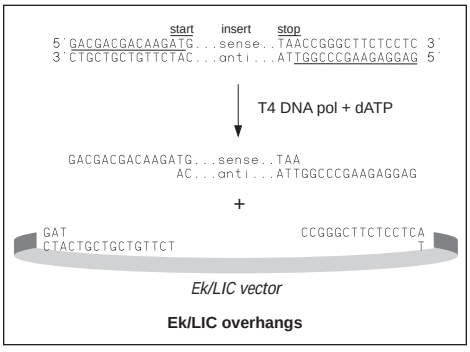
Developed through collaboration between Novagen and CBD Technologies, Inc.

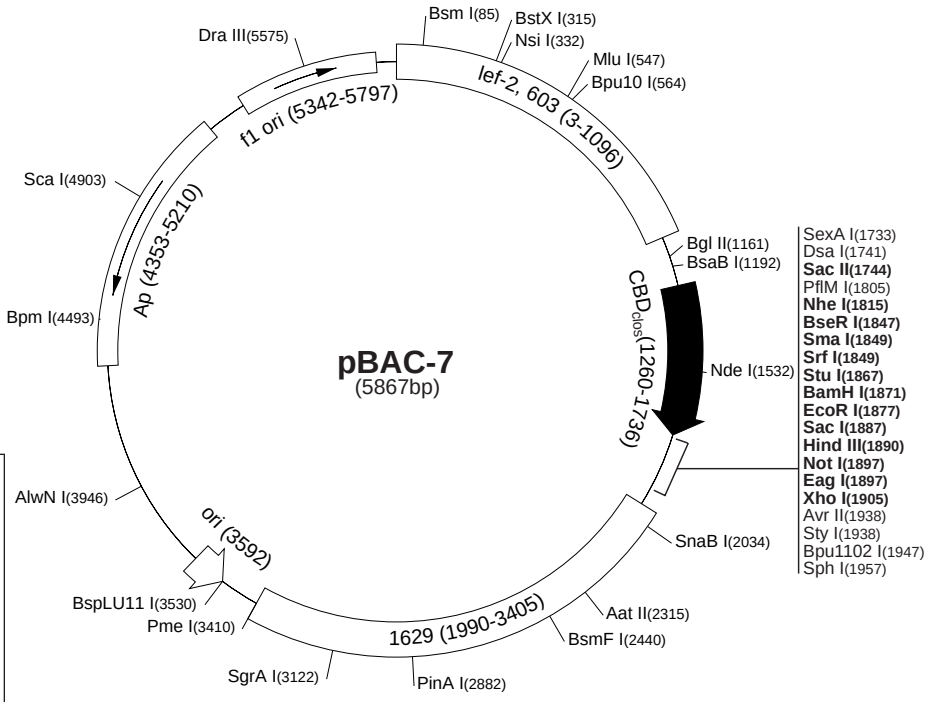
pBAC™-7 is a baculovirus transfer plasmid (Cat. No. 70104-3) compatible with BacVector™ -1000, -2000, or -3000 Triple Cut Virus DNA for low background transfection and efficient utilization of the polh promoter. Cloning sites are provided for the creation of N-terminal fusions of an insert with CBD_{clos}•Tag™ and/or S•Tag™ sequences. pBAC-7 provides an ATG start codon at the optimal position relative to native polyhedrin translation signals. A Ligation-Independent Cloning (LIC) version of the vector is available (Cat. No. 70116-3) for rapid, directional cloning of PCR products adjacent to the enterokinase cleavage site. Unique restriction sites are indicated on the circle map. The cloning/expression region of the coding strand transcribed from the polh promoter is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the coding strand. Single stranded sequencing of phage-derived DNA can be performed using the 1629DWN primer (Cat. No. 70011-3).

