# Identification of *Nectarine stem pitting-associated* virus (NSPaV) by Next-Generation Sequencing (NGS) in Hungary

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## *Introduction*

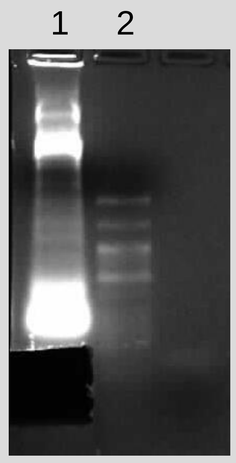
- NSPaV (genus *Luteovirus*) first described in the USA in imported nectarine by NGS (Bag *et al.,* 2015)
- Later identified in peach, nectarine and *Prunus mume* (Villamor *et al*., 2016, Lu *et al*., 2017, Candresse et al., 2017)
- Peach leaves showing severe yellowing symptoms collected from *Prunus persica* 'Baby Gold' tree from a 13-year-old organic orchard in Szob (Pest County) in May 2011 in Hungary

Peach tree showing strong yellowing symptoms (Szob, Hungary)

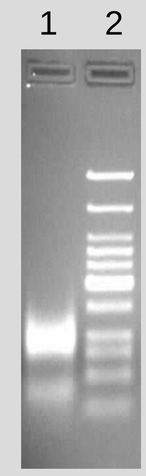
## *Methods*

### *'In house' small RNA library preparation and sequencing on Ion Torrent PGM*

- Total RNA extraction by TRI Reagent; run, excise and purify small RNA from 3% non-denaturing agarose gel
- Adenylation of ION P1B DNA adapter (Song *et al*., 2015)
- Adenylated P1B DNA adapter ligation to small RNA 3'-end
- ION RNA adapter ligation to small RNA 5'-end
- Reverse transcription and PCR amplification
- Run, excise and purify amplified library from 3% non-denaturing agarose gel
- Qubit HS library quantification
- Run emulsion PCR (Ion One Touch 2 system)
- Template Ion Sphere Particles enrichment (Ion One Touch ES system)
- Sequencing on ION 314 Chip (Ion Torrent Personal Genome Machine)
- Sequence analysis by VirusDetect pipeline (Zheng *et al*., 2017)



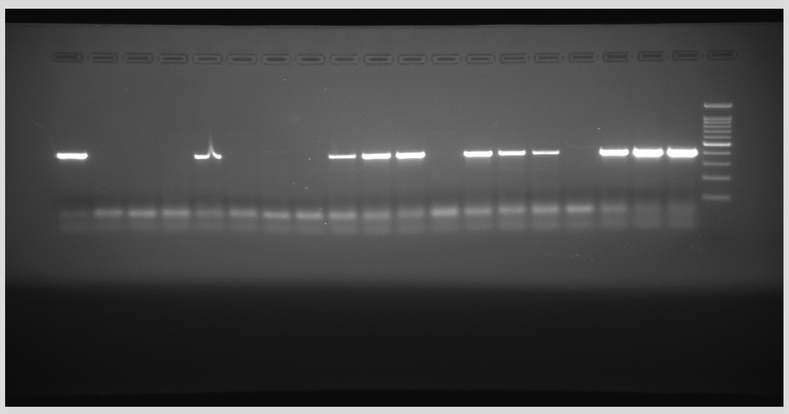

1. Total RNA extracted from infected peach leaves by TRI Reagent; excised small RNA
2. Low Molecular Weight DNA Ladder



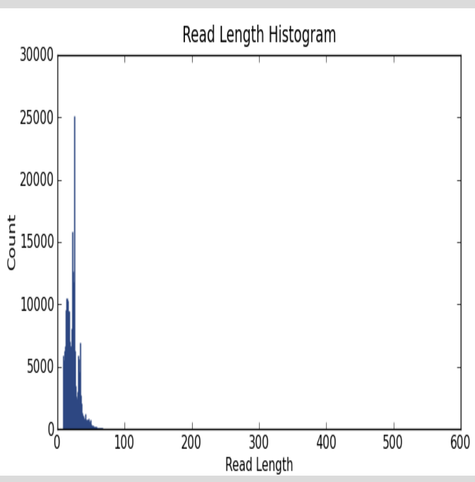

1. Amplified small RNA library by A/P1B Ion Torrent platform-specific primers
2. Low Molecular Weight DNA Ladder (25, 50, 75, 100, 150, 200, 250, 300, 350, 500, 766 bp)

