

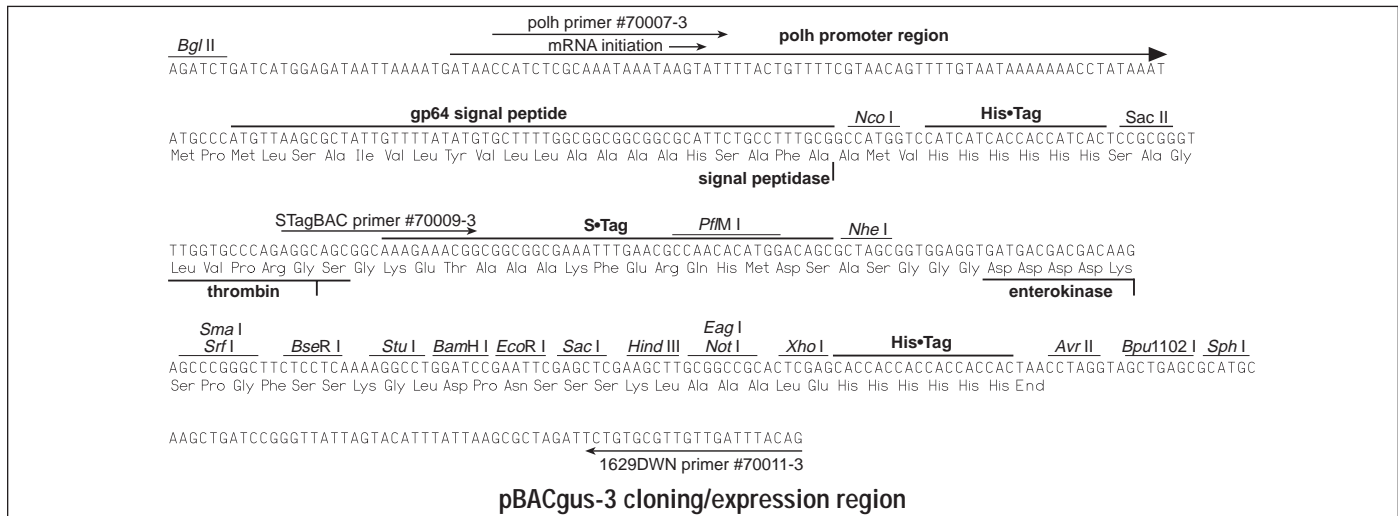
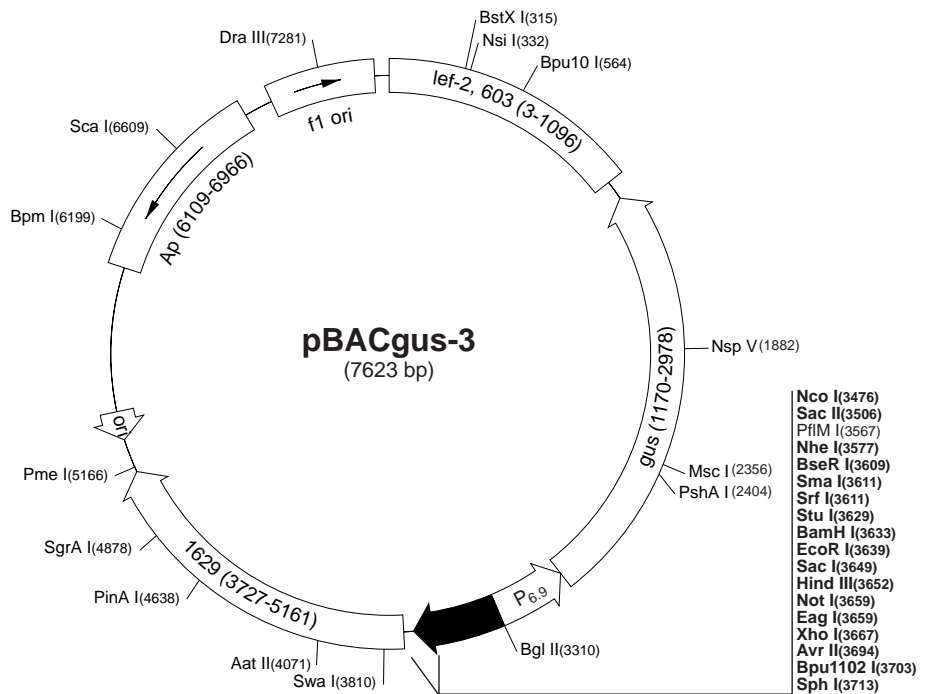
# pBACgus-3 Transfer Plasmid

<b>b</b>	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

pBACgus-3 is a baculovirus transfer plasmid (Cat. No. 70089-3) 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. pBACgus-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).

