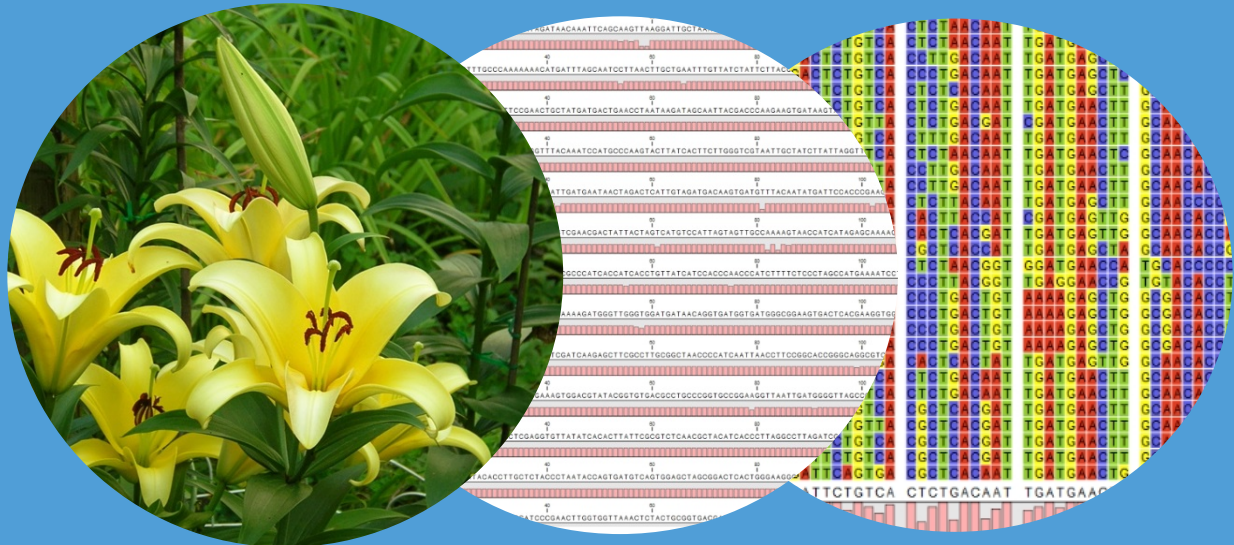


NGS applications in plant pathogen diagnostics

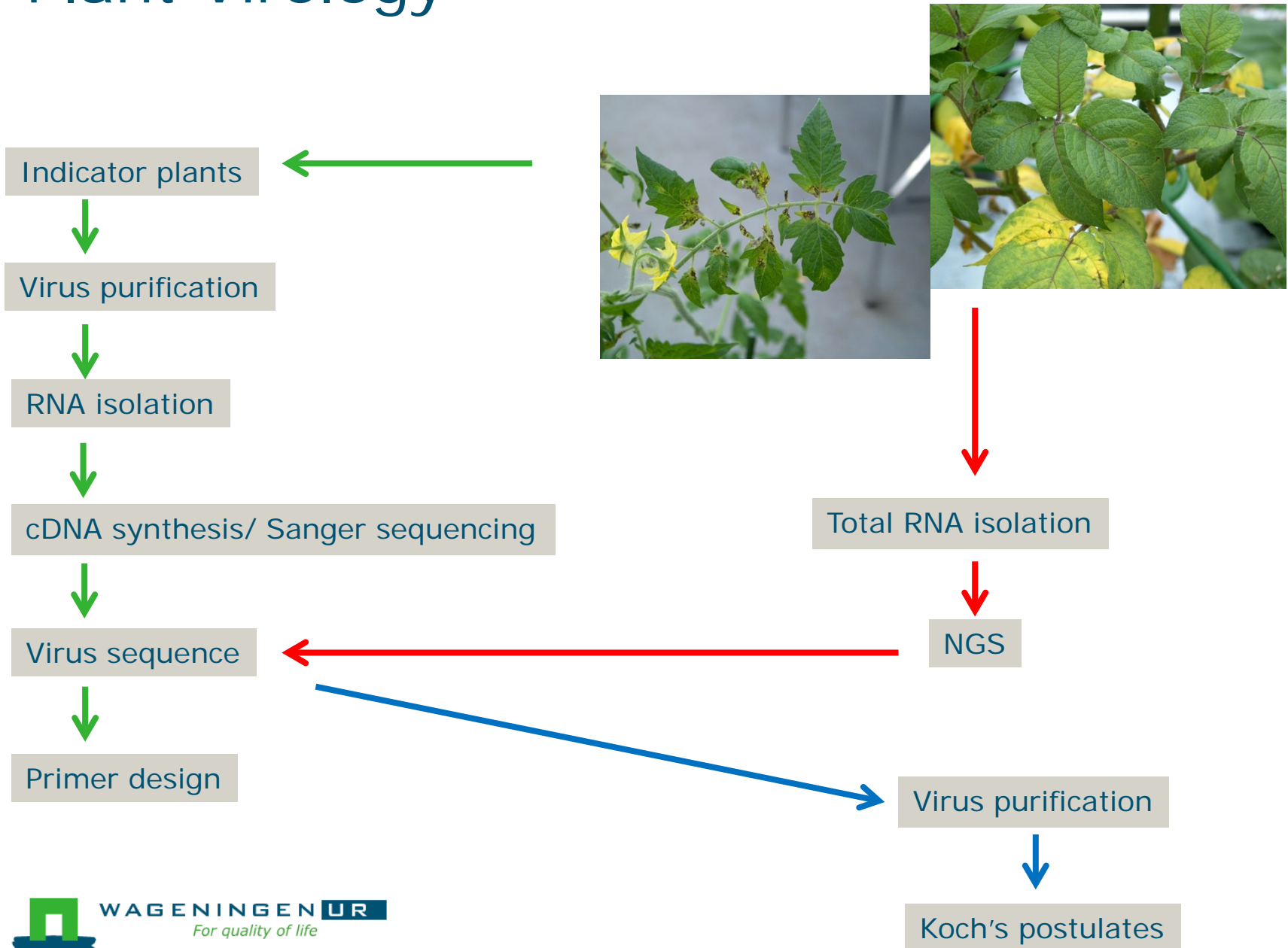
Annette Dullemans,

