

## pAcP(+)*IE1-3* Transfer Plasmid

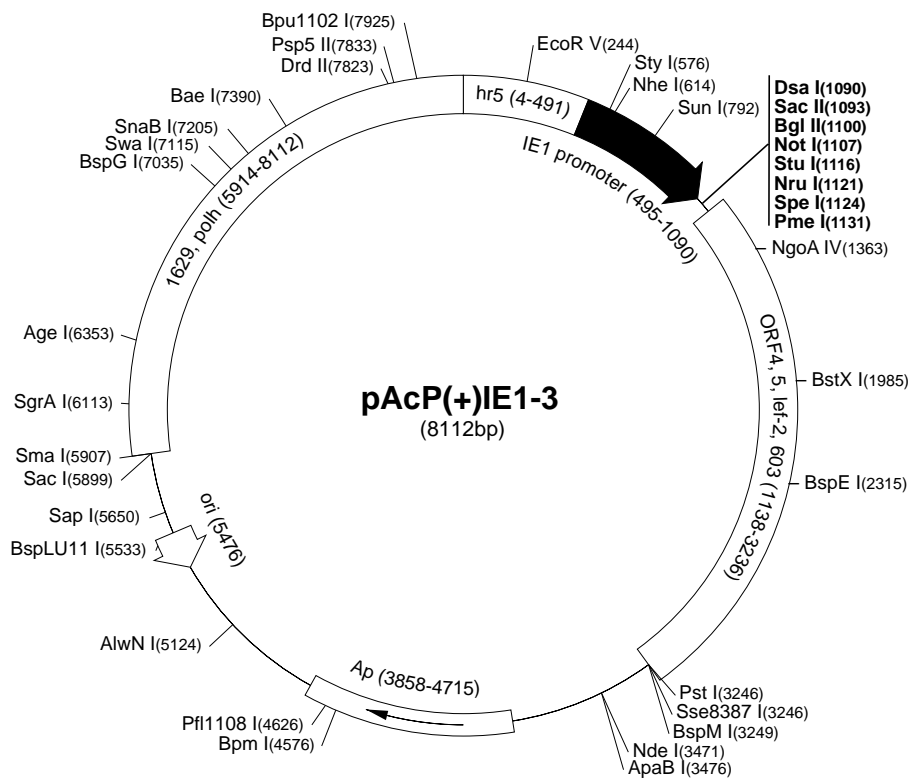
<b>b</b>	Promoter	AcNPV <i>IE1</i>
	Cloning options	polylinker

The pAcP(+)*IE1-3* transfer plasmid (Cat. No. 69094-3) is designed for the production of recombinant baculoviruses containing foreign genes under the control of the baculovirus *ie1* promoter (1). The P(+) designation indicates that this transfer plasmid carries the intact *polyhedrin* gene and will therefore produce occlusion-positive baculoviruses when used with appropriate *occ*<sup>-</sup> baculovirus DNA, e.g., BacVector™-1000 Triple Cut Virus DNA (Cat. No. 70059-3). Inserts must carry their own ATG translation start codon. The *hr5* element, *ie1* promoter, and *mcs* sequences are targeted for insertion into the polyhedrin locus of the viral genome. Unique restriction sites are indicated on the circle map. The cloning/expression region of the coding strand transcribed from the *ie1* promoter is shown below. Note that cleavage at the *Xba*I site at 1097 is blocked by *dam* methylation unless plasmid is prepared from a *dam*<sup>-</sup> host strain.

1. Jarvis, D.L., Weinkauff, C. and Guarino, L.A. (1996) *Prot. Exp. Pur.* **8**, 191-203.

### pAcP(+)*IE1-3* sequence landmarks

<i>hr5</i> enhancer	4-491
<i>ie1</i> promoter + 5'UTR	495-1090
Multiple cloning sites ( <i>Sac</i> II - <i>Pme</i> I)	1090-1135



E1 promoter primer #69103-3 → **IE1 promoter region** → *Dsa* I / *Sac* II    *Bgl* II    *Not* I    *Stu* I / *Nru* I    *Spe* I / *Pme* I

