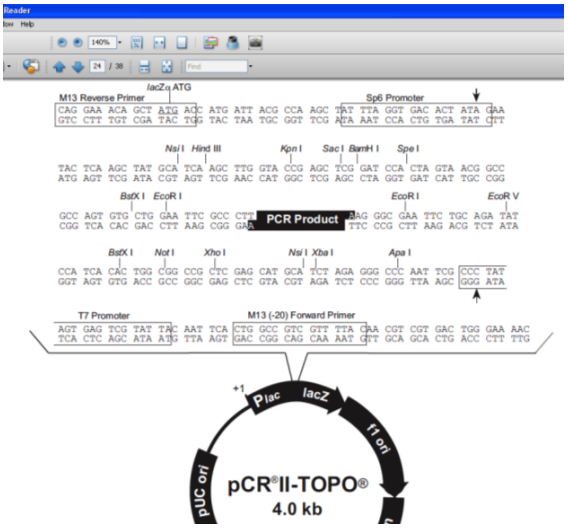
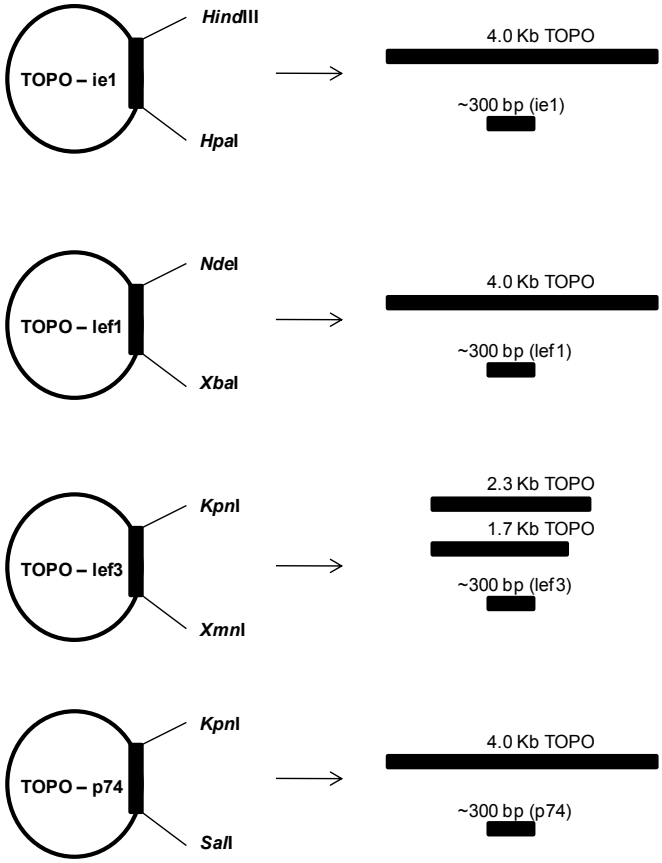
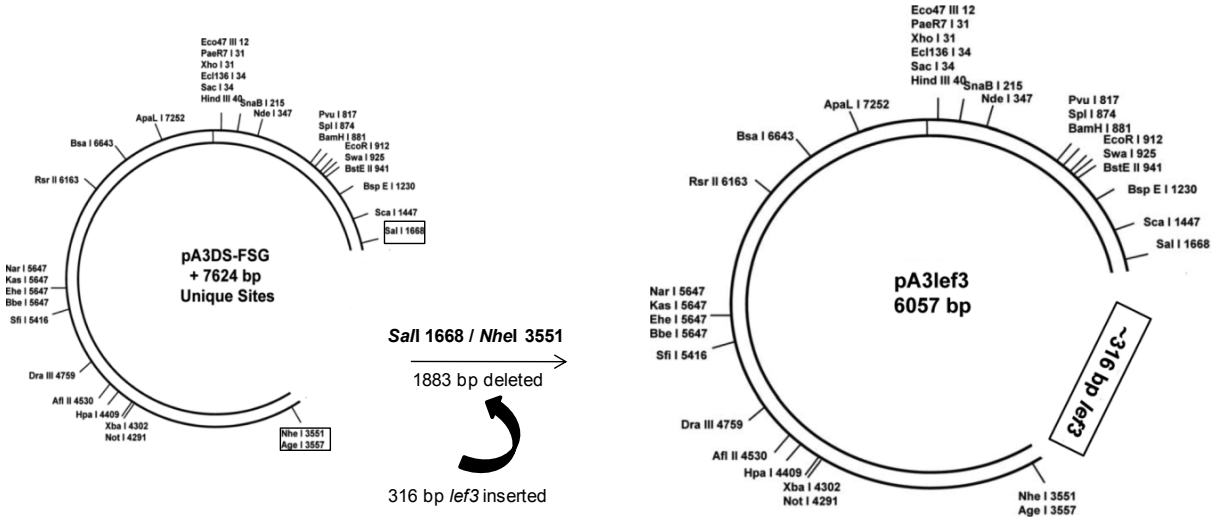
Cloning of baculoviral genes (*ie1*, *lef1*, *lef3* and *p74)* into TOPO vector

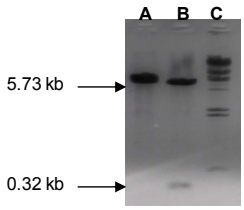




A 316 bp fragment of *lef3* was cloned into pA3DS-FSG vector backbone using *Sal*I and *Nhe*I sites to generate 1-gene construct, pA3Δlef3

