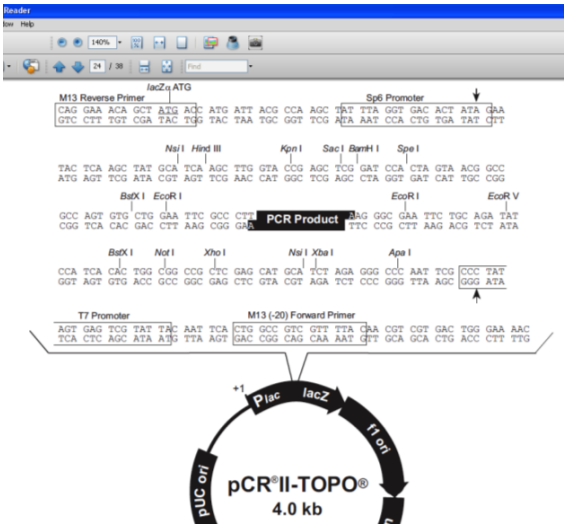
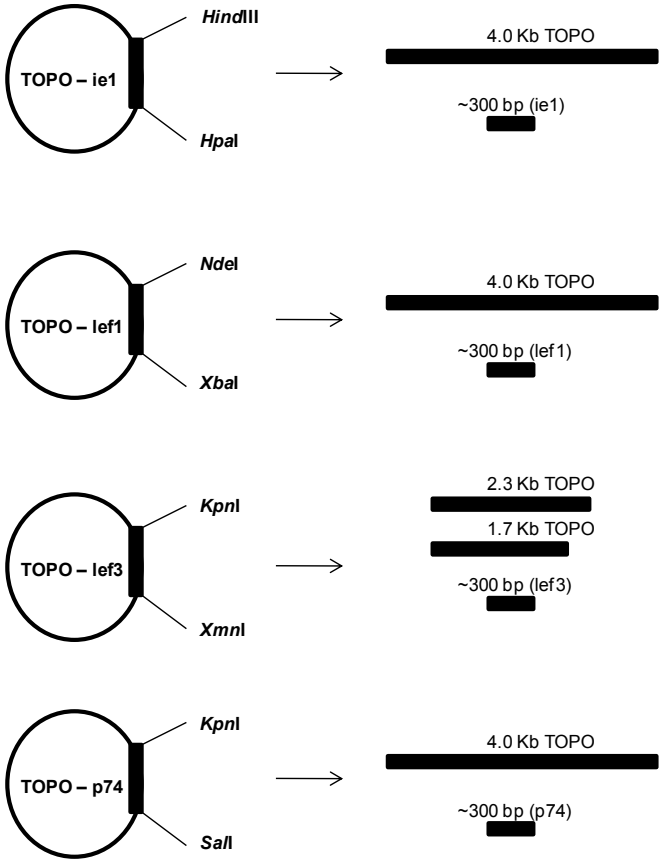
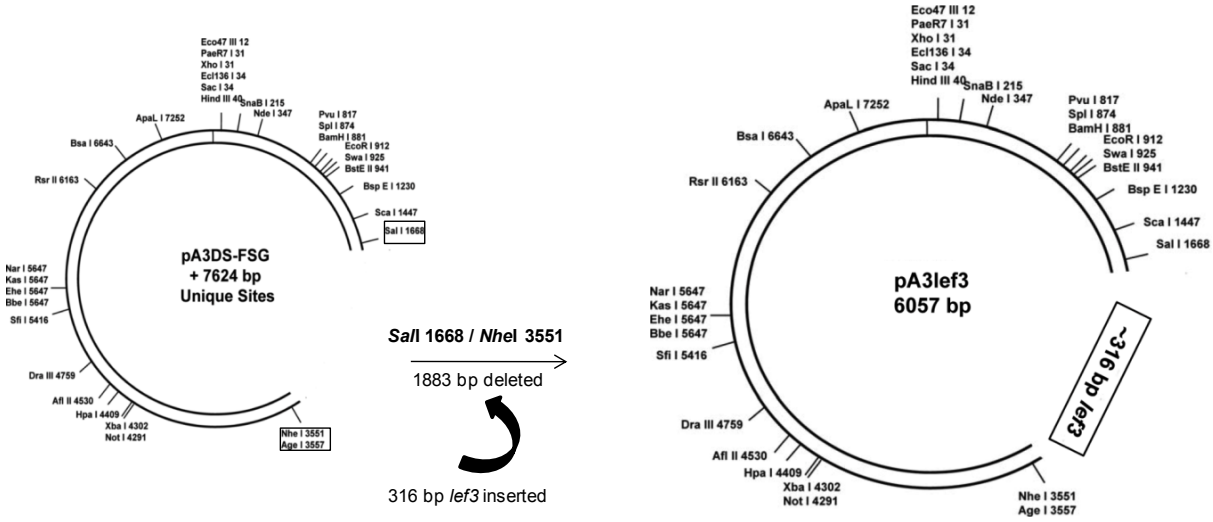
Cloning of baculoviral genes (*ie1*, *lef1*, *lef3* and *p74)* into TOPO vector

