Construction of full-length cDNA clones of *Apple chlorotic leaf spot virus* in two different methods

Lei Zhang[1], Wilhelm Jelkmann[1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim
Email of corresponding author: lei.zhang@jki.bund.de

Apple chlorotic leaf spot virus is a flexuous filamentous particle of approximately 680 to 780 nm in length and 12 nm in width, and is the type species of genus *Trichovirus* within the family *Betaflexiviridae*. The genome size of *Apple chlorotic leaf spot virus* is about 7.5 kb, of which the 3'- and 5'- ends are well conserved, and till now there are 14 complete genome sequences of different strains of the virus are available in the nucleotide database of National Center for Biotechnology Information.

For constructing a clone of a certain gene or DNA fragment, there are several different methods, generally including TA cloning, cloning using restriction enzymes and ligation-independent cloning. The TA cloning should be the simplest one, but for constructing infectious clones of plant viruses, the latter two are always preferred. According to publications, by the way of cloning using restriction enzymes, the infectious cDNA clones of *Apple chlorotic leaf spot virus* and *Apple stem grooving virus* have been constructed, while ligation-independent cloning methods were successfully used for constructing the cDNA clones of *Apple stem pitting virus* and *Apple stem grooving virus.* In our study, a commercial product of In-Fusin HD Cloning Kit (Takara) and a ligation-independent method of circular polymerase extension cloning (CPEC) are employed.

An isolate of ACLSV from a peach tree was chosen for generating the cDNA clone. Total RNA of 100 mg leaf tissue from an infected peach tree was extracted using RNeasy Plant Mini Kit (Qiagen). The cDNA was generated by RT-PCR with RevertAid Premium Reverse Transcriptase (Thermal Scientific). Full-length fragments of ACLSV (inserts) and linear pV297 (a pBin vector, E. Maiss, Hannover) were produced from PCRs using Precisor High-Fidelity DNA Polymerase (BioCat). Assembly reactions were performed with gel purified inserts and vectors using In-Fusion HD Cloning Kit (Takara) according to the manufacturer's instruction and by a circular polymerase extension cloning (CPEC) method, respectively, followed by transforming NEB 10β competent cells. Possibly successful full-length cDNA clones of the *Apple chlorotic leaf spot virus* were identified with PCRs using three different primer pairs and sequent XbaI digestion with isolated plasmids (Qiangen Miniprep Kit). Finally, *Agrobacterium* strain ATHV was transformed and used for infecting tobacco plants (*N. occidentails* 37b).