**pBACgus-3 sequence landmarks**

polh promoter region	3326-3408
polh transcription start	3360
wt polh 5'UTR -1 position	3408
signal peptide coding region	3409-3474
His•Tag coding sequence	3484-3501
S•Tag coding sequence	3532-3576
Multiple cloning sites ( <i>Sma</i> I - <i>Xho</i> I)	3609-3672
His•Tag coding sequence (C-terminal)	3673-3690



# pBACgus-3 Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AatII	1	4071	DrdI	2	5394 7375	SmaI	1	3611		
AccI	3	107 392 3305	DsaI	3	2411 3476 3503	SnaBI	2	2593 3790		
Acil	85		EaeI	4	2354 3473 3659 6567	SphI	1	3713		
AflIII	9	547 1418 1662 1680 2378	EagI	1	3659	SrfI	1	3611		
		4141 4291 4396 5286	EarI	7	199 695 1413 1851 1977	Sspl	10	155 573 1708 3023 3114		
AhdI	2	647 6179			6974 7612			3884 4081 4954 6983 7536		
AluI	25		Eco47III	4	3423 3576 3748 4931	StuI	1	3629		
AlwI	19		Eco57I	3	1958 5834 6846	StyI	2	3476 3694		
Alw26I	8	433 1097 4577 4640 5076	EcoRI	1	3639	Swal	1	3880		
		5135 6240 7016	EcoRII	8	1502 2429 2832 3629 5312	TaiI	20			
AlwNI	2	2138 5702			5433 5446 7620	TaqI	24			
ApaLI	4	1715 2040 5600 6846	EcoRV	2	2189 2420	TfiI	6	594 2061 2264 2469 3753		
ApoI	15		FauI	16				5261		
AvaI	2	3609 3667	Fnu4HI	54		ThaI	29			
Avall	4	2972 3480 6317 6539	FokI	6	1553 2178 3212 6145 6326	TseI	27			
AvrII	1	3694			6613	Tsp45I	8	1781 2214 2406 2622 2703		
BamHI	1	3633	FspI	3	807 6401 7574			6435 6646 7150		
BanI	8	3513 4593 4620 4824 4875	HaeII	11		Tsp509I	50			
		4887 6127 7287	HaeIII	20		TspRI	14			
BanII	3	3610 3649 7257	HgaI	19		UbaEI	2	1656 2360		
BbsI	2	646 4249	HhaI	40		VspI	6	20 128 2027 3019 4275		
BbvI	27		HincII	5	108 393 1838 2372 3306			6351		
BcgI	5	1188 1872 2049 4605 6684	HindIII	1	3652	XhoI	1	3667		
BcgI'	5	1222 1906 2083 4571 6718	HinfI	13		XmnI	2	4331 6778		
BclI	3	1156 2884 3315	HphI	26		Enzymes that do not cut pBACgus-3:				
Bfal	11		MaeIII	21		AflIII	Apal	AscI	BspEI	BssHII
BglI	3	3472 6299 7567	MbolI	20		Bst1107I	BstEII	Bsu36I	EcoNI	EcoO109I
BglII	1	3310	MluI	4	547 1662 1680 2378	FseI	HpaI	KpnI	NdeI	PacI
Bpml	1	6249	MnlI	35		PmlI	Psp5II	PstI	PvuII	RsrII
Bpu10I	1	564	MscI	1	2356	SanDI	SapI	SexAI	SfiI	SgfI
Bpu1102I	1	3703	MseI	52		SpeI	Sse8387I	SunI	Tth111I	XbaI
Bsal	2	433 6240	MslI	13		XcmI				
BsaAI	4	2074 2593 3790 7328	MspI	25						
BsaBI	4	1383 1477 2322 3341	MspA1I	8	803 3505 3527 4713 4893					
BsaHI	6	643 4068 4268 4621 4888			5628 5873 6814					
		6716	MunI	6	2843 3164 3779 4124 4685					
BsaJI	7	2411 2429 3476 3503 3609			5050					
		3694 5446	MwoI	30						
BsaWI	7	981 1735 2162 4638 5492	NarI	2	4621 4888					
		5639 6470	NciI	11						
BseRI	1	3609	NcoI	1	3476					
BsgI	4	1458 4435 4729 4883	NgoAIV	2	929 7223					
BsiEI	7	1258 3662 5202 5626 6549	NheI	1	3577					
		6698 7595	NlaIII	22						
BsiHKAI	7	1719 2044 3649 3674 5604	NlaIV	20						
		6765 6850	NotI	1	3659					
BsII	15		NruI	2	1260 2904					
BsmI	4	85 2922 3038 3459	NsiI	1	332					
BsmBI	2	1097 4640	NspI	5	114 1422 2405 3713 5290					
BsmFI	2	2266 4196	NspV	1	1882					
Bsp1286I	12		PfiMI	1	3567					
BspLU11I	2	1418 5286	PinAI	1	4638					
BspMI	2	1641 2371	PleI	7	298 4123 5180 5665 6168					
BsrI	20				7384 7392					
BsrBI	6	3237 4743 5038 5219 7020	PmeI	1	5166					
		7184	PshAI	1	2404					
BsrDI	3	227 6240 6414	Psp1406I	3	6405 6778 7541					
BsrFI	6	929 4638 4830 4878 6259	PvuI	2	6549 7595					
		7223	RcaI	3	2089 6006 7014					
BsrGI	5	197 916 1427 1853 4120	RsaI	17						
BssSI	2	5459 6843	SacI	1	3649					
BstXI	1	315	SacII	1	3506					
BstYI	11		Sall	3	106 391 3304					
Cac8I	29		Sau3AI	33						
Clal	2	4762 5085	Sau96I	10	2010 2144 2972 3480 6221					
CviJI	87				6300 6317 6539 7334 7602					
Ddel	9	564 1824 3233 3703 4644	Scal	1	6659					
		5561 5970 6136 6676	ScrFI	19						
Dpnl	33		SfaNI	16						
Dral	7	45 577 3880 5166 6045	Sfcl	6	2963 5123 5551 5742 6420					
		6064 6756			7105					
DrallI	1	7331	SgrAI	1	4878					