

Table 1 Suppl. Examples of epitopes fused to coat proteins of plant filamentous viruses. FLT - fresh leaf tissue, CPV - canine parvovirus; HIV-1 - human immunodeficiency virus type 1; gp - glycoprotein; BNYVV - beet necrotic yellow vein virus; ep - epitope; NP - nucleoprotein; HCV - hepatitis C virus; HPV - human papillomavirus; aa - amino acids.

| Epitope                          | Epitope sequence                               | Epitope length (aa) | Plant virus | Placement on CP                   | Plant host                                                                | Yield of CPVs                                          | References                               |
|----------------------------------|------------------------------------------------|---------------------|-------------|-----------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------|------------------------------------------|
| CPV VP2                          | VQPDGGQPAVRNERA                                | 15                  | PPV         | N-terminus                        | <i>N. clevelandii</i>                                                     | -                                                      | Fernández-Fernández <i>et al.</i> (1998) |
| 2x CPV VP2                       | 2x VQPDGGQPAVRNERA                             | 30                  | PPV         | N-terminus                        | <i>N. clevelandii</i>                                                     | -                                                      | Fernández-Fernández <i>et al.</i> (1998) |
| <i>S. aureus</i> FnBP D2         | GQNNGNQSFEEDETEKDKPKYEQG 38<br>GNIIDIDFDSVPHIH | 38                  | PVX         | N-terminus                        | <i>N. benthamiana</i>                                                     | -                                                      | Brennan <i>et al.</i> (1999)             |
| HIV-1 gp 41                      | ELDKWA                                         | 6                   | PVX         | N-terminus                        | <i>N. benthamiana</i>                                                     | 400 - 600 µg g <sup>-1</sup> of FLT                    | Marusic <i>et al.</i> (2001)             |
| BNYVV ep4                        | RTSPPGQ                                        | 7                   | PVX         | N-terminus                        | <i>N. benthamiana</i>                                                     | up to 115 µg g <sup>-1</sup> of FLT                    | Uhde <i>et al.</i> (2005)                |
| BNYVV ep6                        | SANVRRD                                        | 7                   | PVX         | N-terminus                        | <i>N. benthamiana</i>                                                     | up to 115 µg g <sup>-1</sup> of FLT                    | Uhde <i>et al.</i> (2005)                |
| BNYVV ep4ep6                     | RTSPPGQSANVRRD                                 | 14                  | PVX         | N-terminus                        | <i>N. benthamiana</i>                                                     | up to 115 µg g <sup>-1</sup> of FLT                    | Uhde <i>et al.</i> (2005)                |
| CSFV E2 <sup>1</sup>             | Region between 790-860 aa                      | 70                  | PVX         | N-terminus; 6xHis; via FMDV 2a    | <i>N. benthamiana</i>                                                     | 120 µg g <sup>-1</sup> of FLT                          | Marconi <i>et al.</i> (2006)             |
| CSFV E2 <sup>2</sup>             | Region between 854-894 aa                      | 40                  | PVX         | N-terminus; 6xHis; via FMDV 2a    | <i>N. benthamiana</i>                                                     | -                                                      | Marconi <i>et al.</i> (2006)             |