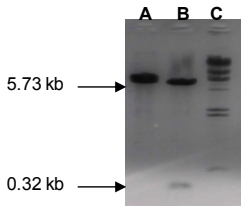




A 316 bp fragment of *lef3* was cloned into pA3DS-FSG vector backbone using *Sal*I and *Nhe*I sites to generate 1-gene construct, pA3Δlef3

Step 1



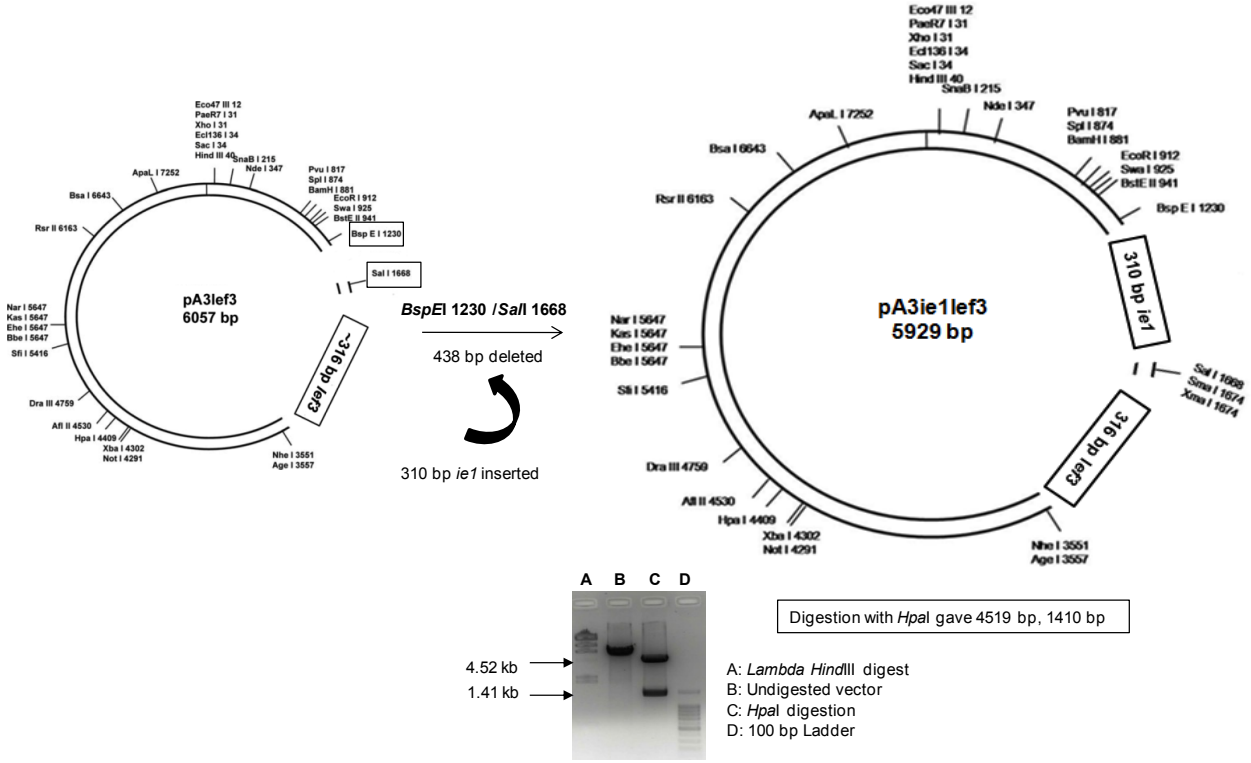