Els Verstappen, Ilse Houwers, Martin Verbeek, Theo van der Lee, René van der Vlugt and Peter Bonants

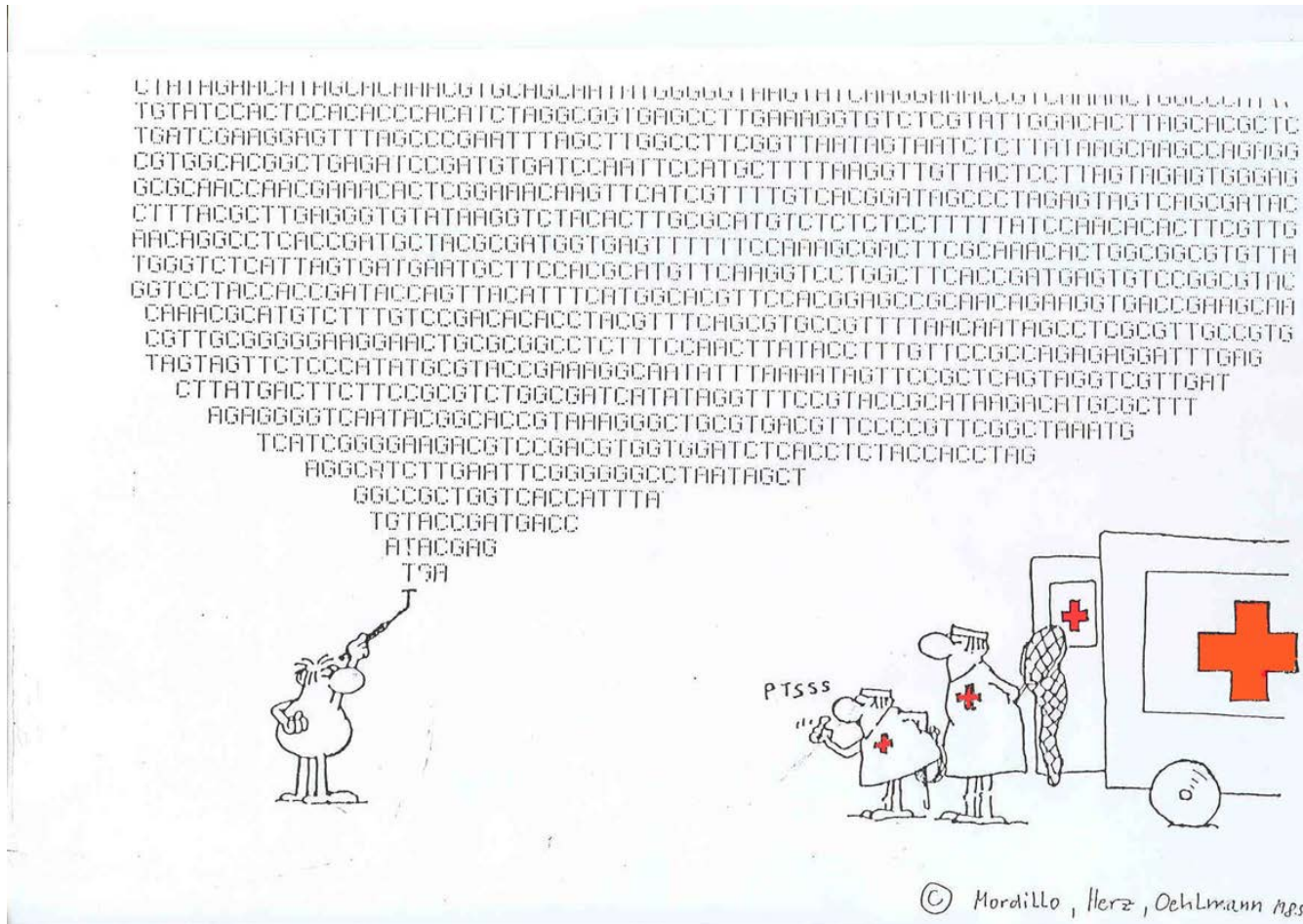
- EPPO-TESTA meeting Angers, 2 December 2015