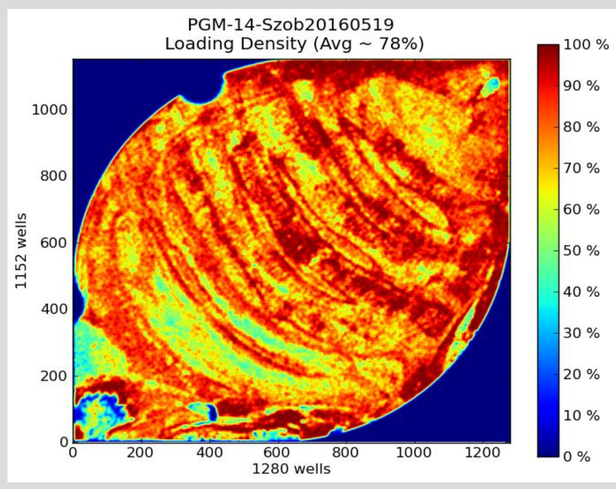
## *Results*

- 18 contigs were assembled from 227.878 reads showing 95 to 100% homology to NSPaV sequences in GenBank (NC_027211, KT273409 and KT273410)
- 75 contigs revealed 95 to 100% similarity to *Plum pox virus* (PPV)
- The presence of NSPaV in the sample was confirmed by specific primers based on the contig sequences and also by NSPaVF-NSPaVR primers (Bag *et al.,* 2015)
- RT-PCR products of two NSPaV isolates from the same orchard were Sanger-sequenced and deposited in GenBank (KY626337.1 and KY829024.1) that shared 96 to 98% nucleotide identity with NSPaV sequences in GenBank
- NSPaV and PPV mixed infection was detected in all the additionally tested 13 peach samples showing the same symptoms from the same place
- NSPaV and PPV mixed infection was detected in 9 peach samples from another orchard showing no typical yellowing symptoms
- NSPaV was detected also in symptomless trees (11 out of 19 samples)
- NSPaV seems to be very frequent in peach in Hungary (36 out of 46 tested samples)
- NSPaV association with the yellowing disease remains uncertain

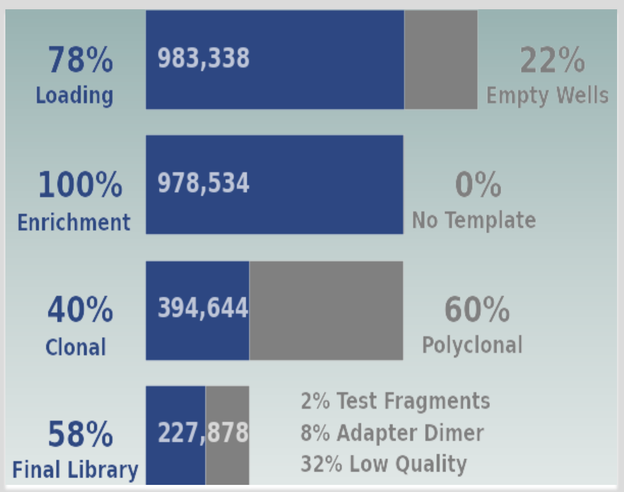


Detection of NSPaV in symptomless peach samples by RT-PCR




Loading density of the ION 314 Chip




Wells-beadogram of the loaded ION 314 Chip

## *References*

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