Step 1



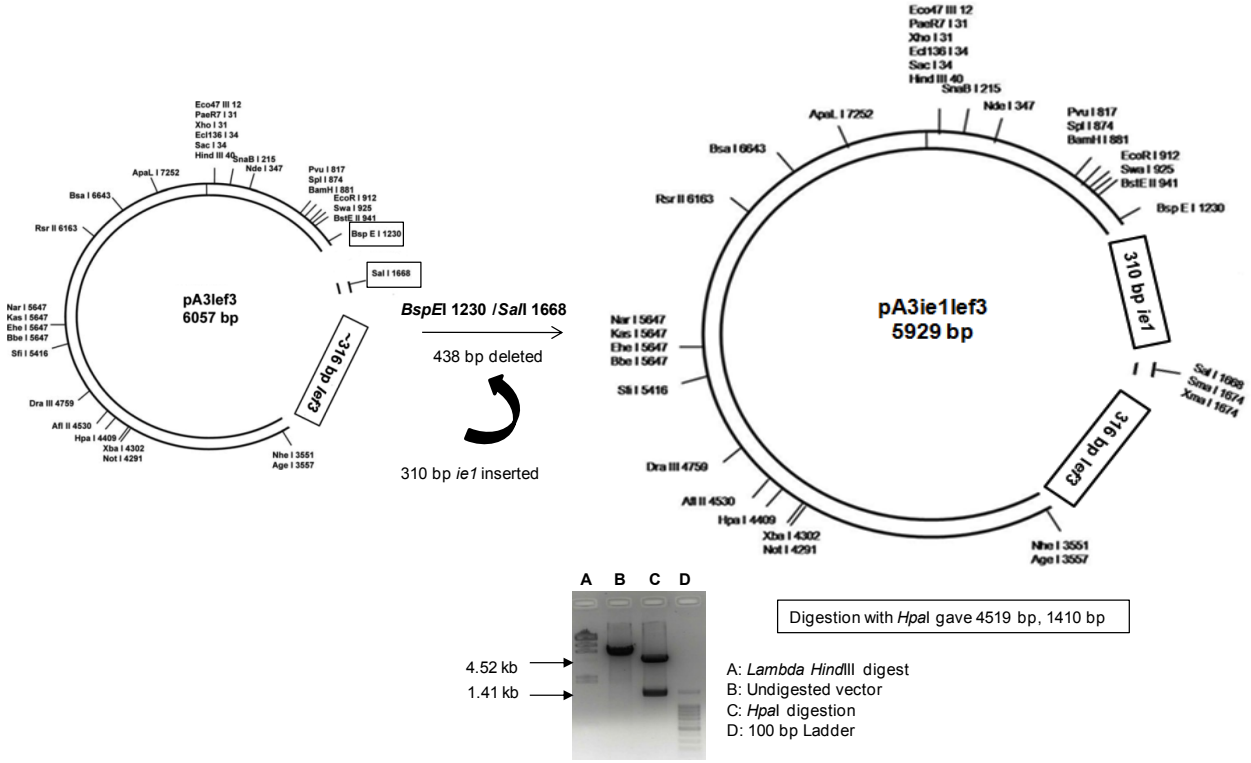


Digestion with *Xmn*I and *Sca*I gave 5734 bp and 323 bp fragments

A: *Xmn*I digestion
B: *Xmn*I and *Sca*I digestion
C: *Lambda Hind*III digest

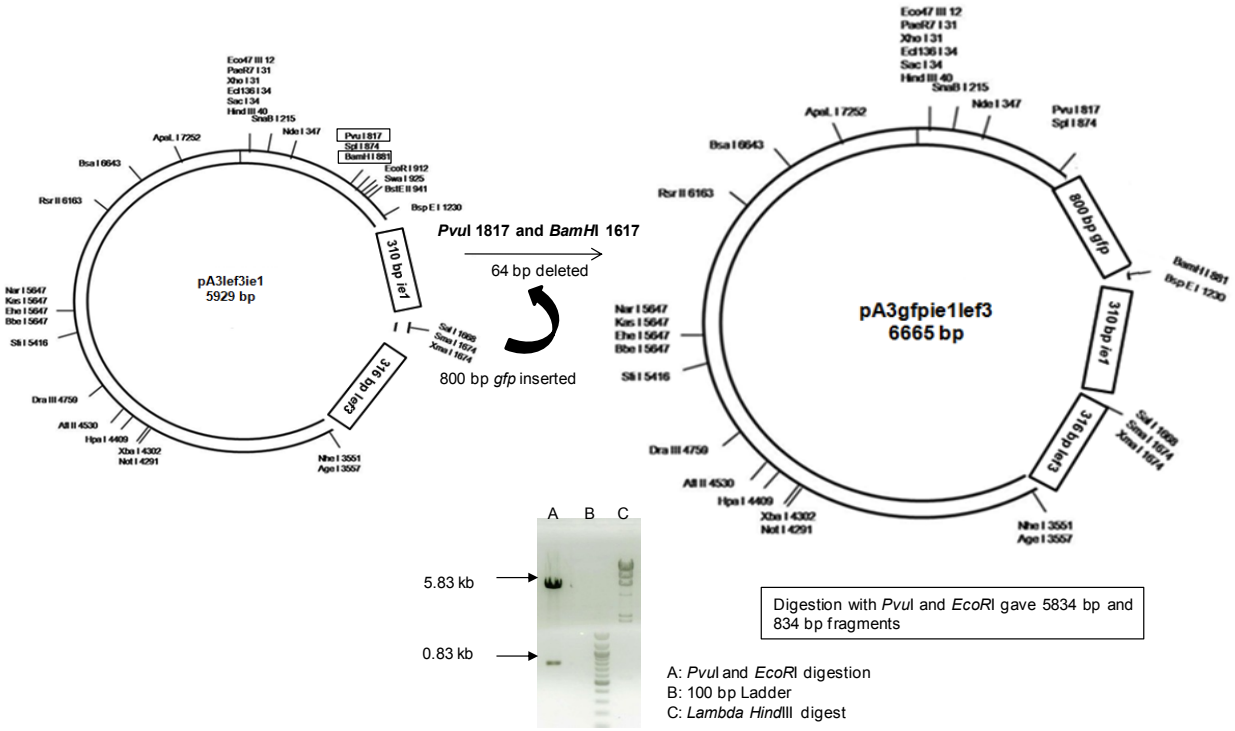
Step 2

A 310 bp fragment of *ie1* was cloned into pA3Δlef3 vector backbone using *Bsp*EI and *Sal*I sites to generate 2-gene construct, pA3Δie1lef3



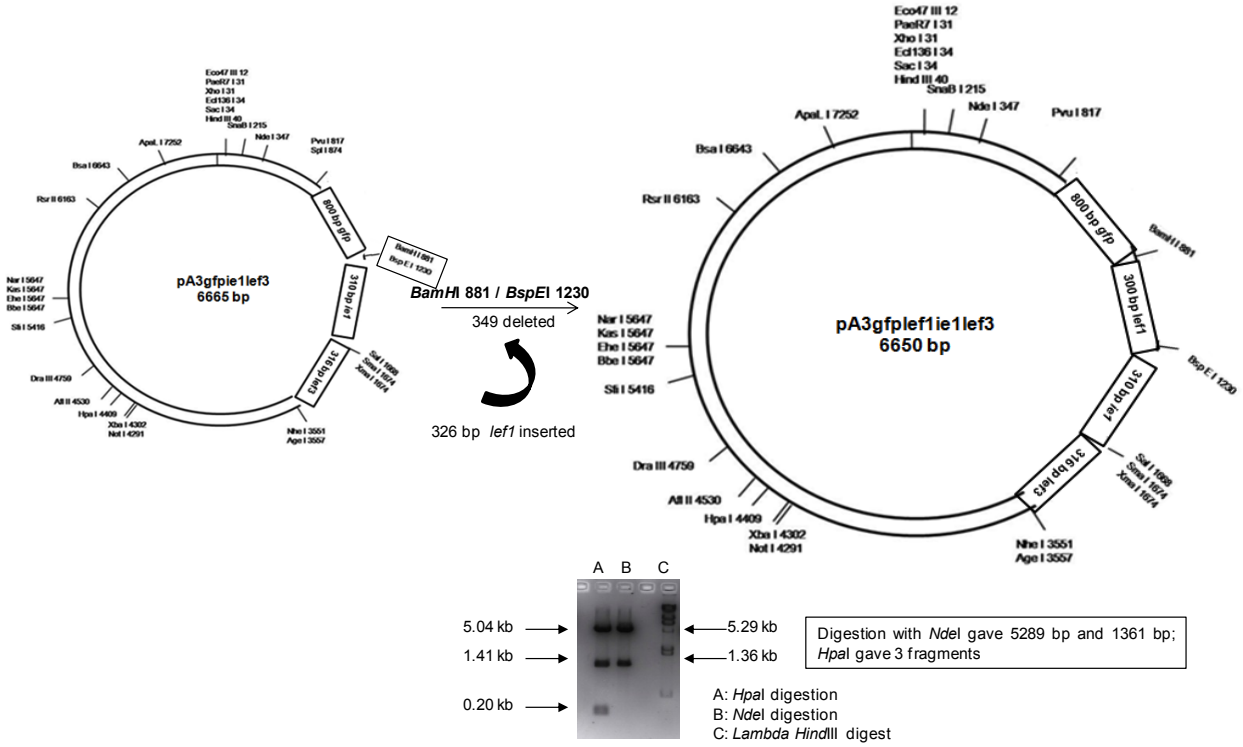
Step 3

A 800 bp fragment of *gfp* was cloned into pA3Δie1lef3 vector backbone using *Pvu*I and *BamH*I sites to generate 3-gene construct, pA3Δgfpie1lef3