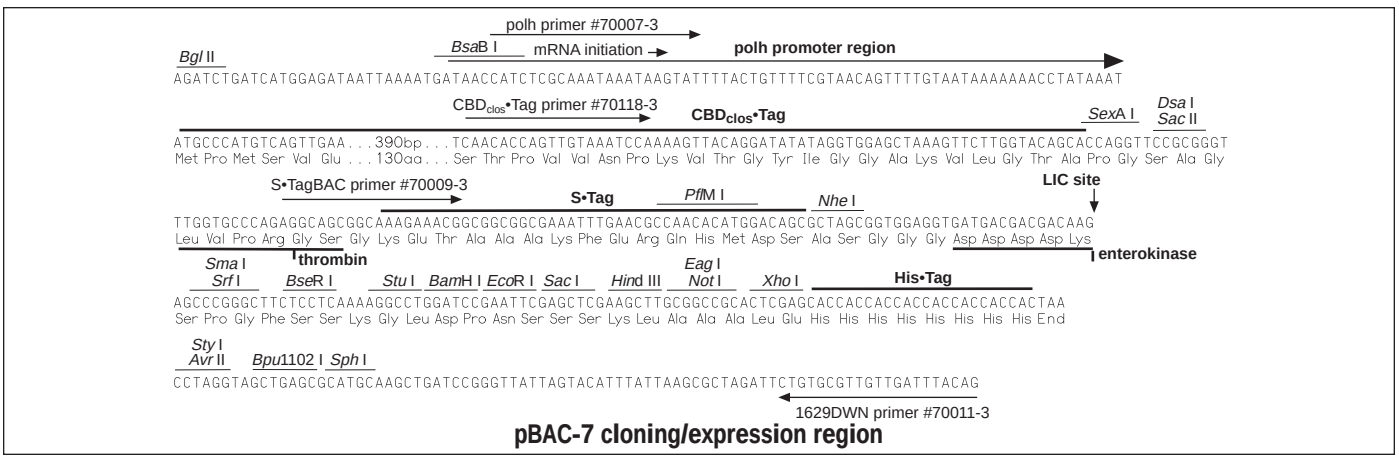
pBAC-7 sequence landmarks

polh promoter region	1177-1259
polh transcription start	1210-1211
wt polh 5'UTR –1 position	1259
CBD_{clos}•Tag coding seq.	1260-1736
S•Tag coding sequence	1770-1814
Multiple cloning sites (*Srf* I - *Xho* I)	1846-1910
His•Tag® coding sequence	1911-1934

Notes: The *Stu* I site is modified by *dcm* methylation, so the plasmid must be grown in *dcm*⁻ hosts to use this site for cloning. The *Srf* I and *Sma* I sites are destroyed during Ligation Independent Cloning. Primer sequence extensions required for LIC compatibility are underlined in the diagram below.