Digestion with *Xmn*I and *Sca*I gave 5734 bp and 323 bp fragments

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

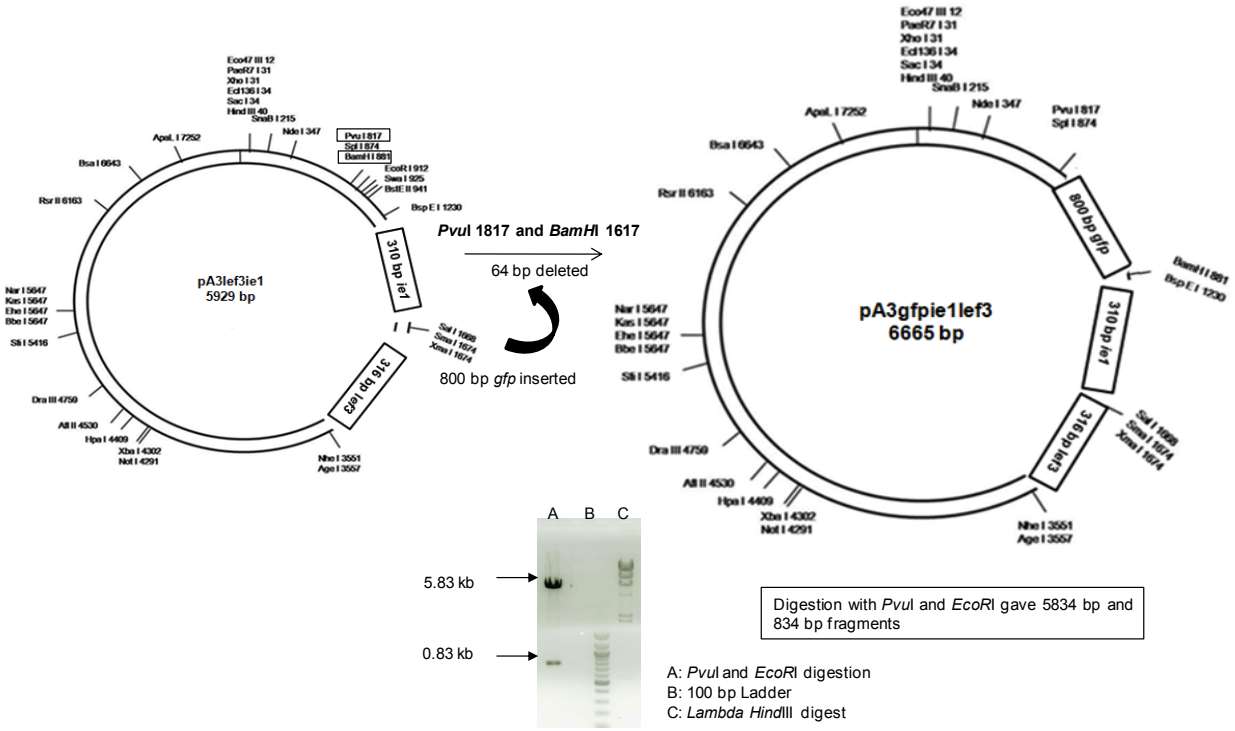
Step 2

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



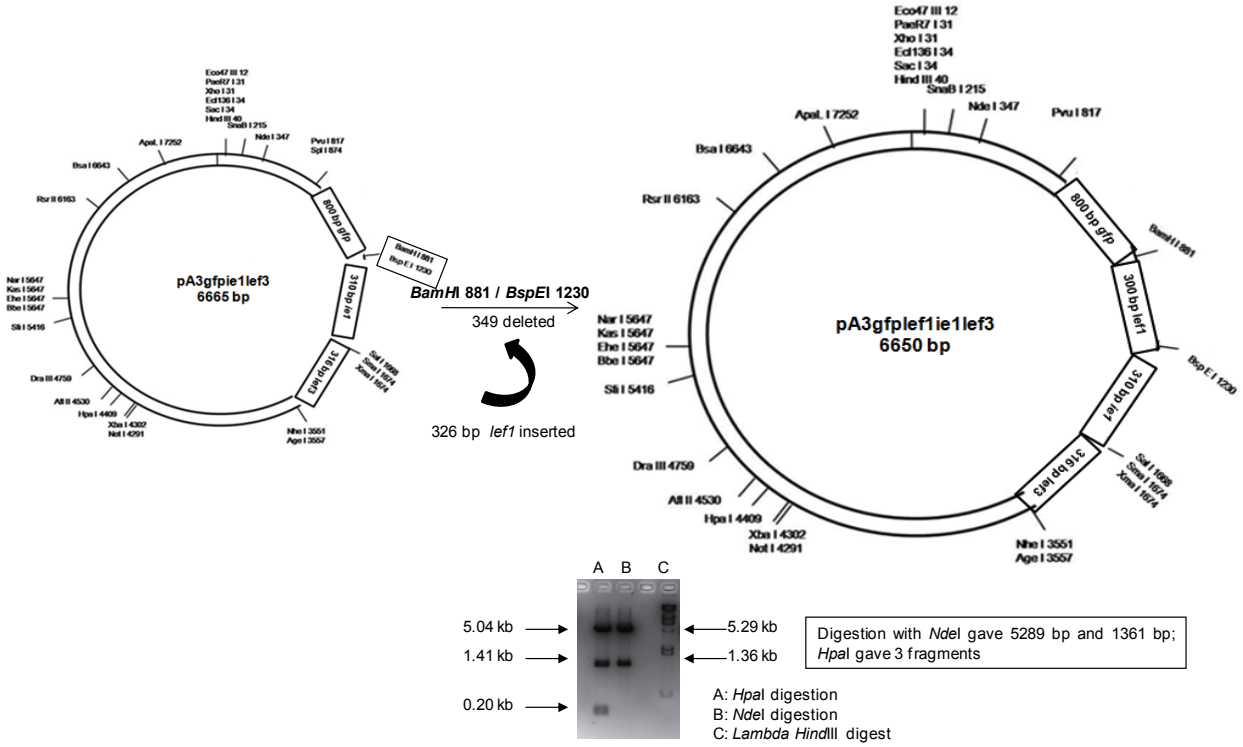
Step 3

