pBAC-2cp Transfer Plasmid

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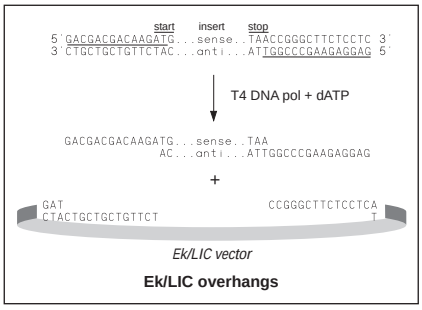
Locus	polh
Promoter	polh
N-terminal fusion	His•Tag, S•Tag
C-terminal fusion option	His•Tag
Cloning options	LIC, polylinker

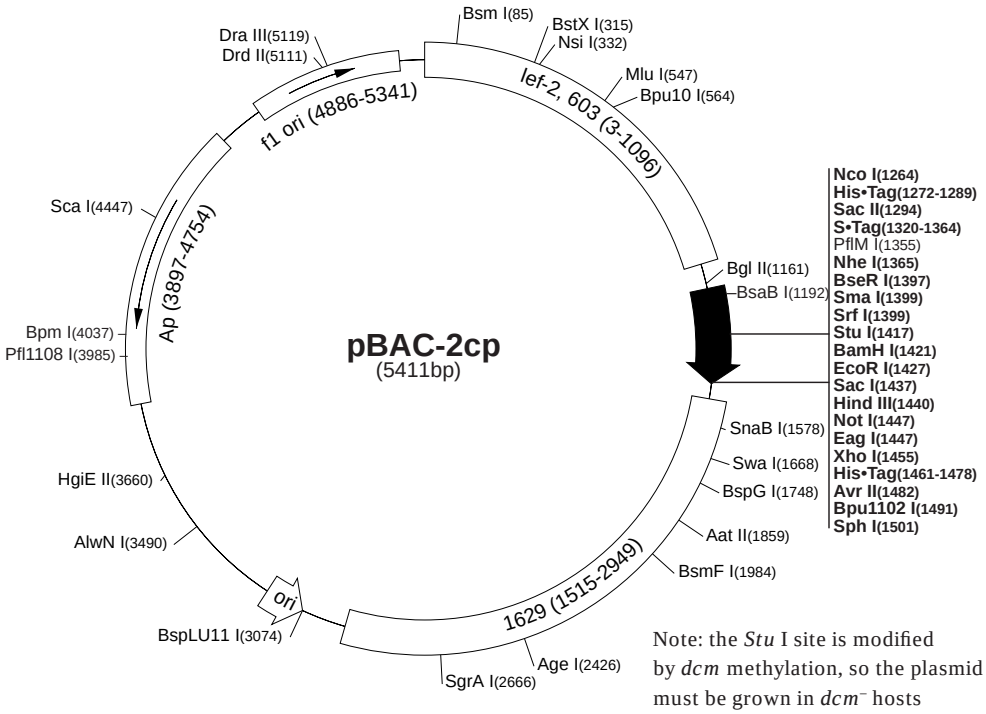
pBAC™-2cp is a baculovirus transfer plasmid (Cat. No. 70004-3) designed for simplified cloning and expression of target genes in insect cells. The plasmid is compatible with BacVector™-1000, -2000 or -3000 Triple Cut Virus DNA for low background transfection and efficient utilization of the polh promoter. pBAC-2cp provides an ATG start codon at the optimal position relative to native polyhedrin translation signals. Cloning sites are provided for the creation of N-terminal fusions of an insert with His•Tag® and/or S•Tag™ sequences. A Ligation-Independent Cloning (LIC) version of the vector is available (Cat. No. 70021-3) for rapid, directional cloning of PCR products adjacent to the enterokinase cleavage site. The vector provides optional expression of a C-terminal His•Tag fusion sequence by allowing read-through of inserts in the proper reading frame. 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-2cp sequence landmarks

