(a)

pC86_T36CA_4430-8106

PCR product 1

pGemT36_JP1

PCR product 2

pGemT36_JP2

SacI digestion
Gel-purification
Ligation
Long-range PCR

PCR product 3

pT36-GFP-JP

SmaI / MauBI

pT36CAori-full

(b)

Overlap PCR

pGemT363'GFP-JP

pGemT36p23GFP

ApaI / HindIII

pGemT36p23GFPnos

PstI / SwaI

pCAMT36p23GFP

pT36CAori-full-3'GFP

pGemT36Δp33

Overlap PCR

XmaI (an isoschizomer of SmaI) / PmeI

pT36CA-ori

Figure S4. Schematic procedure for cloning the pT36CA-ori construct/prototype vector. A two-part procedure was used to engineer pT36CA-ori. (a) Part I of the cloning procedure generated the intermediate binary plasmids, pHyb1 and pT36CAori-full. (b) Part II of the cloning procedure led to the construction of the binary plasmid/prototype vector pT36CA-ori. Gray and white boxes represent the CTV open reading frames (ORFs) that originated from the C86 (pT36FL) binary plasmid (El-Mohtar and Dawson, 2014) and T36CA cDNA clones (Chen *et al.,* 2018), respectively. Names of ORFs are as described in Fig. 1. Black boxes represent the region containing a ribozyme sequence and nopaline synthase (NOS) terminator. The cauliflower mosaic virus 35S promoter (35S) and a coding region of green fluorescence protein (GFP) are as illustrated. Bars in purple and dark green represent the controller element of the beet yellows virus (BYV) and citrus tristeza virus (CTV) major coat protein, respectively. The restriction enzymes used for cloning and their locations are as shown. The name of each cloned DNA (made up of a combination of letter and numerical identifiers preceded by the letter "p") or PCR product is indicated at the bottom of, or next to, each illustration.