TGGATATTGTTTCAGTTGCAAGTTGACACTGGCGGCACAAAGATCGTGAACCAACCAAGTGACCCGGGATCTAGATCTGCGCCGCAGGCCTCGGACTAGTTTTAAACCCATCAGCAA

CTATATATTGATAGACATTTCCAGTTTGTGATATTAGTTTGTGCGTCTCATTACA ← TV14AS primer #69105-3

### pAcP(+)*IE1-3* cloning/expression region

# pAcP(+)<sup>IE1-3</sup> Restriction Sites

Enzyme	# Sites	Locations						Enzyme	# Sites	Locations						Enzyme	# Sites	Locations						
AatII	2	3726	6928					BsrDI	4	2071	2988	4411	4585					NciI	8	3573	3608	4109	4460	5156
AccI	2	1902	2187					BsrFI	5	1363	4560	6113	6161	6353										
AccIII	5	700	3599	4341	5581	6535		BsrGI	6	1376	2095	2302	2955	3137										
Acil	80									6871														
AflIII	12							BssHII	2	510	771													
AgeI	1	6353						BstXI	1	1985														
AluI	34							BstYI	10	1094	1100	3998	4015	4783										
AlwI	16									4795	4881	4892	5909	7845										
Alw21I	7	2672	2683	3480	3977	4062		Cac8I	35															
		5223	5899					CjeI	34															
Alw44I	5	2668	2679	3476	3973	5219		CjePI	16															
AlwNI	1	5124						Clal	3	212	6231	7434												
ApaBI	1	3476						CviJI	96															
ApoI	20							CviRI	37															
AvaI	2	2867	5905					DdeI	13															
Avall	8	596	631	2500	4281	4503		DpnI	34															
		7293	7721	7833				Drat	7	1131	1719	2251	4067	4759										
BaeI	1	7390						DrdI	2	3562	5431													
BamHI	2	5909	7845					DrdII	1	7823														
BanI	11							Dsal	1	1090														
BanII	2	169	5899					EaeI	6	850	1107	3201	3265	4252										
BbsI	3	1646	6742	7699						5694														
BbvI	25							EagI	2	850	1107													
BccI	17							Eam1105I	3	1650	2516	4645												
Bce83I	5	4041	4909	5150	5448	5939		EarI	7	2094	3358	3846	5650	7376										
BceII	10	71	739	2275	2949	3253				7800	7895													
		5034	6385	6426	6540	7957		Ecil	8	910	2285	4489	5317	5463										
Bcgl	8	3030	3064	3223	3257	4107				6466	6472	6478												
		4141	6392	6426				Eco47III	2	6064	7247													
BcII	2	786	2901					Eco57I	3	3979	4991	7357												
Bfal	14							EcoO109I	2	3665	7833													
BglI	3	1003	3409	4527				EcoRI	6	86	190	297	373	480										
BglII	1	1100								5889														
Bmgl	2	6119	6619					EcoRII	9	3299	5372	5385	5506	5794										
Bpml	1	4576								7341	7506	7611	7836											
Bpu10I	2	1729	7850					EcoRV	1	244														
Bpu1102I	1	7925						FauI	13															
Bsal	2	1859	4579					FokI	11															
BsaAI	5	198	381	792	7205	7978		FspI	5	662	1489	3052	3399	4422										
BsaBI	4	1099	2724	7560	8087			GdiII	6	850	1107	3201	3265	4252										
BsaHI	9	1651	3218	3419	3723	4105				5694														
		6105	6372	6725	6925			HaeI	4	1116	5059	5511	5522											
BsaJI	8	576	1090	3299	5373	5794		HaeII	8	3422	5293	5663	6066	6108										
		5905	7342	7836						6210	6375	7249												
BsaWI	6	1311	2315	4349	5180	5327		HaeIII	18															
		6353						Hgal	16															
BsaXI	4	795	1993	2366	5679			HgiEII	2	3471	4951													
Bsbl	6	1432	2738	3537	6410	6599		HhaI	46															
		7606						Hin4I	14															
BscGI	13							HincII	7	1052	1903	2188	2294	3222										
BseRI	3	789	1618	7358				HindIII	3	3254	6838	7766												
BsgI	3	6114	6268	6562				Hinfl	17															
Bsil	5	801	2653	3669	3976	5360		HpaI	2	2294	7690													
BsiEI	12							HphI	12															
BsII	15							KpnI	2	5905	7389													
BsmI	3	783	2213	2466				Maell	28															
BsmAI	9	1195	1859	3608	3650	3803		MaeIII	22															
		4579	5915	6351	6414			MbolI	18															
BsmBI	4	1195	3608	3650	6351			MluI	3	880	1745	2664												
BsmFI	4	6795	7657	7819	8011			MmeI	5	1008	5140	5324	6292	7782										
BsoFI	48							MnlI	42															
Bsp24I	8	3713	3745	4839	4871	5017		MseI	59															
		5049	6762	6794				MslI	7	1198	2226	2733	3874	4233										
Bsp1286I	10	169	2672	2683	3480	3977				4392	6126													
		4062	5223	5899	6121	6621		MspI	23															
BspEI	1	2315						MspAII	13															
BspGI	1	7035						MunI	4	5941	6306	6867	7212											
BspLU11I	1	5533						MwoI	33															
BspMI	1	3249						NarI	3	3419	6105	6372												
BsrI	16																							
BsrBI	8	871	2443	3189	3803	5604																		
		5845	5957	6252																				

 Enzymes that do not cut pAcP(+)<sup>IE1-3</sup>:

AflIII	Apal	AscI	AvrII	Bst1107I
BstEII	Bsu36I	DrallI	EcoNI	FseI
MscI	NcoI	Pacl	PflMI	PmlI
PshAI	RsrII	SexAI		