# FULL GENOME SEQUENCING OF A DIVERGENT ISOLATE OF LChV-1 AND MONITORING OF THE VIRUS DERIVED siRNAs IN SWEET CHERRY OVER FOUR SEASONS

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## Introduction

*Little cherry virus* 1 (LChV-1) is a member of the newly proposed genus *Velarivirus* in the family *Closteroviridae* and infects several *Prunus* species, including sweet cherry in which it seems widespread. The virus exhibits high genetic diversity and so far several isolates belonging to different phylogenetic groups have been characterized (1). The aim of the present work was to obtain the full genome sequence of a divergent variant of LChV-1 using high-throughput sequencing of siRNAs and to monitor the fluctuation of the virus derived siRNAs over the four seasons of the year.

## Materials and Methods

In winter of 2013, small interfering RNAs (siRNAs) extracted from a sweet cherry tree infected by the G15-3 isolate of LChV-1 were subjected to NGS using the Ion Torrent platform (Ion 318 chip). The generated data were used to reconstruct the virus genome using Geneious. The genome was further confirmed by Sanger sequencing. In addition, siRNAs were isolated (mainly from stems) of the same tree in April, July and October and further sequenced using the same NGS approach.

## Results and Discussion

Mapping of the siRNA reads to LChV-1 reference genome generated large sequence fragments with several gaps. Primers were designed based on these sequences and used in RT-PCR assays. The obtained amplicons were subjected to Sanger sequencing allowing to close the gaps. The complete genome of the G15-3 isolate has a total length of 16.880 nt and shows 26-27% nucleotide sequence divergence with other fully sequenced LChV-1 isolates (ITMAR, UW2, V2356). The highest levels of variation were observed in the p4, CPm and p27 proteins. The monitoring of virus derived siRNAs throughout the year showed a fluctuation in their levels with the maximum and minimum being observed in spring and summer, respectively. These results correlate with those of RT-qPCR assays conducted on RNA extracted from stems of the same tree during the four seasons.

## References

1. Katsiani et al., 2015. *Plant Pathology* 64: 817-824.