Plant Virology



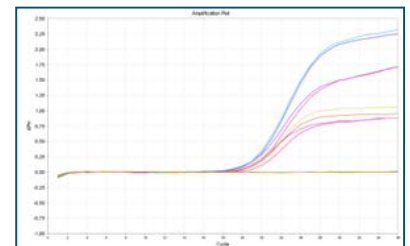
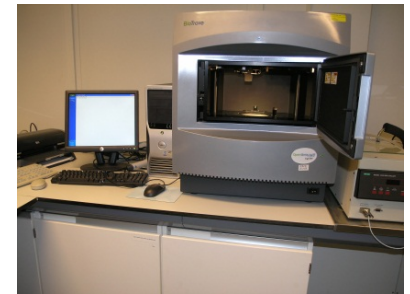
a DNA sequence in itself is useless



Plant Virology

■ Applications for NGS:

- Identification of (new) viruses
- Quality control of virus collection material
- Study of diagnostic samples
(presence: yes/no & sequence variation)
 - Developing primers for detection strategies:
 - (Real time)-(RT)-PCR
 - Luminex: xTAG
 - On-site: LAMP



Strawberry Latent Ringspot Virus (SLRSV)

- Unassigned species in the family *Secoviridae*
- Worldwide distribution
- Many hosts: Strawberry, Raspberry, Blackberry, Impatiens, Anemone, Tibouchina, Bean, Pepino, Mint, Rose, Prunus Robinia, Quinoa, Lily,.....
- 1 full length sequence: Golden Ginger Mint
- 2 RNA segments : 7.5 + 3.8 kb



SLRSV in Lily

- Symptomless
- Screening bulbs for export
- Detection by ELISA
 - Antiserum available:
 - Bioreba & Prime Diagnostics
 - Different reaction with different isolates
- RT-PCR (Tang et al, 2012)
 - Not in accordance with ELISA results