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



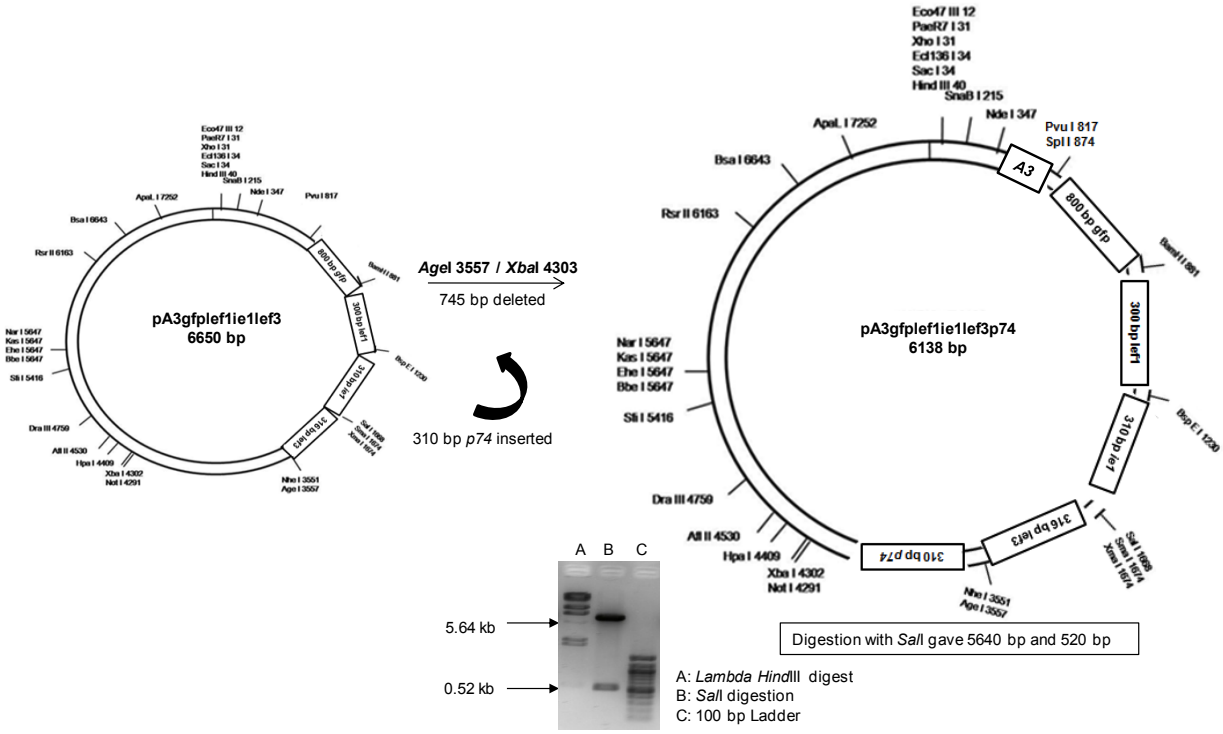
A 326 bp fragment of *lef1* was cloned into pA3Δgfpie1lef3 vector backbone using *Bam*HI and *Bsp*EI sites to generate 4-gene construct, pA3Δgfplef1ie1lef3

