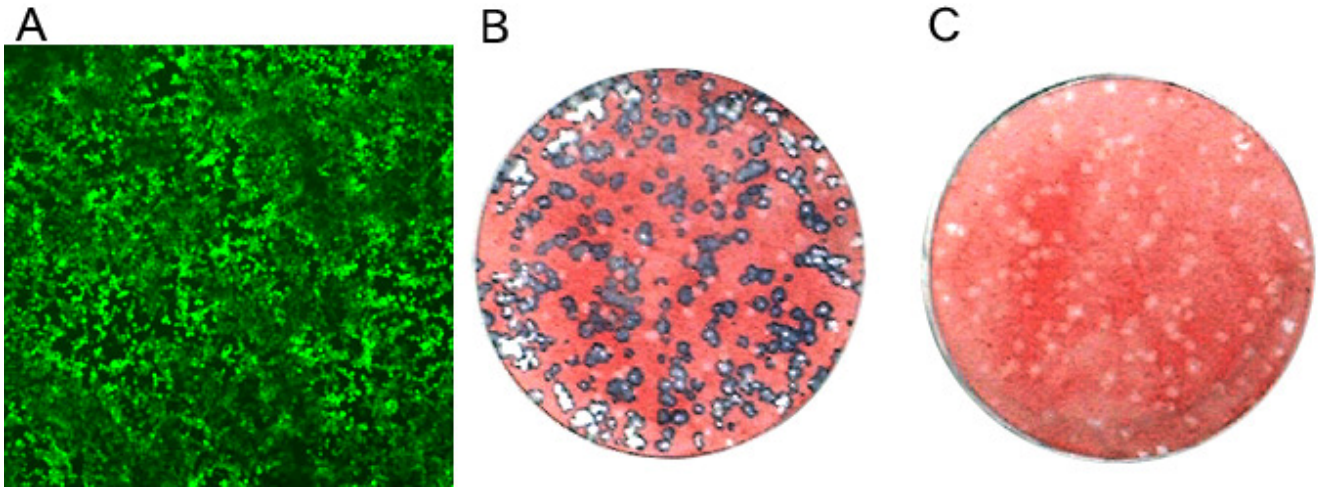
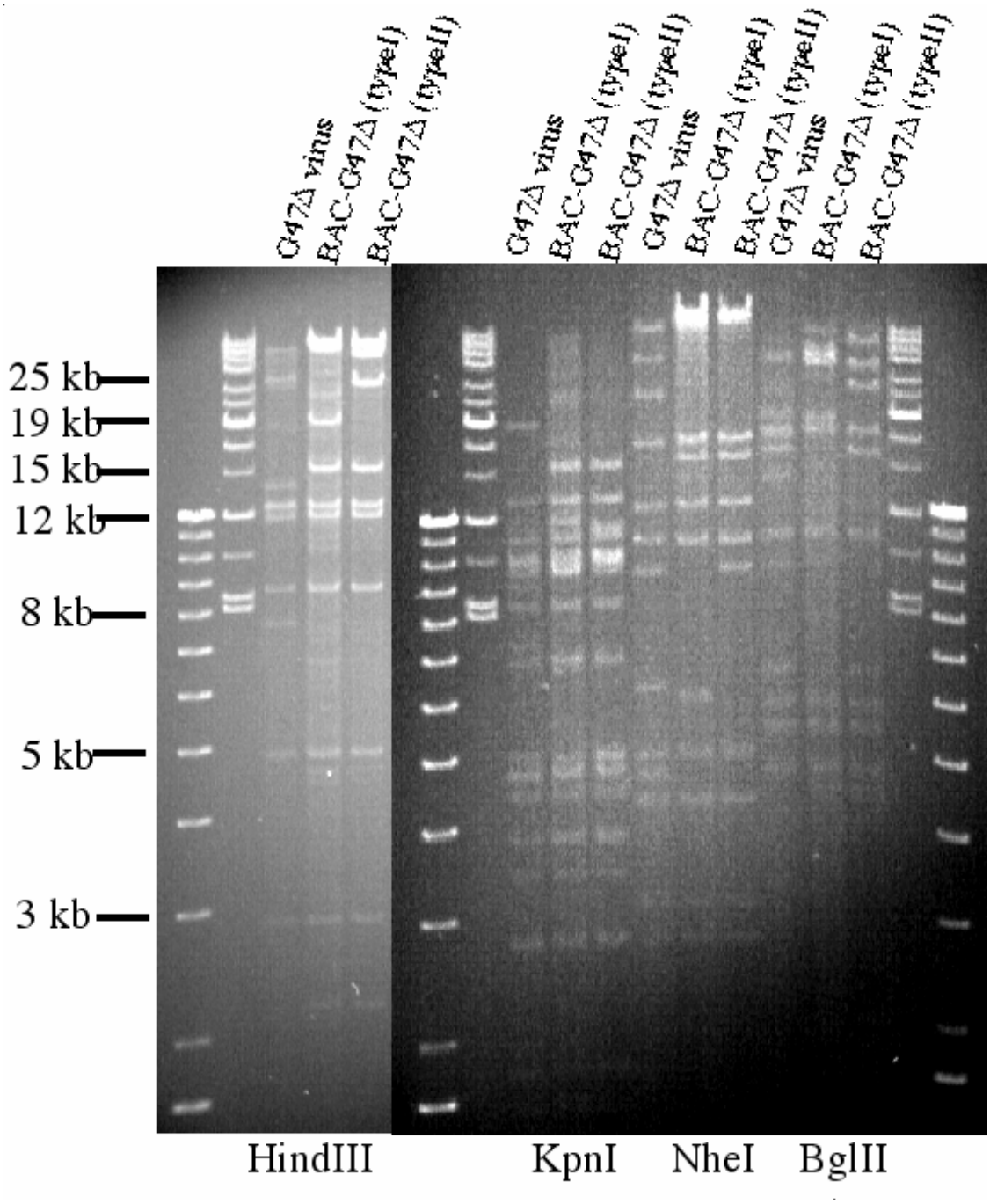
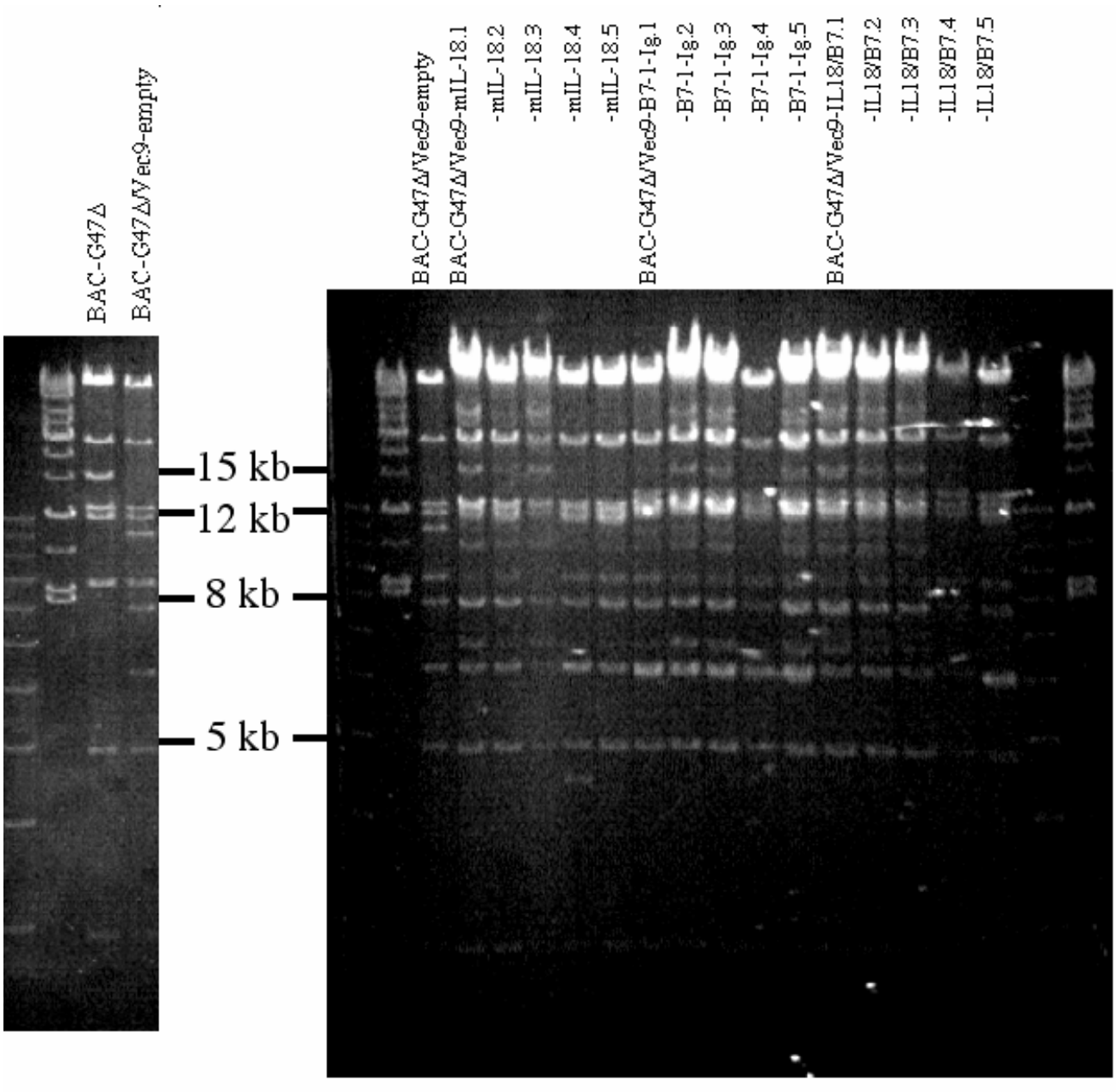
Supplementary Figure 1. Generation of BAC-G47delta plasmid. A homologous recombination of G47Δ DNA and pBAC-ICP6EF, a plasmid that contains the insertion sequences flanked by ~1.2 kb fragments from both ends of the *ICP6* coding region, results in formation of GFP positive and lacZ negative virus plaques on Vero cells. At a 30 -50% cytopathic effect, GFP positive plaques were screened and selected using an inverted fluorescence microscope (A). X-gal staining of Vero cells infected