Procedure

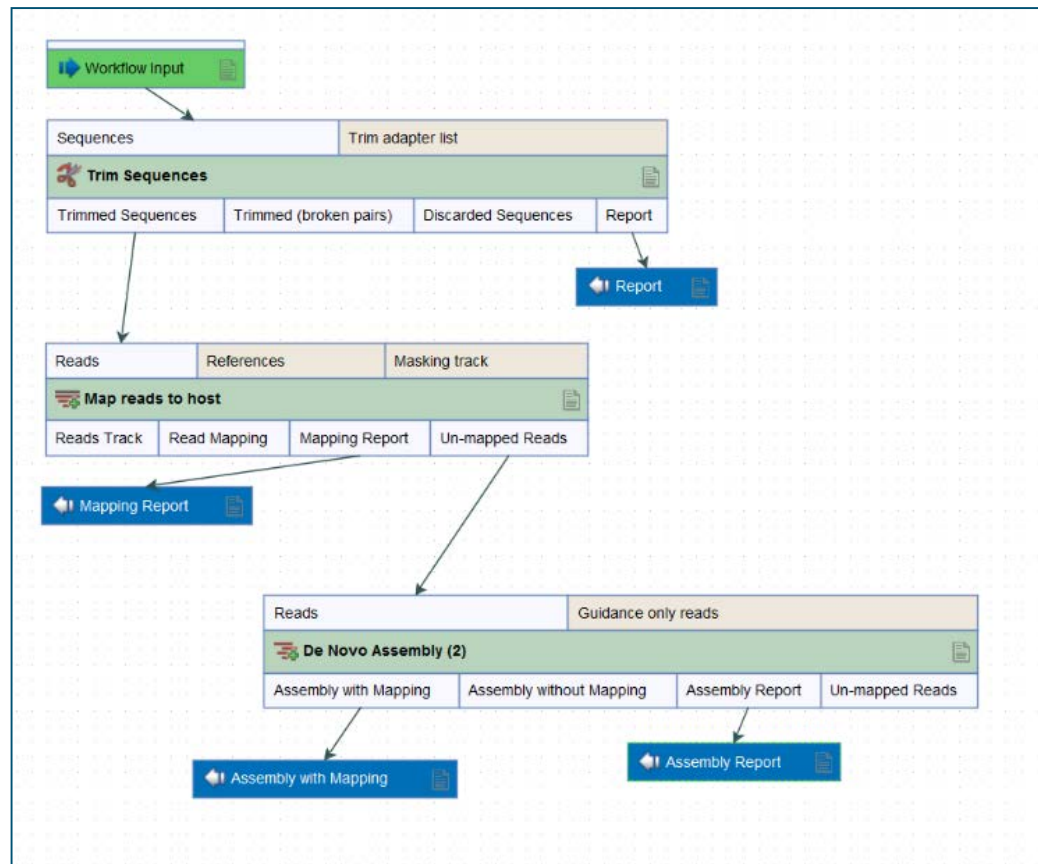
- Total RNA isolated
 - collection material (indicator plants)
 - Lily bulbs
- DNase treatment
- RiboZero TrueSeq Illumina HiSeq library preparation
 - MiD labeling, maximum 24 samples/lane
 - Paired-end reads, 125 nts