| HPV-16 L2 and E7                 | LVEETSFIDAGAP and QAEPDRAHYNIVTFCKK            | 13 and 17           | PVA         | N- and C-terminus                 | transgenic <i>N. benthamiana</i> 28 - 43 µg g <sup>-1</sup> of FLT HC-Pro | -                                                      | Čerovska <i>et al.</i> (2008)            |
| HPV-16 L2 and E7                 | LVEETSFIDAGAP and QAEPDRAHYNIVTFCKK            | 13 and 17           | PVA         | N- and C-terminus                 | <i>N. benthamiana</i> and <i>Brassica rapa</i>                            | 10 and 4 µg g <sup>-1</sup> of FLT                     | Hoffmeisterová <i>et al.</i> (2008)      |
| Influenza A virus NP             | ASNENMETM                                      | 9                   | PVX         | N-terminus                        | <i>N. benthamiana</i>                                                     | 1100 µg g <sup>-1</sup> of FLT                         | Lico <i>et al.</i> (2009)                |
| HCV-E2 R9                        | QTTVVGGSQSHTVRGLTSLFSPGA 27<br>SQN             | 27                  | PVX         | N-terminus; via FMDV 2a           | <i>N. benthamiana</i>                                                     | 75 - 125 µg g <sup>-1</sup> of FLT                     | Uhde-Holzem <i>et al.</i> (2010)         |
| HPV-16 L2                        | LVEETSFIDAGAP                                  | 13                  | PVX         | N- or C-terminus                  | transgenic <i>N. benthamiana</i> 3H                                       | 170 or 8 µg g <sup>-1</sup> of FLT                     | Cerovska <i>et al.</i> (2012)            |
| VEGFR-3                          | LASEEFEQIESRRHQESGFR                           | 20                  | TuMV        | N-terminus                        | <i>A. thaliana</i>                                                        | 15 - 45 µg g <sup>-1</sup> of FLT                      | Sánchez <i>et al.</i> (2013)             |
| VEGFR-3                          | LASEEFEQIESRRHQESGFR                           | 20                  | TuMV        | N-terminus                        | <i>B. juncea</i>                                                          | 15 - 30 µg g <sup>-1</sup> of FLT                      | Sánchez <i>et al.</i> (2013)             |
| HPV-16 E7                        | QAEPDRAHYNIVTFCKK                              | 17                  | PVX         | Internal loops; 6xHis or StreptII | <i>N. benthamiana</i> (wt and HC-Pro)                                     | -                                                      | Vaculik <i>et al.</i> (2015)             |
| ZP3 contraceptive vaccine        | ZP3 aa 331-343                                 | 13                  | TMV         | between aa 154-155 at C terminus  | <i>N. tabacum</i> Xanthi                                                  | 100 µg cm <sup>-2</sup> of leaf                        | Fitchen <i>et al.</i> (1995)             |
| Malaria B cell epitope (QGPGAP)2 |                                                | 15                  | TMV         | leaky stop at C terminus          | <i>N. tabacum</i> Xanthi                                                  | 1.2 mg g <sup>-1</sup> FLT (about 25% of wt TMV)       | Turpen <i>et al.</i> (1995)              |
| Murine hepatitis Virus           | PLLGIGSTCAEDGN                                 | 15                  | TMV         | between aa 154-155 at C terminus  | <i>N. tabacum</i> Xanthi                                                  | high concentration in plants, some versions insoluble. | Koo <i>et al.</i> (1999)                 |
| Rabies virus glycoprotein        | DDGDDAVYTRIMMNGGRLKR                           | 20                  | TMV         | between aa 154-155 at C terminus  | <i>N. tabacum</i> Xanthi                                                  | 1-3 mg g <sup>-1</sup> of FLT                          | Bendahmane <i>et al.</i> (1999)          |

