

Figure S2. Generation dsDNA inserts coding for SFV nsP12 P1' variants by random mutagenesis. Cloning steps are numbered from 1 to 5. The first step of cloning was the hybridization of complementary, *E. coli*-optimized oligonucleotide primers that coded for the SFV nsP12 cleavage site sequence (red). The primers were flanked by BamHI and NheI 'sticky' ends (green and blue, respectively), and contained a PacI restriction endonuclease recognition site prior to the coding sequence of cleavage site (purple) (1). After linearization of pT7-Blue-3 vector by BamHI and NheI restriction endonucleases (2), the annealed oligonucleotide primer pair was inserted into pT7-Blue-3 plasmid by ligation (3). Point mutations were introduced to the cleavage sites by random mutagenesis using an oligonucleotide primer degenerated at the codon corresponding to SFV nsP12 P1' residue (yellow) (4). The short dsDNAs coding for different SFV nsP12 P1' variants were released from the pT7-Blue-3 plasmid by digestion using PacI and NheI restriction enzymes (5), followed by cloning of the short dsDNAs into pDEST-His₆-MBP-mEYFP plasmids.