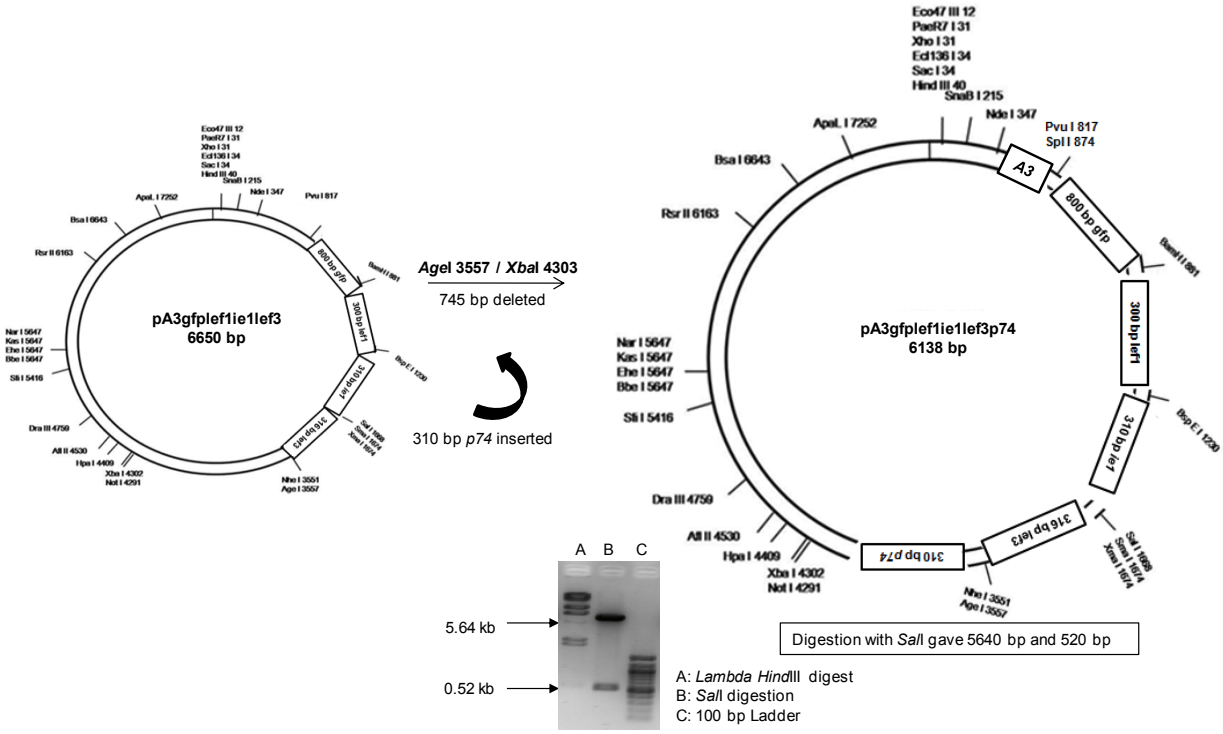
A 326 bp fragment of *lef1* was cloned into pA3Δgfpie1lef3 vector backbone using *Bam*HI and *Bsp*EI sites to generate 4-gene construct, pA3Δgfplef1ie1lef3

Step 4



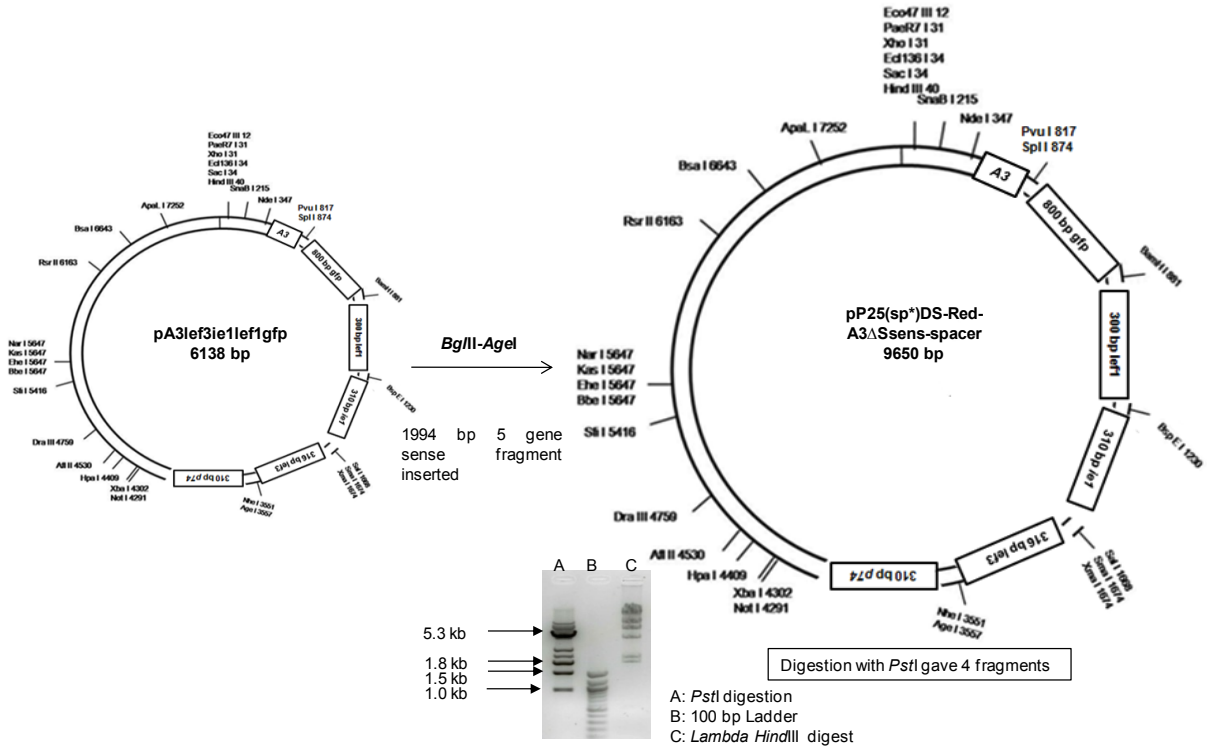
Step 5

A 310 bp fragment of *p74* was cloned into pA3Δgfplef1ie1lef3 vector backbone using *Age*I and *Xba*I sites to generate 5-gene construct, pA3Δgfplef1ie1lef3p74



