

pAcP(+)*IE1-2* Transfer Plasmid



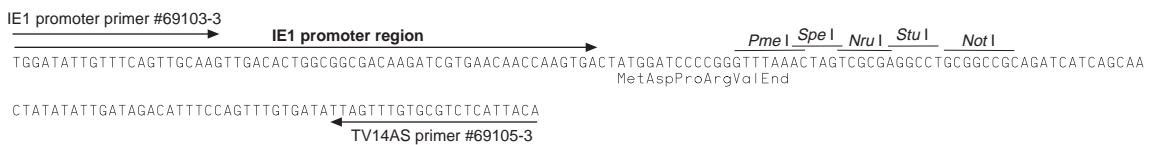
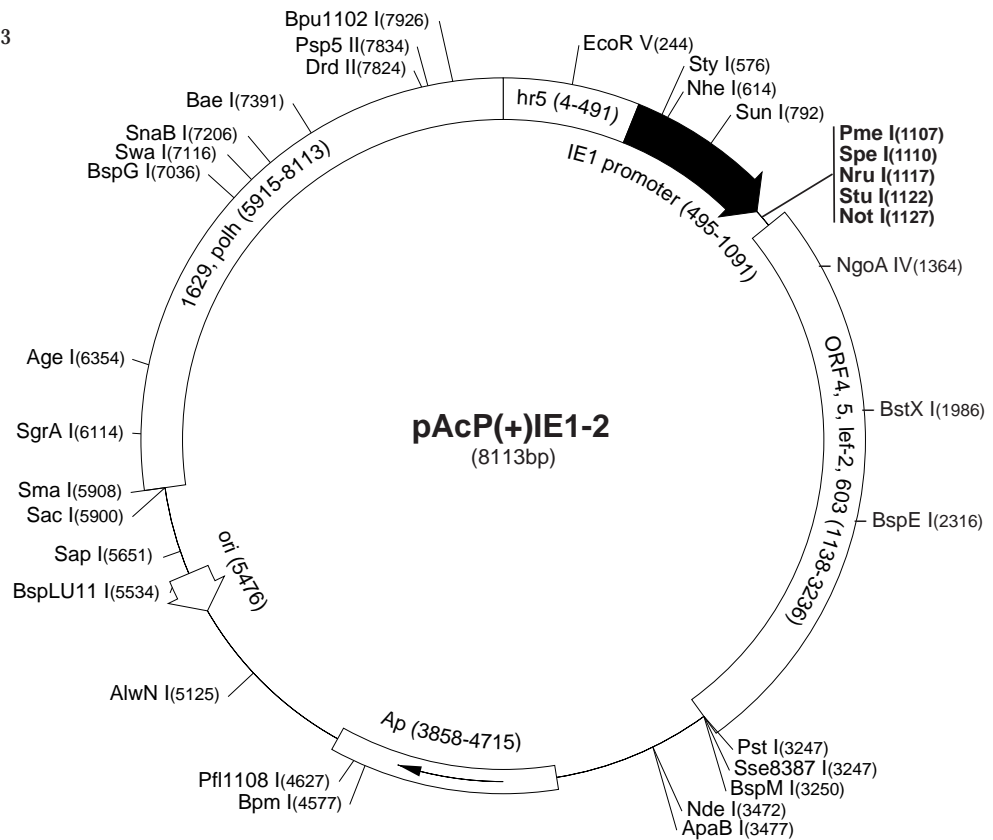
Promoter	AcNPV <i>IE1</i>
N-terminal fusion	<i>IE1</i> ATG
Cloning options	polylinker

The pAcP(+)*IE1-2* transfer plasmid (Cat. No. 69093-3) 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-* baculovirus DNA, e.g., BacVector™-1000 Triple Cut Virus DNA (Cat. No. 70059-3). pAcP(+)*IE1-2* carries the natural *ie1* translation initiation site, which can be used to express fusion proteins when the *Pme I* cloning site is used. 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.

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

pAcP(+)*IE1-2* sequence landmarks

<i>hr5</i> enhancer	4-491
<i>ie1</i> promoter + 5'UTR	495-1091
<i>ie1</i> translation initiation	1092
Multiple cloning sites (<i>Pme I</i> - <i>Not I</i>)	1104-1133