|                                                      |                   |      |     |                                                                 |                               |                                 |
|------------------------------------------------------|-------------------|------|-----|-----------------------------------------------------------------|-------------------------------|---------------------------------|
| <i>Pseudomonas aeruginosa</i> outer membrane protein | TDAYNQKLSERRAN    | 14   | TMV | between aa 154-155 at C <i>N. tabacum</i> Xanthi terminus       | -                             | Staczek <i>et al.</i> (2000)    |
| FMDV                                                 |                   | 8-10 | TMV | between aa 154-155 at C <i>N. tabacum</i> Xanthi terminus       | 5 mg g <sup>-1</sup> of FLT   | Wu <i>et al.</i> (2003)         |
| Rabbit papillomavirus L2                             | VGPLDIVPEVADPGGPT | 17   | TMV | between aa 154-155 at C <i>N. tabacum</i> terminus              |                               | Palmer <i>et al.</i> (2006)     |
| Various xeno and self epitopes                       |                   | 8-19 | TMV | chemical linkage to mutant TMV carrying reactive lysine residue | 7.3 mg g <sup>-1</sup> of FLT | Mc Cormick <i>et al.</i> (2006) |

Table 2 Suppl. Examples of antigens fused to coat proteins of plant filamentous viruses. SIT - systemically infected tissue, TSP - total soluble protein, FLT - fresh leaf tissue.

| Antigen       | Antigen length (aa) | Plant virus | Placement on CP                       | Plant host                                | Yield                              | References                      |
|---------------|---------------------|-------------|---------------------------------------|-------------------------------------------|------------------------------------|---------------------------------|
| Rotavirus VP6 | 397                 | PVX         | N-terminus; via FMDV 2a               | <i>N. benthamiana</i>                     | 1 $\mu\text{g VP6 g}^{-1}$ of SIT  | O'Brien <i>et al.</i> (2000)    |
| HPV-16 E7     | 98                  | PVX         | N-terminus                            | <i>N. tabacum</i> cv. <i>Petit Havana</i> | 0,5% E7CP relative to TSP          | Morgenfeld <i>et al.</i> (2009) |
| HPV-16 E7ggg  | 98                  | PVX         | N- or C-terminus                      | transgenic <i>N. benthamiana</i> HC-Pro   | HC-instability during purification | Plchova <i>et al.</i> (2011)    |
| HPV-16 E6GT   | 158                 | PVX         | C-terminus                            | transgenic <i>N. benthamiana</i> K7       | 5 $\mu\text{g CPV g}^{-1}$ of FLT  | Cerovska <i>et al.</i> (2013)   |
| Protein A     | 133                 | TMV         | C-terminus, via 15 aa flexible linker | <i>N. benthamiana</i>                     | 3 mg $\text{g}^{-1}$ of FLT        | Werner <i>et al.</i> (2006)     |

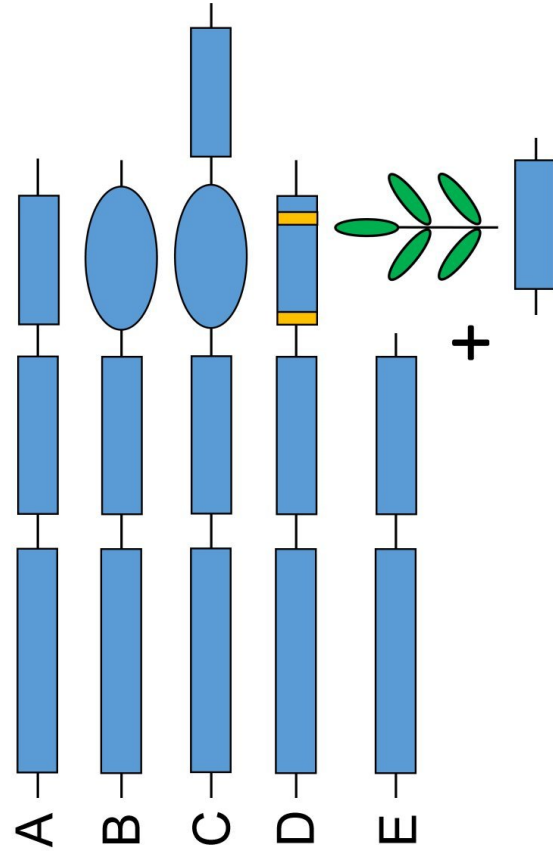


Fig. 1 Suppl. Different strategies for plant viral vector design. *A* - native virus, *B* - exchange of genes, *C* - gene insertion, *D* - epitope display, *E* - deconstructed virus. Genes of viral genome are schematically depicted as *rectangles*, *ellipses* represents gene for foreign protein, *yellow rectangles* demonstrate foreign epitopes. The plant symbol emblemizes the transgenic plants bearing the part of the deconstructed viral genome