Step 4



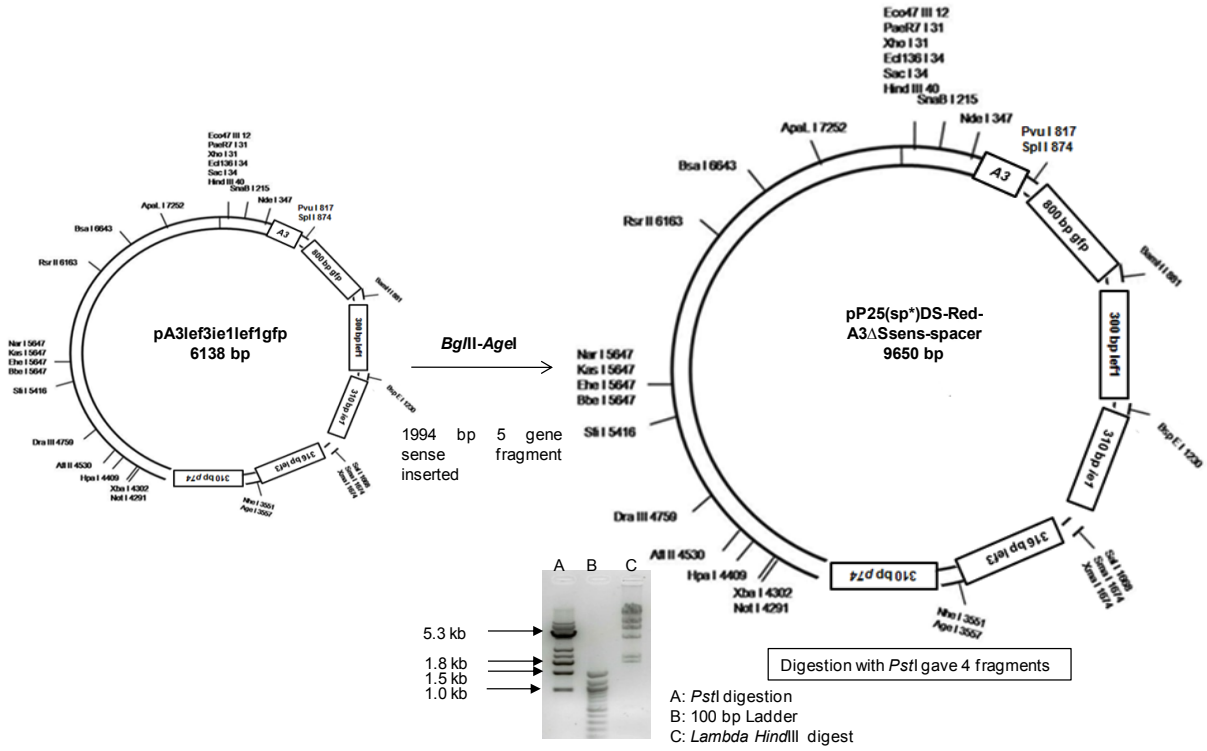
Step 5

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



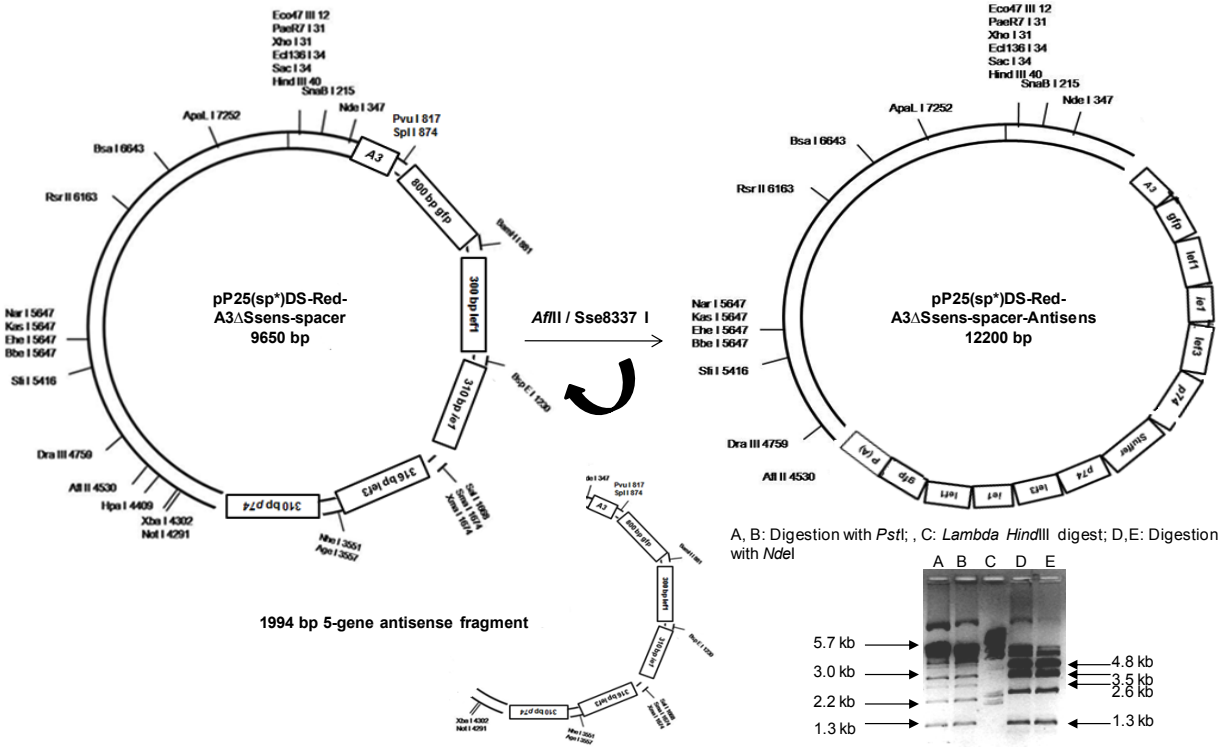
Step 6

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

