pBACsurf-1 Transfer Plasmid

TB148 06/00

Locus	polh
Promoter	polh
N-terminal fusion	gp64 signal sequence
C-terminal fusion option	gp64 mature domain
Cloning options	polylinker

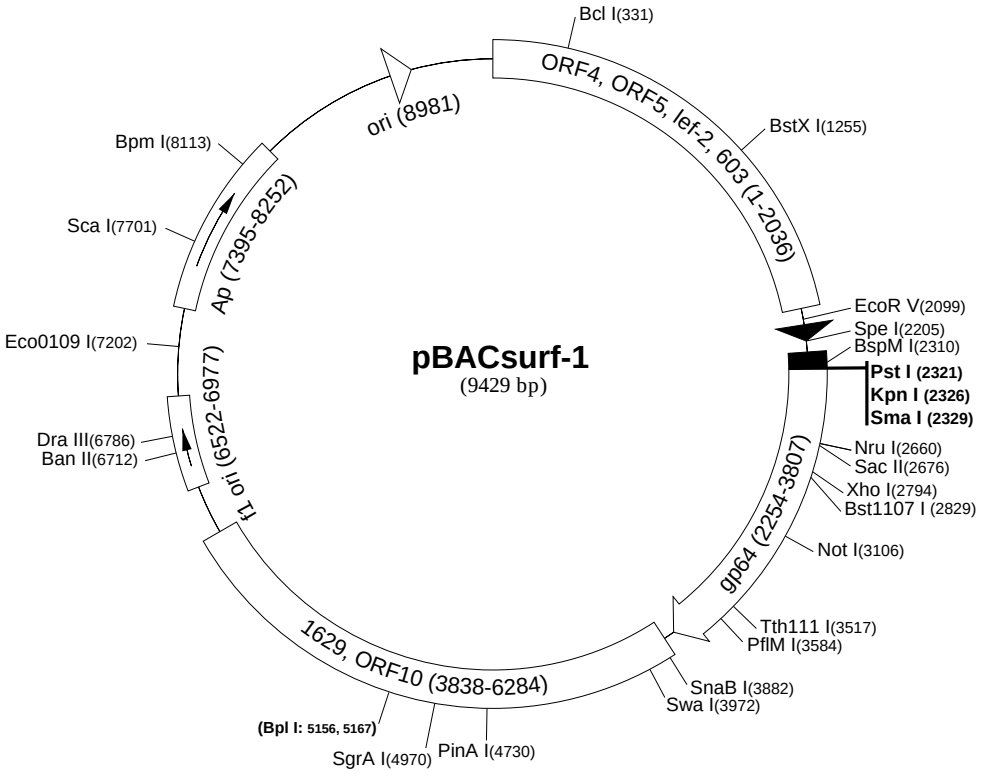
pBACsurf-1 is a baculovirus transfer plasmid (Cat. No. 70055-3) designed for expression of target proteins on the virion surface (1). For virus display, inserts are cloned in-frame between the upstream AcNPV gp64 signal sequence and downstream gp64 mature domain. The transmembrane region of the mature domain anchors the fusion protein in the membrane of the baculovirus nucleocapsid. By including a translation termination codon in the insert, target proteins can be secreted from the cell. 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. 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 non-coding strand. Single stranded sequencing of phage-derived DNA can be performed using the gp64 signal primer (Cat. No. 70008-3).

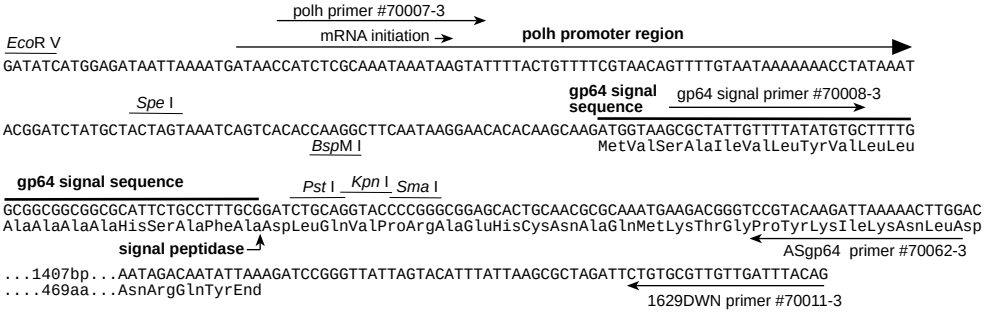
Note: Before transfection of insect cells with pBACsurf-1 derived transfer vectors, it is highly recommended to linearize the vector with the enzyme BplI (Fermentas). This treatment prevents a frame-shift mutation from occuring within the ORF 1629 open reading frame.

pBACsurf-1 sequence landmarks

polh promoter region	2121-2191
polh transcription start	2142-2143
gp64 signal sequence	2254-2325
Multiple cloning sites (*Pst* I - *Sma* I)	2321-2329
gp64 mature domain	2332-3807