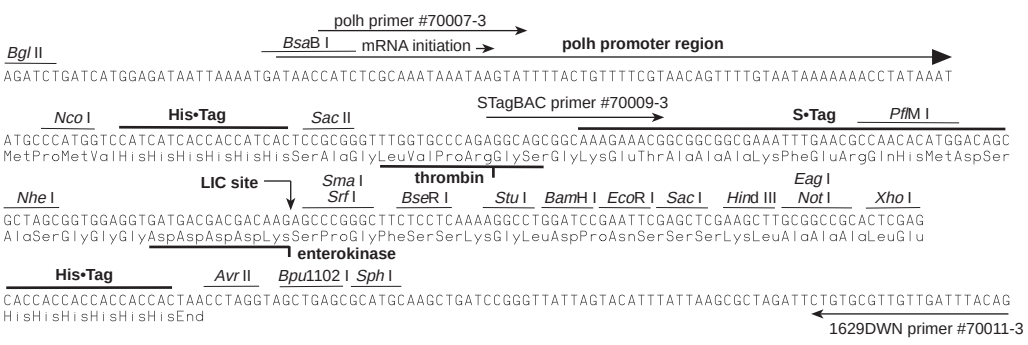
polh promoter region	1177-1259
polh transcription start	1210-1211
wt polh 5'UTR –1 position	1259
His•Tag coding sequence	1272-1289
S•Tag coding sequence	1320-1364
Multiple cloning sites (*Sma* I - *Xho* I)	1397-1460
His•Tag coding sequence	1461-1478



Ek/LIC overhangs



Note: the *Stu* I site is modified by *dcm* methylation, so the plasmid must be grown in *dcm*⁻ hosts to use this site for cloning.