pBAC-7 cloning/expression region

pBAC-7 Restriction Sites

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Enzyme	# Sites	Locations
AatII	1	2315
AccI	2	107 392
AciI	63	
AflIII	5	547 2385 2535 2640 3530
AhdI	2	647 4423
AluI	26	
AlwI	14	
Alw26I	8	433 1097 2821 2884 3320 3379 4484 5260
AlwNI	1	3946
ApaLI	2	3844 5090
ApoI	15	
AvaI	2	1847 1905
AvaII	2	4561 4783
AvrII	1	1938
BamHI	1	1871
BanI	8	1751 2837 2864 3068 3119 3131 4371 5531
BanII	3	1848 1887 5501
BbsI	2	646 2493
BbvI	20	
BcgI	2	2849 4928
BcgI'	2	2815 4962
BclI	2	1156 1166
BfaI	11	
BglI	2	4543 5811
BglII	1	1161
BpmI	1	4493
Bpu10I	1	564
Bpu1102I	1	1947
BsaI	2	433 4484
BsaAI	2	2034 5572
BsaBI	1	1192
BsaHI	6	643 2312 2512 2865 3132 4960
BsaJI	4	1741 1847 1938 3690
BsaWI	5	981 2882 3736 3883 4714
BseRI	1	1847
BsgI	3	2679 2973 3127
BsiEI	6	1900 3446 3870 4793 4942 5839
BsiHKAI	5	1887 1912 3848 5009 5094
BsiI	10	190 412 1805 2201 3552 3570 3736 4015 5353 5679
BsmI	1	85
BsmBI	2	1097 2884
BsmFI	1	2440
Bsp1286I	10	1756 1848 1887 1912 2622 3122 3848 5009 5094 5501
BspLU11I	1	3530
BsrI	13	
BsrBI	5	2987 3282 3463 5264 5428
BsrDI	3	227 4484 4658
BsrFI	6	929 2882 3074 3122 4503 5467
BsrGI	3	197 916 2364
BssSI	2	3703 5087
BstXI	1	315
BstYI	8	1161 1871 4171 4182 4268 4280 5048 5065
Cac8I	26	
ClaI	2	3006 3329
CviJI	67	
DdeI	7	564 1947 2888 3805 4214 4380 4920
DpnI	23	
DraI	9	45 577 1357 1363 2124 3410 4289 4308 5000
DraIII	1	5575
DrdI	2	3638 5619
DsaI	1	1741
EaeI	2	1897 4811
EagI	1	1897
EarI	4	199 695 5218 5856
Eco47III	3	1814 1992 3175
Eco57I	2	4078 5090
EcoRI	1	1877
EcoRII	6	1733 1867 3556 3677 3690 5864
FauI	10	1736 2579 2593 2741 2950 2980 3342 5364 5433 5837
Fnu4HI	40	
FokI	3	4389 4570 4857
FspI	3	807 4645 5818
HaeI	9	1816 1994 2868 3033 3135 3177 3778 5417 5425
HaeIII	13	
HgaI	9	294 651 1114 2094 2520 3641 4219 4949 5350
HhaI	30	
HincII	2	108 393
HindIII	1	1890
HinfI	10	304 594 1997 2373 3430 3505 3901 4418 5620 5642
HphI	10	331 1027 1839 2798 4266 4493 4909 5115 5150 5572
MaeIII	16	
MboII	16	
MluI	1	547
MnlI	28	
MseI	47	
MslI	8	70 1098 1435 3113 3377 4675 4834 5193
MspI	17	
MspA1I	8	803 1743 1765 2957 3137 3872 4117 5058
MunI	4	2023 2368 2929 3294
MwoI	19	
NarI	2	2865 3132
NciI	6	1848 1849 1968 3910 4606 4957
NdeI	1	1532
NgoAIV	2	929 5467
NheI	1	1815
NlaIII	14	
NlaIV	20	
NotI	1	1897
NsiI	1	332
NspI	3	114 1957 3534
PflMI	1	1805
PinAI	1	2882
PleI	7	298 2367 3424 3909 4412 5628 5636
PmeI	1	3410
Psp1406I	3	4649 5022 5785
PvuI	2	4793 5839
RcaI	2	4250 5258
RsaI	13	
SacI	1	1887
SacII	1	1744
SalI	2	106 391
Sau3AI	23	
Sau96I	6	4465 4544 4561 4783 5578 5846
ScaI	1	4903
ScrFI	12	
SexAI	1	1733
SfaNI	7	339 1646 2779 3627 4679 4870 5119
SfcI	5	3367 3795 3986 4664 5349
SgrAI	1	3122
SmaI	1	1849
SnaBI	1	2034
SphI	1	1957
SrfI	1	1849
SspI	7	155 573 2128 2325 3198 5227 5780
StuI	1	1867
StyI	1	1938
SwaI	3	1357 1363 2124
TaiI	16	
TaqI	16	
TfiI	3	594 1997 3505
ThaI	15	
TseI	20	
Tsp45I	5	1425 1478 4679 4890 5394
Tsp509I	47	
TspRI	11	
VspI	4	20 128 2519 4595
XhoI	1	1905
XmnI	2	2575 5022

Enzymes that do not cut pBAC-7:

AflII	ApaI	AscI	BspEI	BspMI
BssHII	Bst1107I	BstEII	Bsu36I	EcoNI
EcoO109I	EcoRV	FseI	HpaI	KpnI
MscI	NcoI	NruI	NspV	PacI
PmlI	PshAI	Psp5II	PstI	PvuII
RsrII	SanDI	SapI	SfiI	SgfI
SpeI	Sse8387I	SunI	Tth111I	UbaEI
XbaI	XcmI			