1. Boublik, Y., Di Bonito, P. and Jones, I.M. (1995) *Bio/Technology* **13**, 1079–1084.





pBACsurf-1 cloning/expression region

pBACsurf-1 Restriction Sites

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Enzyme	# Sites	Locations				
Enzyme	# Sites	Locations				
AatII	3	3521	4163	7263		
AccI	6	1047	1332	2828	2920	5250
		6239				
AciI	92					
AflIII	15					
AhdI	3	721	1587	8182		
AluI	37					
AlwI	19					
Alw26I	14					
AlwNI	2	3584	8661			
ApaLI	7	553	564	3384	5970	7013
		7510	8756			
ApoI	15					
AvaI	5	365	2327	2794	2983	6890
AvaII	4	733	2362	7818	8040	
BamHI	2	2517	3530			
BanI	12					
BanII	1	6712				
BbsI	4	1586	2362	4341	6115	
BbvI	27					
BcgI	3	208	4697	7678		
BcgI'	3	174	4663	7644		
BclI	1	331				
BfaI	12					
BglI	3	3117	6470	8064		
BplI	2	5156	5167			
BpmI	1	8113				
Bpu10I	2	1504	2843			
BsaI	2	1373	8116			
BsaAI	4	2741	3047	3882	6783	
BsaBI	4	512	2124	5402	5452	
BsaHI	12					
BsaJI	13					
BsaWI	7	917	1921	4730	5999	7886
		8717	8864			
BseRI	2	1620	3205			
BsgI	4	3256	4527	4821	4975	
BsiEI	9	718	2628	3109	6086	6441
7664	7813	8736	9160			
BsiHKAI	10	557	568	2339	3092	3388
		5974	7017	7514	7599	8760
BsiI	18					
BsmI	3	772	1025	2298		
BsmBI	6	2037	2434	2882	4732	7145
		7187				
BsmFI	2	4288	5740			
Bsp1286I	13					
BspEI	2	917	5999			
BspLU11I	2	5257	9070			
BspMI	1	2310				
BsrI	19					
BsrBI	8	47	793	4835	5130	6639
		7340	9141	9382		
BsrDI	5	250	1167	3478	7948	8122
BsrFI	8	1869	3396	4730	4922	4970
		6078	6678	8097		
BsrGI	8	95	277	930	1137	1856
		2783	4212	5442		
BssSI	5	579	6196	7206	7513	8897
Bst1107I	1	2829				
BstXI	1	1255				
BstYI	11					
Cac8I	39					
ClaI	2	4854	5177			
CviJI	104					
DdeI	11					
DpnI	33					
DraI	9	985	1517	3972	5297	5306
		6172	7604	8296	8315	
DraIII	1	6786				
DrdI	5	3283	3517	6830	7099	8968

Enzyme	# Sites	Locations				
DsaI	5	2673	2867	3290	3626	3680
EaeI	11					
EagI	2	3106	6083			
EarI	5	1139	2609	6419	7383	9187
Eco47III	4	2262	3840	5023	5839	
Eco57I	3	3707	7516	8528		
EcoO109I	1	7202				
EcoRII	8	2486	2801	2822	6360	8909
		8922	9043	9331		
EcoRV	1	2099				
FauI	16					
Fnu4HI	60					
FokI	7	267	693	6380	7100	7743
		8030	8211			
FspI	7	184	1747	2732	3245	5402
		6460	7959			
HaeII	14					
HaeIII	24					
HgaI	17					
HhaI	52					
HincII	8	14	942	1048	1333	3738
		5251	6061	6191		
HindIII	2	4245	5291			
HinfI	22					
HpaI	4	942	3738	6061	6191	
HphI	17					
KpnI	1	2326				
MaeIII	24					
MboII	20					
MluI	3	568	1487	2529		
MnlI	38					
MscI	3	3295	3454	5472		
MseI	75					
MslI	13					
MspI	25					
MspA1I	14					
MunI	5	3506	3871	4216	4777	5142
MwoI	36					
NarI	4	3120	4713	4980	6480	
NciI	8	2328	2329	3816	7110	7145
		7646	7997	8693		
NcoI	2	3626	3680			
NgoAIV	3	1869	3396	6678		
NlaIII	30					
NlaIV	26					
NotI	1	3106				
NruI	1	2660				
NsiI	6	772	1272	3073	3182	3242
		3478				
NspI	8	210	234	937	1054	3244
		5261	7157	9074		
NspV	3	156	2776	5905		
PflMI	1	3584				
PinAI	1	4730				
PleI	14					
PmlI	2	2741	3047			
Psp1406I	7	220	273	3128	6187	6996
		7580	7953			
PstI	1	2321				
PvuI	2	6441	7813			
PvuII	3	5828	6410	9250		
RcaI	4	3326	7237	7342	8350	
RsaI	31					
SacII	1	2676				
SalI	3	1046	1331	5249		
SapI	2	2609	9187			
Sau3AI	33					
Sau96I	10	733	2362	2670	6429	6789
		7202	7818	8040	8057	8136
ScaI	1	7701				
ScrFI	16					
SfaNI	22					

Enzyme	# Sites	Locations				
SfcI	6	2317	5215	6560	7936	8614
		8805				
SgrAI	1	4970				
SmaI	1	2329				
SnaBI	1	3882				
SpeI	1	2205				
SphI	2	234	3244			
SspI	11					
StyI	5	2223	2399	3296	3626	3680
SwaI	1	3972				
TaiI	44					
TaqI	32					
TfiI	8	727	1534	2904	3845	5448
		5461	9096	9236		
ThaI	32					
TseI	27					
Tsp45I	12					
Tsp509I	71					
TspRI	20					
Tth111I	1	3517				
VspI	7	960	1068	3754	4367	8007
		9242	9301			
XhoI	1	2794				
XmnI	3	4423	6007	7582		

Enzymes that do not cut pBACsurf-1:

AflII	ApaI	ApaBI	AscI	AvrII
BaeI	BglII	Bpu1102I	BssHII	BstEII
Bsu36I	EcoNI	EcoRI	FseI	NdeI
NheI	PacI	PmeI	PshAI	Psp5II
RsrII	SacI	SexAI	SfiI	SgfI
SrfI	Sse8387I	StuI	SunI	XbaI
XcmI				