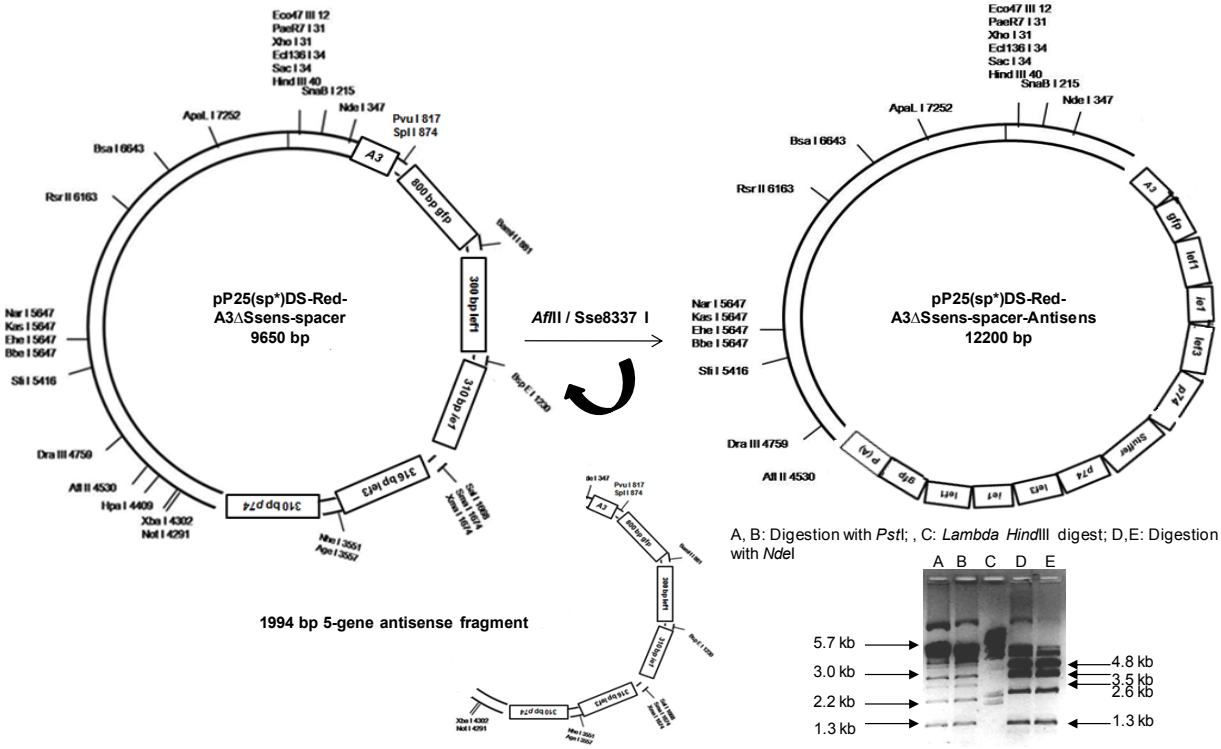
Step 6

1994 bp 5-gene sense fragment cloned to pP25(sp*)DsRed vector backbone using *Bgl*II and *Age*I sites to generate pP25(sp*)DsRed-A3ΔSsens-Spacer



Step 7

Antisense fragment of five genes was cloned into intermediary form of sense-spacer vector backbone using *Afl*II – *Sse8337*I sites to generate pP25(sp*)DsRed-A3ΔS Sense.Spacer.Antisense-Poly A region



A, B: Digestion with *Pst*I; , C: *Lambda Hind*III digest; D,E: Digestion with *Nde*I

Step 8

pP25(sp*)DsRed-A3ΔSSense.Spacer.Antisense-Poly A construct and PiggyP25IL2-(3xP3 DsRed2) TQ backbone vector were digested using *Bgl*II – *Sfi*I sites to generate PiggyA3ΔSSense-Spacer-Antisense(3xP3.DsRed2) multiple flip-flop piggyBac vector

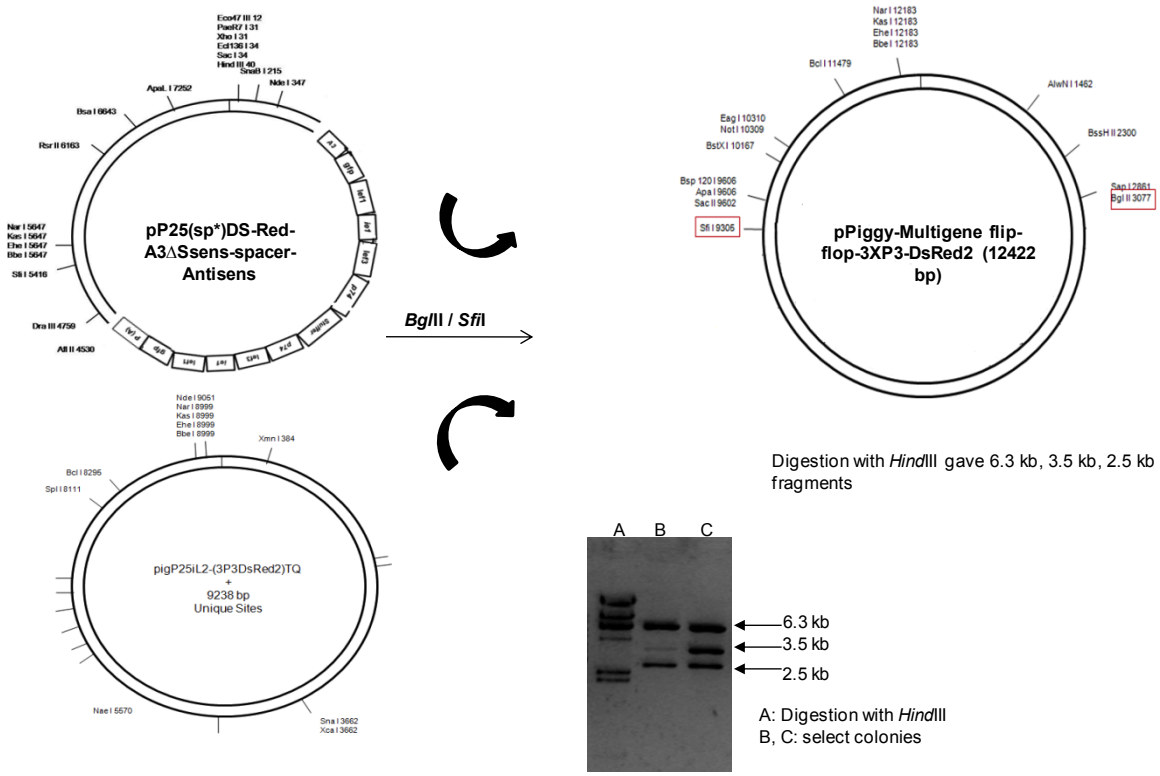


Figure S1 The four baculoviral genes *ie1*, *lef1*, *lef3* and *p74* were initially cloned into pCRII TOPO vector (Invitrogen). The resultant plasmids were labeled as Topo-*ie1*, Topo-*lef1*, Topo-*lef3*, and Topo-*p74*.These vectors having right inserts were confirmed by restriction digestions and DNA sequencing for later use in cloning steps. The various steps involved in the construction of *pPiggyMG(+)3XP3-GFP*, *pPiggyMG(-)3XP3-DsRed2* and *pPiggyMG(+/-)3XP3-DsRed2* were schematically represented. The stepwise description is provided in the text.