pBAC-2cp cloning/expression region

pBAC-2cp Restriction Sites

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Enzyme	# Sites	Locations
AatII	1	1859
AccI	2	107 392
AceIII	4	2244 3026 4266 5272
AciI	62	
AflIII	5	547 1929 2079 2184 3074
AgeI	1	2426
AluI	23	
AlwI	14	
Alw21I	5	1437 1462 3392 4553 4638
Alw44I	2	3388 4634
AlwNI	1	3490
ApoI	12	
AvaI	2	1397 1455
AvaII	3	1268 4105 4327
AvrII	1	1482
BamHI	1	1421
BanI	8	1301 2381 2408 2612 2663 2675 3915 5075
BanII	3	1398 1437 5045
BbsI	2	646 2037
BbvI	19	
BccI	12	
Bce83I	5	2846 3165 3463 3704 4572
BcefI	7	20 1342 2242 2356 2397 3576 5089
BcgI	4	2359 2393 4472 4506
BclI	2	1156 1166
BfaI	10	639 952 1366 1483 1538 1808 3569 3822 4157 4963
BglI	2	4087 5355
BglII	1	1161
BmgI	3	1304 2164 2664
BpmI	1	4037
Bpu10I	1	564
Bpu1102I	1	1491
BsaI	2	433 4028
BsaAI	2	1578 5116
BsaBI	1	1192
BsaHI	6	643 1856 2056 2409 2676 4504
BsaJI	5	1264 1291 1397 1482 3234
BsaWI	5	981 2426 3280 3427 4258
BsaXI	3	303 1286 5168
BsbI	5	864 1352 2184 2373 5209
BscGI	7	2593 3401 3747 3968 3992 4514 5022
BseRI	1	1397
BsgI	3	2223 2517 2671
BsiI	2	3247 4631
BsiEI	6	1450 2990 3414 4337 4486 5383
BsiI	10	190 412 1355 1745 3096 3114 3280 3559 4897 5223
BsmI	1	85
BsmAI	8	433 1097 2365 2428 2864 2923 4028 4804
BsmBI	2	1097 2428
BsmFI	1	1984
BsoFI	39	
Bsp24I	6	1994 2026 3567 3599 3745 3777
Bsp1286I	10	1306 1398 1437 1462 2166 2666 3392 4553 4638 5045
BspGI	1	1748
BspLU11I	1	3074
BsrI	11	
BsrBI	5	2531 2826 3007 4808 4972
BsrDI	3	227 4028 4202
BsrFI	6	929 2426 2618 2666 4047 5011
BsrGI	3	197 916 1908
BstXI	1	315
BstYI	8	1161 1421 3715 3726 3812 3824 4592 4609
Cac8I	24	
CjeI	20	
CjePI	26	
ClaI	2	2550 2873
CviJI	63	
CviRI	15	
DdeI	7	564 1491 2432 3349 3758 3924 4464
DpnI	23	
DraI	7	45 577 1668 2954 3833 3852 4544
DraIII	1	5119
DrdI	2	3182 5163
DrdII	1	5111
DsaI	2	1264 1291
EaeI	2	1447 4355
EagI	1	1447
Eam1105I	2	647 3967
EarI	4	199 695 4762 5400
EciI	7	11 2305 2311 2317 3148 3294 4122
Eco47III	3	1364 1536 2719
Eco57I	2	3622 4634
EcoRI	1	1427
EcoRII	5	1417 3100 3221 3234 5408
FauI	10	1286 2123 2137 2285 2494 2524 2886 4908 4977 5381
FokI	3	3933 4114 4401
FspI	3	807 4189 5362
GdiII	2	1447 4355
HaeI	4	1417 3089 3100 3552
HaeII	9	1366 1538 2412 2577 2679 2721 3322 4961 4969
HaeIII	13	
HgaI	9	294 651 1114 1638 2064 3185 3763 4493 4894
HgiEII	1	3660
HhaI	30	
Hin4I	10	270 646 1630 1765 2229 2265 2512 2857 3966 4040
HincII	2	108 393
HindIII	1	1440
HinfI	10	304 594 1541 1917 2974 3049 3445 3962 5164 5186
HphI	11	
MaeII	15	
MaeIII	13	
MboII	15	
MluI	1	547
MmeI	4	2493 3289 3473 5141
MnlI	28	
MseI	43	
MslI	7	70 1098 2657 2921 4219 4378 4737
MspI	17	
MspA1I	8	803 1293 1315 2501 2681 3416 3661 4602
MunI	4	1567 1912 2473 2838
MwoI	18	
NarI	2	2409 2676
NciI	6	1398 1399 1512 3454 4150 4501
NcoI	1	1264
NgoAIV	2	929 5011
NheI	1	1365
NlaIII	13	
NlaIV	19	
NotI	1	1447
NsiI	1	332
NspI	3	114 1501 3078
Pfl1108I	1	3985
PflMI	1	1355
PleI	7	298 1911 2968 3453 3956 5172 5180
PmeI	1	2954
Psp1406I	3	4193 4566 5329
PvuI	2	4337 5383
RcaI	2	3794 4802
RleAI	2	305 2649
RsaI	11	
SacI	1	1437
SacII	1	1294
SalI	2	106 391
Sau96I	7	1268 4009 4088 4105 4327 5122 5390
Sau3AI	23	
ScaI	1	4447
ScrFI	11	
SfaNI	6	339 2323 3171 4223 4414 4663
SfcI	5	2911 3339 3530 4208 4893
SgrAI	1	2666
SmaI	1	1399
SnaBI	1	1578
SphI	1	1501
SrfI	1	1399
SspI	7	155 573 1672 1869 2742 4771 5324
StuI	1	1417
StyI	2	1264 1482
SwaI	1	1668
TaqI	16	
TaqII	7	307 2976 4315 4500 4653 4670 5214
TfiI	3	594 1541 3049
ThaI	15	
TseI	19	
Tsp45I	3	4223 4434 4938
Tsp509I	41	
Tth111II	5	1061 1732 3664 3671 3703
UbaJI	20	
VspI	4	20 128 2063 4139
XhoI	1	1455
XmnI	2	2119 4566

Enzymes that do not cut pBAC-2cp:

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