

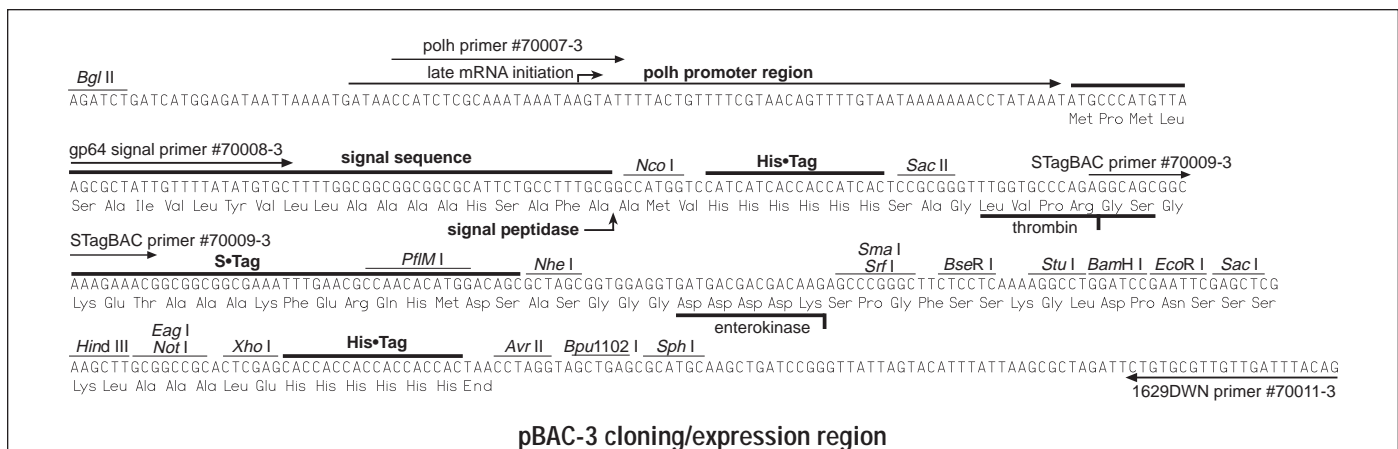
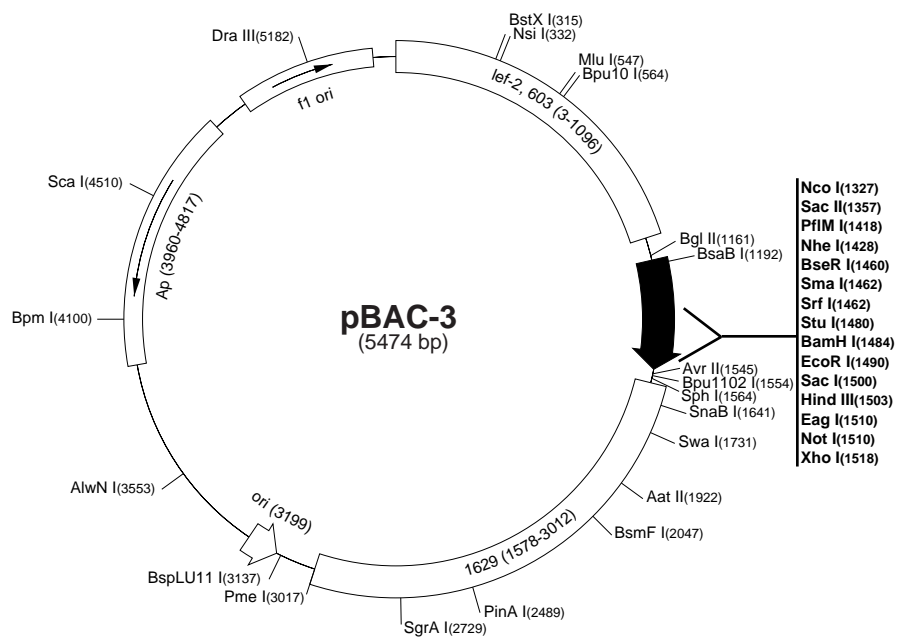
# pBAC-3 Transfer Plasmid

	Locus	polh
	Promoter	polh
	secretion signal	gp64 signal peptide
	N-terminal fusion	His•Tag, S•Tag
	C-terminal fusion option	His•Tag
	Cloning options	polylinker

pBAC™-3 is a baculovirus transfer plasmid (Cat. No. 70088-3) compatible with BacVector™ -1000, -2000, or -3000 Triple Cut Virus DNA for low background transfection and efficient utilization of the polh promoter and expression of secreted proteins. A 20 amino acid gp64 signal peptide sequence is capable of directing high levels of protein into the secretory pathway of infected insect cells. Cloning sites are provided for the creation of N-terminal fusions of an insert with His•Tag® and/or S•Tag™ sequences. pBAC-3 provides an ATG start codon at the optimal position relative to native polyhedrin translation signals. 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-3 sequence landmarks

polh promoter region	1168-1259
polh transcription start	1211
wt polh 5'UTR -1 position	1259
signal peptide coding region	1260-1325
His•Tag coding sequence	1335-1349
S•Tag coding sequence	1383-1427
Multiple cloning sites (Sma I - Xho I)	1460-1523
His•Tag coding sequence (C-terminal)	1524-1541