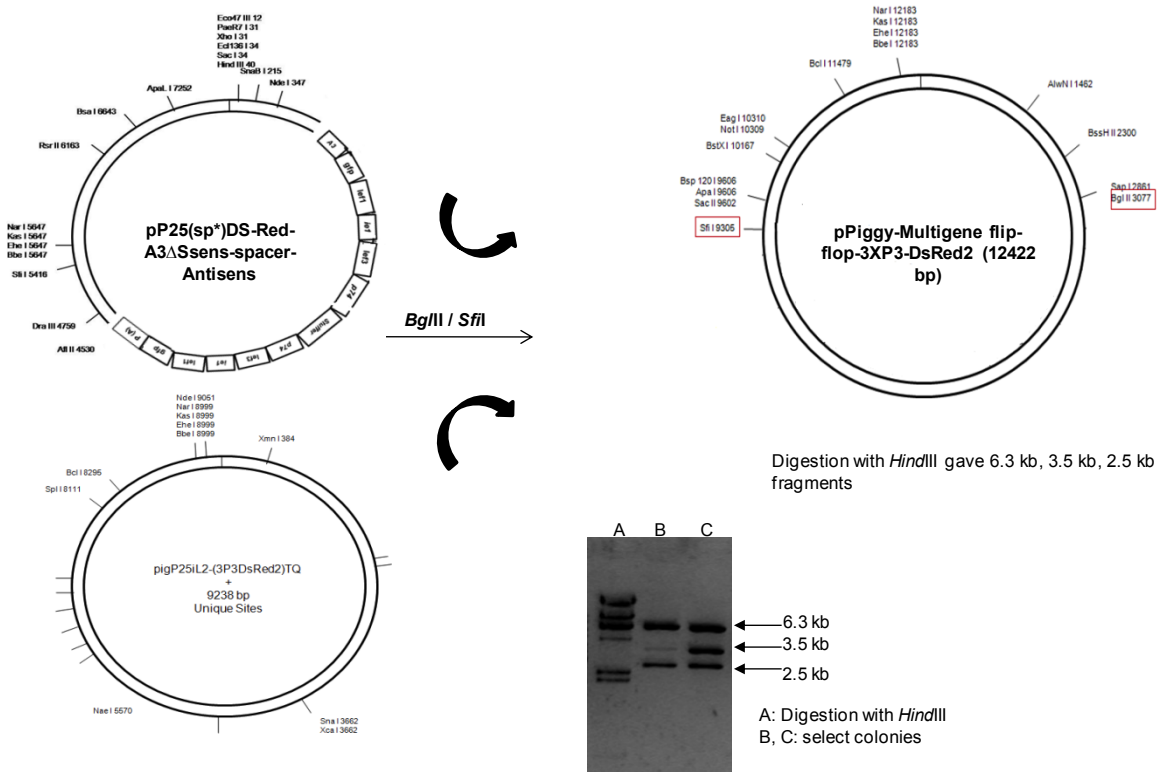


Figure S1 The four baculoviral genes *ie1*, *lef1*, *lef3* and *p74* were initially cloned into pCRII TOPO vector (Invitrogen). The resultant plasmids were labeled as Topo-*ie1*, Topo-*lef1*, Topo-*lef3*, and Topo-*p74*.These vectors having right inserts were confirmed by restriction digestions and DNA 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.