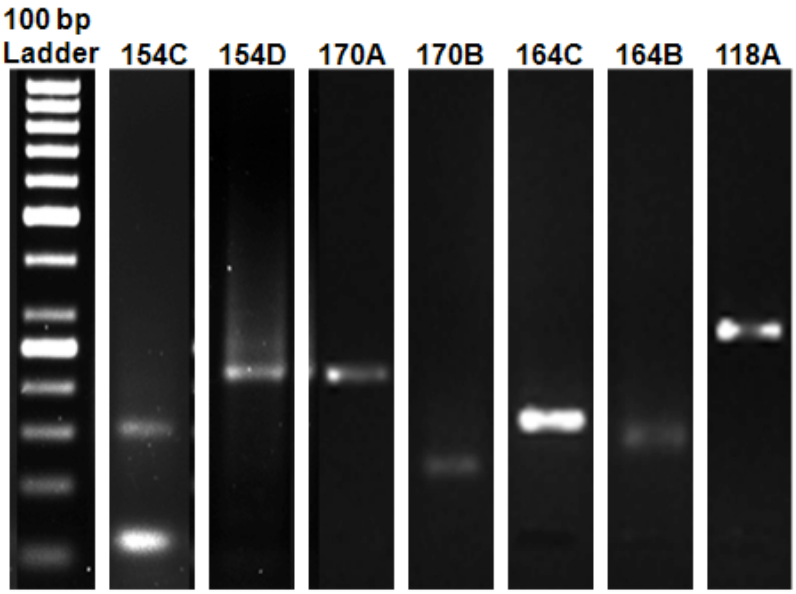


Figure S2 Copy number and site of insertion of transgenes in transgenic lines were characterized using TED assay. Seven Nistari transgenic lines (MG(+/-) 170A, 170B, 164C, 164B, 154C, 154D and 118A) showed single copy insertions.

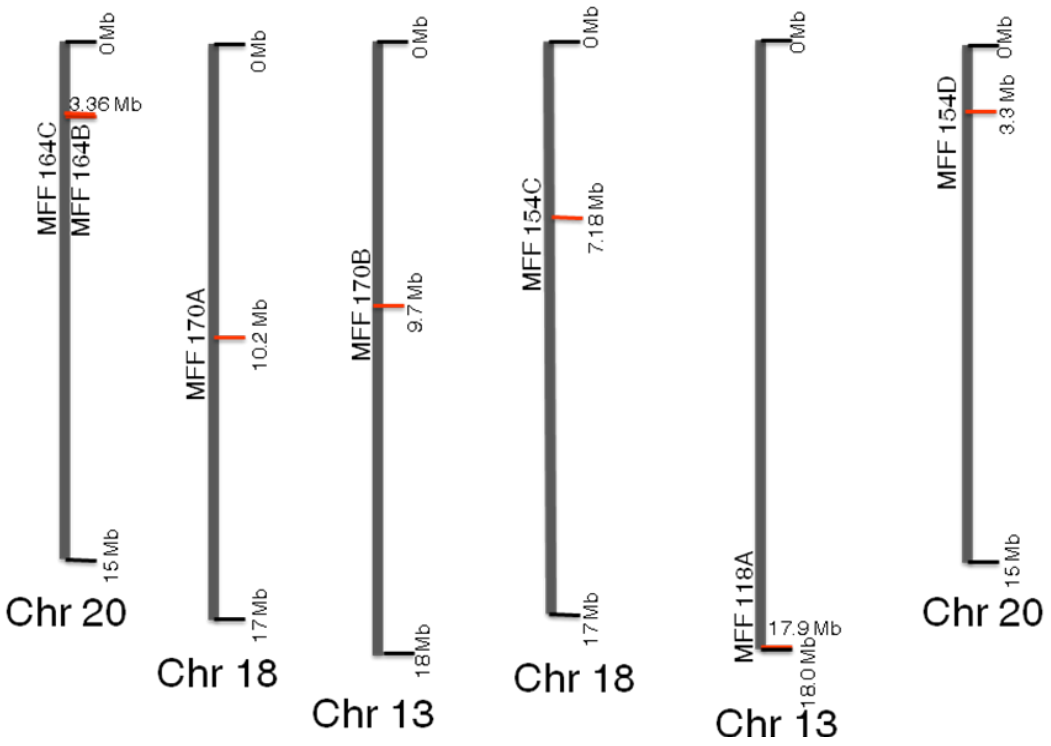


Figure S3 Mapping of transgenes to chromosome locations in different transgenic lines expressing dsRNA for essential baculoviral genes.

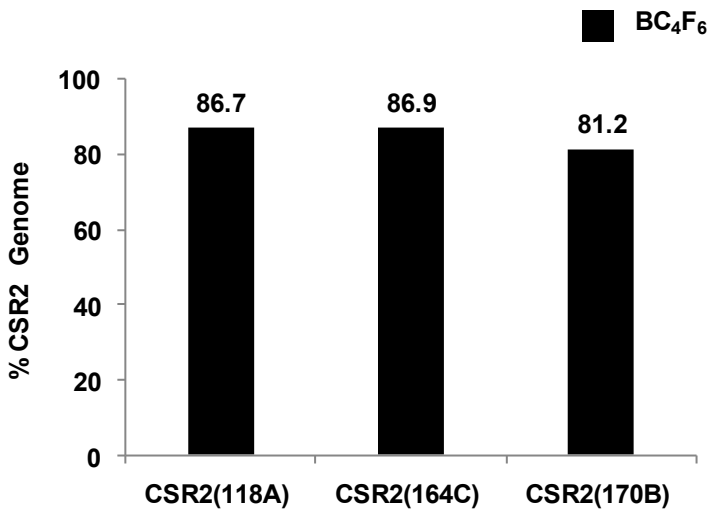


Figure S4 Histogram representing percentage incorporation of the CSR2 genome in the BC_4F_6 population of transgenic lines, CSR2(118A), CSR2(164C) and CSR2(170B) obtained through recurrent backcross strategy, as measured by CSR2-specific SSR alleles from 10 loci.

File S1

ADDITIONAL METHODS

Construction of the vectors: The vectors were constructed by amplifying the portions of *ie1*, *lef1*, *lef3* and *p74* genes encoding the *immediate early 1* gene, late expression factors 1, 3 and envelope protein of BmNPV, respectively, using primers carrying specific restriction enzyme sites. Similarly, *gfp* gene of pPIGA3GFP (Kanginakudru *et al.* 2007) was amplified. The primers used for the amplification of the gens are listed in the TABLE S1**.** The amplicons were cloned into the T-overhangs of pCRII TOPO vector (Invitrogen) (FIGURE S1). The resultant plasmids were labeled as Topo-*ie1*, Topo-*lef1*, Topo-*lef3*, Topo-*p74* and Topo-*gfp.* The targeted regions of *ie1*, *lef1*, *lef3*, and *p74* are as mentioned in File S2. These vectors having right inserts were confirmed by restriction digestions and DNA sequencing. These baculoviral genes produce virus specific proteins and do not share any homology to known insect or human genes.