# pBAC-3 Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AatII	1	1922	DsaI	2	1327 1354	SrfI	1	1462		
AccI	2	107 392	EaeI	3	1324 1510 4418	SspI	7	155 573 1735 1932 2805		
AcII	66		EagI	1	1510			4834 5387		
AflIII	5	547 1992 2142 2247 3137	EarI	4	199 695 4825 5463	StuI	1	1480		
AhdI	2	647 4030	Eco47III	4	1274 1427 1599 2782	StyI	2	1327 1545		
AluI	23		Eco57I	2	3685 4697	Swal	1	1731		
AlwI	14		EcoRI	1	1490	TaiI	15			
Alw26I	8	433 1097 2428 2491 2927	EcoRII	5	1480 3163 3284 3297 5471	TaqI	16			
		2986 4091 4867	FauI	10	1349 2186 2200 2348 2557	TfiI	3	594 1604 3112		
AlwNI	1	3553			2587 2949 4971 5040 5444	ThaI	15			
ApaLI	2	3451 4697	Fnu4HI	43		TseI	19			
ApoI	12		FokI	3	3996 4177 4464	Tsp45I	3	4286 4497 5001		
AvaI	2	1460 1518	FspI	3	807 4252 5425	Tsp509I	41			
Avall	3	1331 4168 4390	HaeII	10	1276 1429 1601 2475 2640	TspRI	10	838 1965 3039 3545 3558		
AvrII	1	1545			2742 2784 3385 5024 5032			3829 3978 4083 4430 4457		
BamHI	1	1484	HaeIII	14		VspI	4	20 128 2126 4202		
BanI	8	1364 2444 2471 2675 2726	HgaI	9	294 651 1114 1701 2127	XhoI	1	1518		
		2738 3978 5138			3248 3826 4556 4957	XmnI	2	2182 4629		
BanII	3	1461 1500 5108	HhaI	32		Enzymes that do not cut pBAC-3:				
BbsI	2	646 2100	HincII	2	108 393	AflII	Apal	AscI	BspEI	BspMI
BbvI	19		HindIII	1	1503	BssHII	Bst1107I	BstEII	Bsu36I	EcoNI
BcgI	2	2456 4535	HinfI	10	304 594 1604 1980 3037	EcoO109I	EcoRV	FseI	HpaI	KpnI
BcgI'	2	2422 4569			3112 3508 4025 5227 5249	MscI	NdeI	NruI	NspV	PacI
BclI	2	1156 1166	HphI	11		PmlI	PshAI	Psp5II	PstI	PvuII
Bfal	10	639 952 1429 1546 1601	MaeIII	13		RsrII	SanDI	SapI	SexAI	SfiI
		1871 3632 3885 4220 5026	MbolI	15		Sgfi	SpeI	Sse8387I	SunI	Tth111I
BglI	3	1323 4150 5418	MluI	1	547	UbaEI	XbaI	XcmI		
BglII	1	1161	MnlI	28						
BpmI	1	4100	MseI	44						
Bpu10I	1	564	MslI	7	70 1098 2720 2984 4282					
Bpu1102I	1	1554			4441 4800					
BsaI	2	433 4091	MspI	17						
BsaAI	2	1641 5179	MspA1I	8	803 1356 1378 2564 2744					
BsaBI	1	1192			3479 3724 4665					
BsaHI	6	643 1919 2119 2472 2739	MunI	4	1630 1975 2536 2901					
		4567	MwoI	21						
BsaJI	5	1327 1354 1460 1545 3297	NarI	2	2472 2739					
BsaWI	5	981 2489 3343 3490 4321	NciI	6	1461 1462 1575 3517 4213					
BseRI	1	1460			4564					
BsgI	3	2286 2580 2734	NcoI	1	1327					
BsiEI	6	1513 3053 3477 4400 4549	NgoAIV	2	929 5074					
		5446	NheI	1	1428					
BsiHKAI	5	1500 1525 3455 4616 4701	NlaIII	14						
BsII	10	190 412 1418 1808 3159	NlaIV	19						
		3177 3343 3622 4960 5286	NottI	1	1510					
BsmI	2	85 1310	Nsil	1	332					
BsmBI	2	1097 2491	NspI	3	114 1564 3141					
BsmFI	1	2047	PfiMI	1	1418					
Bsp1286I	10	1369 1461 1500 1525 2229	PinAI	1	2489					
		2729 3455 4616 4701 5108	PleI	7	298 1974 3031 3516 4019					
BspLU11I	1	3137			5235 5243					
BsrI	11		PmeI	1	3017					
BsrBI	5	2594 2889 3070 4871 5035	Psp1406I	3	4256 4629 5392					
BsrDI	3	227 4091 4265	PvuI	2	4400 5446					
BsrFI	6	929 2489 2681 2729 4110	RcaI	2	3857 4865					
		5074	RsaI	11						
BsrGI	3	197 916 1971	SacI	1	1500					
BssSI	2	3310 4694	SacII	1	1357					
BstXI	1	315	Sall	2	106 391					
BstYI	8	1161 1484 3778 3789 3875	Sau3AI	23						
		3887 4655 4672	Sau96I	7	1331 4072 4151 4168 4390					
Cac8I	24				5185 5453					
Clal	2	2613 2936	Scal	1	4510					
CviJI	64		ScrFI	11						
Ddel	7	564 1554 2495 3412 3821	SfaNI	6	339 2386 3234 4286 4477					
		3987 4527			4726					
Dpnl	23		Sfcl	5	2974 3402 3593 4271 4956					
Dral	7	45 577 1731 3017 3896	SgrAI	1	2729					
		3915 4607	SmaI	1	1462					
DrallI	1	5182	SnaBI	1	1641					
DrdI	2	3245 5226	SphI	1	1564					