with BAC-G47Δ candidates before limiting dilution showed a mixture of lacZ positive and negative plaques (B). After isolation via 3 rounds of limiting dilution, BAC-G47Δ virus plaques were 100% lacZ negative (C).



Supplementary Figure 2. Gel electrophoreses confirming the structures of BAC-G47Δ plasmids. DNA was digested by HindIII and separated on 0.6% agarose gel (left lanes). In place of the 12,496-bp band of G47Δ (virus), 14,986-bp and 2,214-bp bands were observed for correctly structured BAC-G47Δ plasmids. Gel analyses were also performed after KpnI, NheI or BglII digestion.



Supplementary Figure 3. Representative gel electrophoreses confirming the structures of BAC-G47Δ/Vec9 plasmids obtained after the first step (Cre recombination) of the system. DNA of BAC-G47Δ, BAC-G47Δ/Vec9-empty, BAC-G47Δ/Vec9-mIL-18, BAC-G47Δ/Vec9-B7-1-Ig, or BAC-G47Δ/Vec9-mIL18/B7 was digested by HindIII and separated by electrophoresis on 0.6% agarose gels in 1 x Tris-borate-EDTA buffer for 18 h at 2.5 V/cm.



Supplementary Figure 4. X-gal staining of virus plaques of G47Δ-empty candidates. Over 99% of the virus plaques formed after the second step (FLPe recombination) of the system were GFP negative and lacZ positive.