The pTopo clones were transformed into one shot INV110 chemically competent *E. coli* cells (Invitrogen). Blue/White screening was performed on LB agar plates containing 40 µl of X-Gal (40 mg/ml) and 40 µl of isopropyl β-D-thiogalactoside (IPTG, 100 mM). White colonies were picked up for insert analysis through digestion. Colonies that gave right inserts of expected size were picked up. For DNA sequencing, 250 ng of plasmid was used in a sequencing reaction that contained 8 µl of Ready reaction mix (BigDye terminator, BDTv 3.0, Applied Biosystems, Foster City, CA) and 5 picomoles of M13 sequencing primers. The cycling conditions used were as follows: 25 cycles of 96°C for 10 sec, 50°C 5 sec, 60°C 4 min. Samples were ethanol precipitated, washed with 70% ethanol and resuspended in Hi-Di^{TM}formamide (Applied Biosystems). The sequencing was carried out in ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and the intactness of each gene fragment was confirmed. The constructs were obtained in a series of cloning steps as described below:

1. A 316 bp fragment of *lef-3* was cloned into pA3DS-FSG vector backbone (7624 bp) using *Sal*I-*Nhe*I sites to generate single gene construct, pA3ΔS-lef3 (6057 bp). The transformants were verified by *Xmn*I (an internal site of *lef3*) restriction digestion and *Xmn*I/*Sca*I double digestion. *Xmn*I digestion linearizes to 6.05 kb fragment of 1-gene construct and double digestion with *Sca*I (a site in backbone) released 323 bp product or insert size and 5.73 kb fragment as per DNA strider map pattern.
2. A 310 bp fragment of *ie-1* was cloned in to pA3ΔS-lef3 vector using *Bsp*EI-*Sal*I sites to generate pA3ΔS-ie1lef3SG, a two gene construct (5929 bp). The transformants were checked upon sequential digestion using *Xmn*I*/Hpa*I restriction system. *Xmn*I linearization of 2-gene construct gave rise to 5929 bp fragment and *Xmn*I/*Hpa*I sequential digestion gave rise to expected three bands of 339 bp, 1071 bp and 4519 bp fragments.

3. An 800 bp fragment of *gfp* was cloned into pA3ΔS-ie1lef3 vector backbone using *Pvu*I-*Bam*HI sites to generate pA3ΔS-gfpie1lef3, a three gene construct (6665 bp). The transformants were checked upon *Pvu*I/*Eco*RI restriction analysis which gave rise to 834 bp insert and 5834 bp backbone.

4. A 326 bp *lef-1* clone was added to pA3ΔS-gfpie1lef3 vector backbone using *Bsp*EI/*Bam*HI to generate pA3ΔS-gfplef1ie1lef3 (6650 bp) a four gene product. The transformants were confirmed through *Hpa*I and *Nde*I restriction digestions which gave rise to 3 fragments of expected lengths 188 bp, 1410 bp, 5040 bp and 2 fragments of expected length 1361 bp and 5289 bp, respectively.

5. A 310 bp fragment of *p74* was cloned in to pA3ΔS-gfplef1ie1lef3 vector backbone using *Age*I-*Xba*I sites to generate a five gene construct, pA3ΔS-gfple1ie1lef3p74 (6160 bp). The transformants were confirmed through *Sal*I restriction digestion. It gave rise to 520 bp insert and 5640 bp backbone.

The five gene sense oriented construct, pA3ΔS-gfplef1ie1lef3p74 was digested with internal *Nhe*I-*Xba*I sites to generate sense and antisense oriented pool of fragment of five genes. Transformants were screened for antisense oriented plasmids. This was confirmed by *HindIII/XmnI* digestion, wherein the antisense colony gave rise to 1.3 kb fragment whereas sense oriented colony gave rise to 2.2 kb fragment.

Sense construct - The *A3:gfp* fused promoter along with four genes and poly A tail were cloned into *pPiggyP25il2-(3XP3-GFP)TQ* using *BglII-NheI* and *BglII-XbaI* sites to generate *pPiggyMG(+)3XP3-GFP* vector (FIGURE 1).

Antisense construct - The antisense fragment obtained as mentioned above was cloned into *pPiggyP25il2-(3XP3-DsRed2)TQ* to generate *pPiggyMG(-)3XP3-DsRed2* vector (FIGURE 1).

6. Inverted Repeat Construct - The above *A3:gfp* fused promoter along with four genes was cloned into pP25(sp*)DsRed vector backbone using *Bgl*II-*Age*I sites to generate sense fragment with spacer region, an intermediary vector form pP25(sp*)DsRed-A3ΔS Sense.Spacer.

7. The antisense fragment of five genes was cloned into intermediary form of sense-spacer vector backbone using *Sse*8337I-*Afl*II sites to generate flip-flop form of multigene construct, pP25(sp*)DsRed-A3ΔS Sense.Spacer.Antisense.Poly A region.

8. The intermediary shuttle vector, pP25(sp*)Sense-Spacer-Antisense vector was digested with *BglII* and *SfiI* sites to generate inverted repeat. This insert was cloned into *PiggyP25IL2-(3XP3-DsRed2)TQ* using *BglII/SfiI* sites to generate *pPiggyA3gfplef1ie1lef3p74.spacer.p74lef3ie1lef1gfp.3XP3-DsRed2 (pPiggyMG(+/-)3XP3-DsRed2)* vector with *DsRed2* as the selection marker gene (FIGURE 1).

Polymerase Chain Reaction: A typical PCR reaction consisted of 20 µl final volume with 5 pmol each of forward and reverse primers. The PCR amplification was performed in 10 mM Tris-HCl, (pH 8.3 containing 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin and 0.01% Triton X-100), 1 mM dNTPs and 0.5 U of *Taq* DNA Polymerase (NEB, UK) per reaction. Thermal cycling was carried out in a thermal cycler (PE9700, Applied Biosystems) using the following conditions: initial denaturation of 3 min at 95°C; 35 cycles of 30 sec at 94°C; 30 s at 52°C (as per the *Tm* of the respective gene) and 2 min at 72°C, and final extension of 10 min at 72°C. The PCR products were quantified on agarose gel, purified using Qiagen PCR purification columns, according to the manufacturer's protocol.