pAcP(+)*IE1-2* cloning/expression region

pAcP(+)IE1-2 Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AatII	2	3727 6929	BsrFI	5	1364 4561 6114 6162 6354	NgoAIV	1	1364		
AccI	2	1903 2188	BsrGI	6	1377 2096 2303 2956 3138	NheI	1	614		
AceIII	5	700 3600 4342 5582 6536			6872	NlaIII	32			
Acil	78		BssHII	2	510 771	NlaIV	26			
AflIII	12		BstXI	1	1986	NotI	1	1127		
AgeI	1	6354	BstYI	9	1094 3999 4016 4784 4796	NruI	1	1117		
AluI	34				4882 4893 5910 7846	Nsil	2	1969 2469		
AlwI	17		Cac8I	35		NspI	10	622 699 2187 2304 3007		
Alw21I	7	2673 2684 3481 3978 4063	CjeI	34				3031 3253 3621 5538 7653		
		5224 5900	CjePI	16		NspV	2	3079 7447		
Alw44I	5	2669 2680 3477 3974 5220	Clal	3	212 6232 7435	Pfi1108I	1	4627		
AlwNI	1	5125	CviJI	96		PleI	12			
ApaBI	1	3477	CviRI	37		PmeI	1	1107		
ApoI	20		Ddel	13		Psp5II	1	7834		
AvaI	3	1099 2868 5906	Dpnl	34		Psp1406I	4	2962 3015 4044 4417		
Avall	8	596 631 2501 4282 4504	Drall	7	1107 1720 2252 4068 4760	PstI	1	3247		
		7294 7722 7834			4779 7116	PvuI	6	108 212 3238 3381 4277		
BaeI	1	7391	DrdI	2	3563 5432			7435		
BamHI	3	1094 5910 7846	DrdII	1	7824	PvuII	3	784 3350 5714		
BanI	11		EaeI	6	850 1127 3202 3266 4253	RcaI	4	3701 3806 4814 7570		
BanII	2	169 5900			5695	RleAI	2	1995 6138		
BbsI	3	1647 6743 7700	EagI	2	850 1127	RsaI	27			
BbvI	25		Eam1105I	3	1651 2517 4646	SacI	1	5900		
BccI	16		EarI	7	2095 3359 3847 5651 7377	Sall	2	1902 2187		
Bce83I	5	4042 4910 5151 5449 5940			7801 7896	SapI	1	5651		
BceII	10	71 739 2276 2950 3254	Ecil	8	910 2286 4490 5318 5464	Sau96I	13			
		5035 6386 6427 6541 7958			6467 6473 6479	Sau3AI	34			
Bcgl	8	3031 3065 3224 3258 4108	Eco47III	2	6065 7248	Scal	2	4165 7963		
		4142 6393 6427	Eco57I	3	3980 4992 7358	ScrFI	19			
BclI	2	786 2902	EcoO109I	2	3666 7834	SfaNI	14			
Bfal	13		EcoRI	6	86 190 297 373 480	SfiI	5	3243 4400 5078 5269 7735		
BglI	3	1003 3410 4528			5890	SgrAI	1	6114		
BmgI	2	6120 6620	EcoRII	9	3300 5373 5386 5507 5795	SmaI	2	1101 5908		
BpmI	1	4577			7342 7507 7612 7837	SnaBI	1	7206		
Bpu10I	2	1730 7851	EcoRV	1	244	SpeI	1	1110		
Bpu1102I	1	7926	FauI	13		SphI	3	622 3007 3253		
BsaI	2	1860 4580	FokI	11		Sse8387I	1	3247		
BsaAI	5	198 381 792 7206 7979	FspI	5	662 1490 3053 3400 4423	SspI	8	981 1724 2142 3092 3841		
BsaBI	3	2725 7561 8088	GdIII	6	850 1127 3202 3266 4253			6042 6915 7112		
BsaHI	9	1652 3219 3420 3724 4106			5695	StuI	1	1122		
		6106 6373 6726 6926	HaeI	4	1122 5060 5512 5523	StyI	1	576		
BsaJI	9	576 1098 1099 3300 5374	HaeII	8	3423 5294 5664 6067 6109	SunI	1	792		
		5795 5906 7343 7837			6211 6376 7250	Swal	1	7116		
BsaWI	6	1312 2316 4350 5181 5328	HaeIII	18		TaqI	31			
		6354	Hgal	16		TaqII	7	1992 3944 3961 4114 4299		
BsaXI	4	795 1994 2367 5680	HgiEI	2	3472 4952			5638 7555		
Bsbl	6	1433 2739 3538 6411 6600	HhaI	46		TfiI	5	1700 2507 5560 5700 7240		
		7607	Hin4I	14		Thal	33			
BscGI	13		HincII	7	1052 1904 2189 2295 3223	TseI	25			
BseRI	3	789 1619 7359			7655 7691	Tsp45I	7	1085 2547 2602 3286 3597		
BsgI	3	6115 6269 6563	HindIII	3	3255 6839 7767			4173 4384		
Bsil	5	801 2654 3670 3977 5361	Hinfl	17		Tsp509I	59			
BsiEI	12		HpaI	2	2295 7691	Tth111II	6	1238 3071 4911 4943 4950		
BsII	15		HphI	12				7054		
BsmI	3	783 2214 2467	KpnI	2	5906 7390	UbaII	23			
BsmAI	9	1196 1860 3609 3651 3804	Maell	28		VspI	7	839 2167 2275 4471 5706		
		4580 5916 6352 6415	MaeIII	22				5765 6719		
BsmBI	4	1196 3609 3651 6352	Mboll	18		XmnI	3	4046 6665 7915		
BsmFI	4	6796 7658 7820 8012	Mlul	3	880 1746 2665					
BsoFI	48		Mmel	5	1008 5141 5325 6293 7783	Enzymes that do not cut pAcP(+)IE1-2:				
Bsp24I	8	3714 3746 4840 4872 5018	MnII	42		AflII	Apal	AscI	AvrII	BglII
		5050 6763 6795	Msel	59		Bst1107I	BstEII	Bsu36I	DraIII	Dsal
Bsp1286I	10	169 2673 2684 3481 3978	Msil	7	1199 2227 2734 3875 4234	EcoNI	FseI	MscI	NcoI	Pacl
		4063 5224 5900 6122 6622			4393 6127	PfiMI	PmlI	PshAI	RsrII	SacII
BspEI	1	2316	MspI	24		SexAI	Sfil	SgfI	SrfI	Tth111I
BspGI	1	7036	MspAII	12		XbaI	Xcml	XhoI		
BspLU11I	1	5534	MunI	4	5942 6307 6868 7213					
BspMI	1	3250	Mwol	33						
BsrI	16		NarI	3	3420 6106 6373					
BsrBI	8	871 2444 3190 3804 5605	NciI	10	1100 1101 3574 3609 4110					
		5846 5958 6253			4461 5157 5907 5908 7832					
BsrDI	4	2072 2989 4412 4586	NdeI	1	3472					