

Text S1. Design of pDWV-L-GFP and DWV-S-GFP

The full-length infectious cDNA clone of DWV type A, pDWV-304 (GenBank accession number MG831200) [Rybov et al., 2019] was used to design eGFP-expressing viral constructs, pDWV-L-GFP and pDWV-S-GFP (Figure 1; Text S2). Cloning included the following steps. At the first stage, two DNA fragments corresponding to the nucleotide positions 1365 – 5089 of DWV-304 with the insertion of the sequence containing restriction sites *AscI* and *BamHI* at the LP-VP2 were generated by overlap extension PCR using the plasmid pDWV-304 as a template and the primers listed in Table S1. Both fragments had *AscI-BamHI* sequences inserted upstream the sequence coding for the proposed proteolytic cleavage peptide (VQAKPEMDNPNPG) between the LP and VP2 of DWV-304. Towards the 5' end from the *AscI* site, the sequence coding for entire proposed proteolytic cleavage peptide at the DWV LP-VP2 interface (VQAKPEMDNPNPG) was inserted in the fragment destined for the construct pDWV-L-GFP, and the sequence coding for the proposed carboxy-terminal peptide of the DWV LP (VQAKPE) was inserted for fragment used in designing pDWV-S-GFP. As a result, the *AscI-BamHI* sequence was linked with the sequences coding for the potential DWV LP-VP2 proteolytic cleavage peptides (Figure S1). For the pDWV-L-GFP design, the this fragment was generated by overlap extension PCR using mutagenic primers “14-AscBamhi-VQAKPEM-For” and “15-MDNPNPG-BamhiAsci-Rev” and the flanking primers “1-F flank-For” and “2-F flank-Rev”. For the pDWV-S-GFP design, the fragment was generated using mutagenic primers “14-AscIBamhi-VQAKPEM-For” and “16-VQAKPE-BamhiAsci-Rev”, and the flanking primers “1-F flank-For” and “2-F flank-Rev”. The resulting fragments were cloned into pCRII-TOPO vector (Invitrogen) and were used to insert the *AscI-BamHI*-flanked eGFP PCR fragment generated using the primers “17-Asci-GFP-for” and “18-BamH-GFP-Rev” and pAcP(+)*IE1-eGFP* [Shi et al., 2007] as a template to make clones with two types of the LP-eGFP-VP2 insert. Then *Sall-SacII* section of these DWV-LP-eGFP-VP2 clones were used to replace *Sall-SacII* section of the pDWV-304 (positions 1438-4273) to produce pDWV-L-GFP and pDWV-S-GFP (Fig. 1).

References:

- Rybov *et al.* (2019) Dynamic evolution in the key honey bee pathogen deformed wing virus: Novel insights into virulence and competition using reverse genetics. *PLoS Biology* 17, e3000502. DOI: <https://doi.org/10.1371/journal.pbio.3000502>
- Shi, X. *et al.*, (2007). Construction and characterization of new piggyBac vectors for constitutive or inducible expression of heterologous gene pairs and the identification of a previously unrecognized activator sequence in piggyBac. *BMC Biotechnology* 7, 5. <https://doi.org/10.1186/1472-6750-7-5>