File S2

Sequence of the selected viral genes

1. The entire *ie1* of BmNPV is 1755 bp, the targeted region is 141 – 450 bp (310 bp)

ie1 **(Accession Number L33180)**

TGGAGCCGACACGGTAGTATCTGACAGCGAGACTGCAGCAGCTTCAAACTTTTTGGCAAGCGTCAATTCGTTAACTGATGATAACGAT

ATAATGGAATGTTTGCTCAAGACCACTGATAATCTCGGAGAAGCAGTTAGTTCTGCTTATTATTCGGAATCCCTTGAGCTGCCTGTTGC

GGAGCAACCATCGCCCAGTTCTGCTTATAATGCGGAATCTTTTGAGCAGTCTGTTGGTGTGAACCAACCATCGGCAGCTGGAACTAAAC

GGAAGCTGGACGAATACTTGGACGATTCACAAAGTGTGGTGGGC

2. The entire *lef1* of BmNPV is 813 bp, the targeted region is 80 – 405 bp (326 bp)

lef1 **(Accession Number L33180):**

GTCACCGGACCACATCCACCAATTCCATAACCAGGATAGCATTGCTTTAATTTGTCTAGCAATTCCTTTGTTATACAACGAGAAAATTTAGTTC

CCTTATAATTATAGCTGTACGGTGCGCGTATTTGTTTGTTAACGTTACAAAAAATATCCCTGTCCACGTCCGGCCAATACTGCAACGTGAGCGC

GTCCAAGTTTGAATCTTGCATATGCGGAATGTACAAACGTACGGCCTCTCTCACACAATGCGCAAAACTGCCCGGCTGAATGCAATCACTATC

CAACTTTGCAGGTTTTTCGAAGGCCTTGTACCGATGCACGCGAAC

3. The entire *lef3* of BmNPV is 1158 bp, the targeted region is 391 – 706 bp (316 bp)

lef3 **(Accession Number L33180):**

TCTTGCGTTTGTGCAATTTTGCAAATTTGAATAGGCTTCGTTTTCTTTAGCCTCGCACAATTCGATGCGCGTAGAGTTGACCACGTTCCAATTCA

TGTACACGTTTGCTCCATTGAAAATTTGTTGACATTTTATATTGTAAATGGTAAAGATTTGGTTTTCATTGTCTTTTAAATATTTAAACACCTCAT

TGATGTCGTCAGACCCCTTTATATTGTTTTTGAATAGATTCATCAGTGTTTTCGCATTGACAAAACATTCCACTTGAACCACGTCGGAATCGTC

GTTAAAATTTTTGTACGCGACTTCAAAAACA

4. The entire *p74* of BmNPV is 1938 bp, the targeted region is 1243 – 1552 bp (310 bp)

p74 **(Accession Number L33180):**

GTTGGCCAGCATTTTGAAAGTGACAAGAATCGTGTCGCCCAGCACGAATCCGACGAGCGATTCCCACCATCTAAACGAACAGCCGCCGTTGA

ATAGATTTCTGCCGAATCGTCGACAGTAGGCTTCGTTGAATTCGCCTTTAAAGCGTTCGGGAAACAAGGGATCGGGATCGGGCCGAACGTTA

AAAGCCGGCACATCGTCCACGCCCATGATCGTGTGTTCTTCGGTGCGCAAGTATGGGCTGTTAAAGTACATTTTGGACAGCGAGTCCACCAA

GATGCATCTGTTGTCGGGCGTGTATCTAAACTCG

File S3

Genomic DNA sequences of different MG(+/-) transgenic lines surrounding *piggyBac* insertions, with the TTAA duplicated sequence appearing at all 5' and 3' insert boundaries. TTAA sequences which are characteristic of *piggyBac* mediated transposition are shown in red colour.

MG(+/-)118A

ACTATCTTTCTAGGGTTAATGATTGAATGCAAACATCAGCGATATGAGAGTAACAAATCAGCTTTAGCGATAAACCTCTACAAGAACAAACGA
ACTACAATAGGTACTTATTATGTTTTTAATAAACTTTTATTATCTTCACACGTTTATTACTATGTATGTATGTNACGGAAACTTTGAACATGATTT
TCACCCCCTTCAAAACGTCGGACTAACTCAAAATTTGGCATACGTATTTATGATTGATGACAATTCAATATTTTAATCAGTTAGATCCAAGGAG
AGGACAGCGATTCTCGTACANACGGTCGCG

MG(+/-)154C

ATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAAGCAAAAGCGGACGTGCGGGCCACAATAGTCGTGCTCATACAAATTTGTGGG
TAGATAGTATATGGCACGATCCAAGGCGAGGACGGCGATTC

MG(+/-)154D

AGCGATATTTCAAGCATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAAGCAGAAGCGGACGTGCGGGCCACAATAGTCGTGCTC
ATACAAATTTGTGGGTAGATAGTATATGGCACGATCCAAGGCGAGGACCGCGATTCTCGTACAAGGGGGGGG

MG(+/-)164B

GGGCGTTTCGCGCTATTTGGAAAGAGAGAGCAATATTTCAAGAATGCATGCGTCAATTTTACGCAGGACTATCTTTCTAGGGTTAAGCAAAA
GCGGACGTGCGGGCCACAATAGTCGTGCTCATACAAATTTGTGGGTAGATAGTTATATGTCACGATCCAAGGAGAGGACAGCGATTCTCGTA
CACGAGGACAGCGATTCTCGTACAC

MG(+/-)164C

TCGCGCTATTTGGAAAGAGAGAGCAATTATTTCAAGAATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAAGCAAAAGCGGACGT
GCGGGCCACAATAGTCGTGCTCATACAAATTTGTGGGTAGATAGTTATATGTCACGATCCAAGGAGAGGACAGCGATTCTCGTACAAACGGT
TACGATTCGAGAAGGG

MG(+/-)170A

ATTTCAGAATGCATGCGTCATTTTACGCAGACTATCTTTCTAGGGTTAAATAAATATGTTAAATGAACAAAAAACCGAATTCCGAGTTTTCAGT

ACAAATCGGCAATTTCATACTTTAACCCCTTATATTATCGTTACGATAATACATTTATATATAGCAATTATTATAAAATACTAAAATATCTTCAAC

AAAATTTACTTACAGAAATTCAATCGAAAACATATTTCTTTAGATCCAAGGAGAGGACAGCGGATTCTCGTACAGCCGGCGGCGATTCGTGAA

GGG

MG(+/-)170B

CGGGCGGATTCGCGCTATTTAGAAAGAGAGAGCAATATTTCAAGNAATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAATAAGC

GTGTTCACGTTTTGGACATGGTTTGCACTGAAACGAGATCCAAGGAGAGGACAGCGATTCTCGTACAAACGGTTACGATTCGAGAAGGGAG

AG

Table S1 PCR primers used for amplification of the different indicated genes/constructs

Gene/construct	Sequence 5’ to 3’
gfp F	ATCGATCGCTAGCCCCGGGATGGTGAGCAAGGGCGAG
gfp R	CGCGGATCCTTACTTGTACAGCTCGTCCA
3XP3GFP F	CCGAATTCAGATCTTCCCACAATGGTTAATTCGAGCTC
3XP3GFP R	CCGGATCCGCCGGCGTACGCGTATCGATAAGCTTTA
DsRed F	CAAGATCTAATTCGAGCTCGCCCGGGGATCTAATTC
DsRed R	TAGCAGATCTGTACGCGTATCGATAAGCTTTAAG
ie1 gene F	CGCTCCGGAGCCGACACGGTAGTATC
ie1 gene R	ACGCGTCGACGGCCCACCACACTTTGTGAAT
lef1 gene F	CGCGGATCCGGTACAAGGCCTTCGAAA
lef1 gene R	CCGTCCGGACCGGACCACATCCACCAA TTCCAT
lef3 gene F	ACGCGTCGACTTGTTTTTGAAGTCGCGTACA
lef3 gene R	TGCTCTAGACTTGCGTTTGTGCAATTTTGCA
p74 gene F	TCCCCCCGGGTAGATACACGCCCGACAACA
p74 gene R	TGCTCTAGACCTGCAGGACCGGTTGGCCAGCATTTTGAA
Viral *lef3* F	GGAGGAAGGCAAGTGCTATG
Viral *lef3* R	CTCAGGGGGATTGCAGTTT
18S rRNA F	CGATCCGCCGACGTTACTACA
18S rRNA R	GTCCGGGCCTGGTGAGATTT

F – Forward; R - Reverse

Table S2 List of transgenic lines obtained

Vector	Injected embryos[a]	Hatched embryos	Fertile moths[b]	G_1 broods with GFP/DsRed positive larvae[c]	Percent G_0 transformed moths	Name of the transgenic lines
pPiggyMG(+) 3XP3-GFP	800	618	550	6	1.1	2G, 58L, 244E
pPiggyMG(-) 3XP3-DsRed2	1380	806	508	6	1.2	126C, 239A
pPiggyMG(+/-) 3XP3-DsRed2	1500	821	600	8	1.3	118A, 118B, 154C, 154D, 164B, 164C, 170A, 170B

[a] *B. mori* eggs were injected with a mixture of *pHA3PIG* and sense, antisense or MG(+/-) constructs
[b] Hatched larvae (G_0) were allowed to develop to moths
[c] The moths were intercrossed and the resulting G_1 broods were screened for GFP/DsRed expression

Table S3 Location of insertion of transgenes

Transgenic Line	Accession Number	Description
MG(+/-)118A	BAAB01060705.1	*Bombyx mori* DNA, contig26425, whole genome shotgun sequence
MG(+/-)164C	AADK01013361.1	*Bombyx mori* strain Dazao Ctg013361, whole genome shotgun sequence
MG(+/-)164B	AADK01013361.1	*Bombyx mori* strain Dazao Ctg013361, whole genome shotgun sequence
MG(+/-)170A	X58446.1	*Bombyx mori* ErA.3 gene for chorion protein
MG(+/-)170B	AADK01004442.1	*Bombyx mori* strain Dazao Ctg004442, whole genome shotgun sequence

Table S4 Pairwise comparisons using Tukey HSD among groups on percentage mortality (values arcsin transformed) in transgenic lines, and their hybrids with CSR2 upon BmNPV treatment done after post-hoc analysis of the corresponding treatment factor using main effects in a one-way ANOVA.

Lines		Mean Difference	Test Statistics	P level	Significance
Transgenic lines					
Control	IE	17.6925	7.8087	0.0001	***
	MG(+/-)	41.0488	18.1171	0.0001	***
	MG(+)	19.7788	8.7295	0.0001	***
	MG(-)	26.6300	11.7533	0.0001	***
	MG(+) x MG(-)	32.2100	14.2160	0.0001	***
IE	MG(+/-)	23.3563	10.3084	0.0001	***
	MG(+)	2.0863	0.9208	0.9863	
	MG(-)	8.9375	3.9446	0.0791	
	MG(+) x MG(-)	14.5175	6.4074	0.0007	***
MG(+/-)	MG(+)	-21.2700	9.3876	0.0001	***
	MG(-)	-14.4188	6.3638	0.0008	***
	MG(+) x MG(-)	-8.8388	3.9010	0.0848	
MG(+)	MG(-)	6.8512	3.0238	0.2882	
	MG(+) x MG(-)	12.4313	5.4866	0.0047	**
MG(-)	MG(+) x MG(-)	5.5800	2.4628	0.5132	
Hybrids of transgenic lines x CSR2					
Control x CSR2	IE x CSR2	21.5750	7.7516	0.0002	***
	MG(+/-) x CSR2	38.8650	13.9637	0.0001	***

	MG(+) x CSR2	14.9100	5.3570	0.0082	**
	MG(-) x CSR2	21.6433	7.7762	0.0002	***
	MG(+) x MG(-) x CSR2	27.6317	9.9277	0.0001	***
IE x CSR2	MG(+/-) x CSR2	17.2900	6.2121	0.0017	**
	MG(+) x CSR2	-6.6650	2.3946	0.5465	
	MG(-) x CSR2	0.0683	0.0246	1.0000	
	MG(+) x MG(-) x CSR2	6.0567	2.1761	0.6431	
MG(+/-) x CSR2	MG(+) x CSR2	-23.9550	8.6067	0.0001	***
	MG(-) x CSR2	-17.2217	6.1875	0.0018	**
	MG(+) x Mg(-) x CSR2	-11.2333	4.0360	0.0758	
MG(+) x CSR2	MG(-) x CSR2	6.7333	2.4192	0.5357	
	MG(+) x MG(-) x CSR2	12.7217	4.5707	0.0322	*
MG(-) x CSR2	MG(+) x MG(-) x CSR2	5.9883	2.1515	0.6538	

The significance between each of the groups was calculated as a *P* value. *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.

Table S5 Pairwise comparisons using Tukey HSD among groups on viral accumulation rate (values logarithm transformed) in transgenic and control lines upon BmNPV treatment was done after post-hoc analysis of the corresponding treatment factor using main effects in a one-way ANOVA.

Lines		Mean Difference	Test Statistics	P level	Significance
Control	IE	0.5094	9.0023	0.0001	***
	MG(+/-)	1.0496	18.5470	0.0001	***
	MG(+)	0.5066	8.9521	0.0001	***
	MG(-)	0.7257	12.8241	0.0001	***
	MG(+) x MG(-)	0.9304	16.4417	0.0001	***
IE	MG(+/-)	0.5401	9.5448	0.0001	***
	MG(+)	-0.0028	0.0501	1.0000	
	MG(-)	0.2163	3.8219	0.0961	
	MG(+) x MG(-)	0.4210	7.4394	0.0002	***
MG(+/-)	MG(+)	-0.5430	9.5949	0.0001	***
	MG(-)	-0.3239	5.7229	0.0029	**
	MG(+) x MG(-)	-0.1191	2.1053	0.6732	
MG(+)	MG(-)	0.2191	3.8720	0.0888	
	MG(+) x MG(-)	0.4238	7.4896	0.0002	***
MG(-)	MG(+) x MG(-)	0.2047	3.6176	0.1308	

The significance between each of the groups was calculated as a *P* value. ***P* < 0.01; ****P* < 0.001.

Table S6 List of 10 SSR loci with primer sequences, annealing temperature, $MgCl_2$ concentration and corresponding amplicon size used to genotype the BC_4F_6 population.

Marker	Forward Primer 5' to 3'	Reverse Primer 5' to 3'	Tm (°C)	$MgCl_2$ (mM)	Amplicon size (bp)
Bmg sat001	TTAACCGCGATGTGACTGAA	TGACCGGCACTAAATATGGA	55	1.8	1200 - 1304
Bmg sat015	CATAGACGCCGGGGAATC	CGCGTAATTCGTTTCCAAAT	55	1.8	500 - 540
Bmg sat031	AGACACAGATGGCGATGATG	CTCCAAGGACCTTCGCTTC	55	1.8	700 - 730
Bmg sat084	TCGTGGTAGACCAGCGATAG	GATTTGCCCTCGAAGTTGAC	55	1.8	1145 - 1200
Bmg sat095	GGTCTTCTTCACGTGCTGGT	TGCCGGATCCAAGTTCTTTA	55	1.8	780 - 1035
Bmg sat098	CATGCATTTACCCTCTCCAC	GTGAATTCCGTACTGGCAAA	55	1.8	850 - 1000
Bmg sat108	CCAGGTCATCATGAAGAAGG	ACTGGCTCTGGCAGGTGTT	55	1.8	1005 - 1050
Bmg sat114	CGTCTCTATCTGCGGACGTT	TCAGCTTAGGCAATCCAAGG	55	1.8	627 - 700
Bm sat070	ACCCTCTACGTAGTATATG	CAGCCTTTTTCAGGGCTAA	55	1.8	107 - 148
Bm sat102	GCTCGCCATATGCAATCCTC	CGTCATTGCCTTCATTTCAGTTC	55	1.8	143 - 186