Data analysis

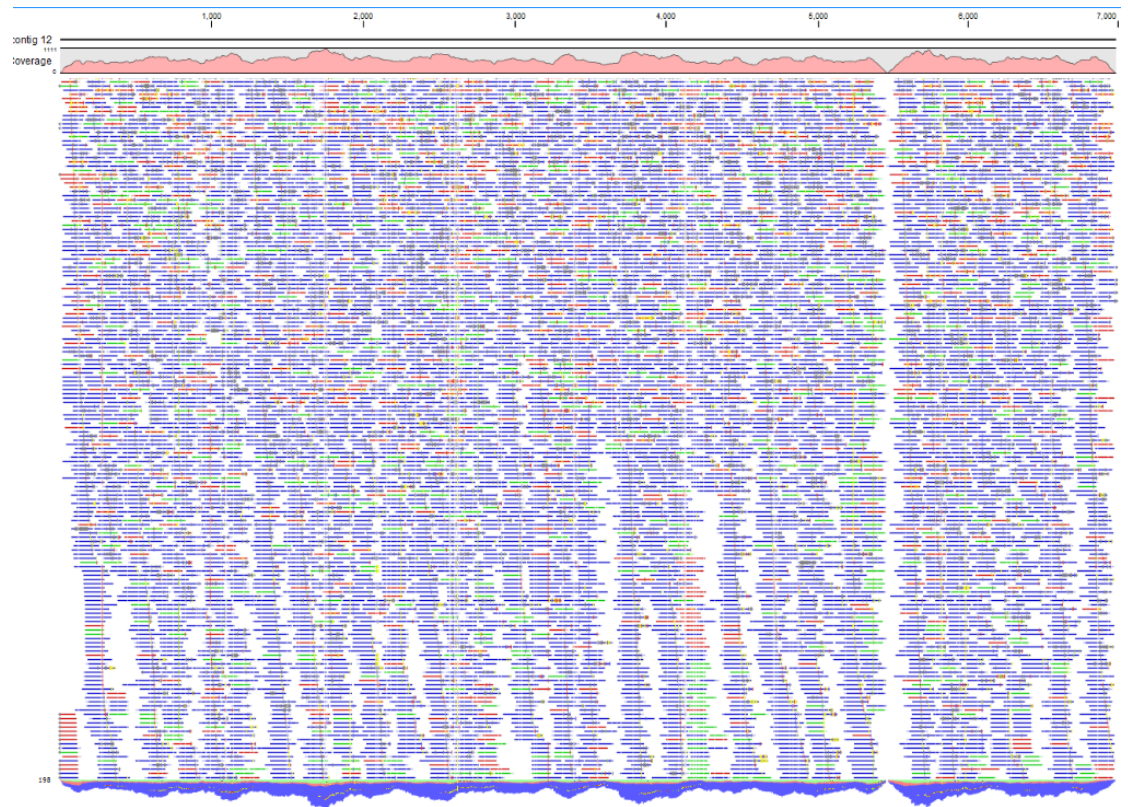


- CLC Genomics Workbench
- In workflow
- In batch



Results

- Contig list
- Select virus contigs by Blast analysis
 - Expected & unexpected virus sequences
- Near full length sequences



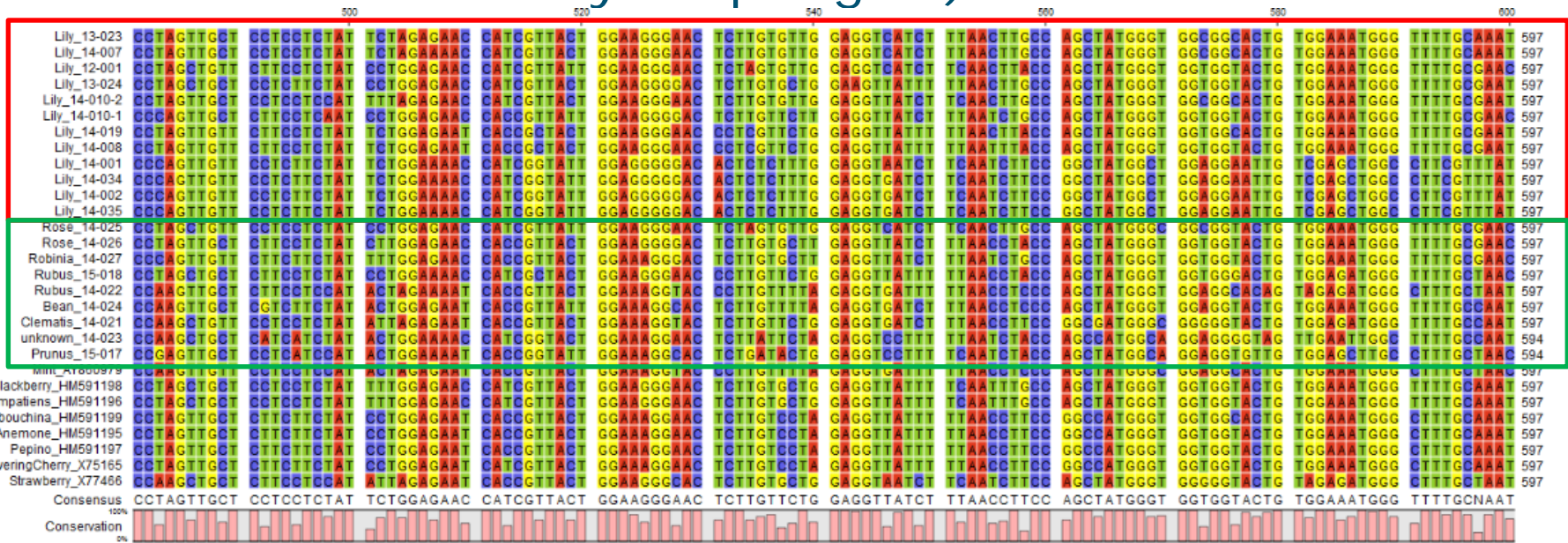
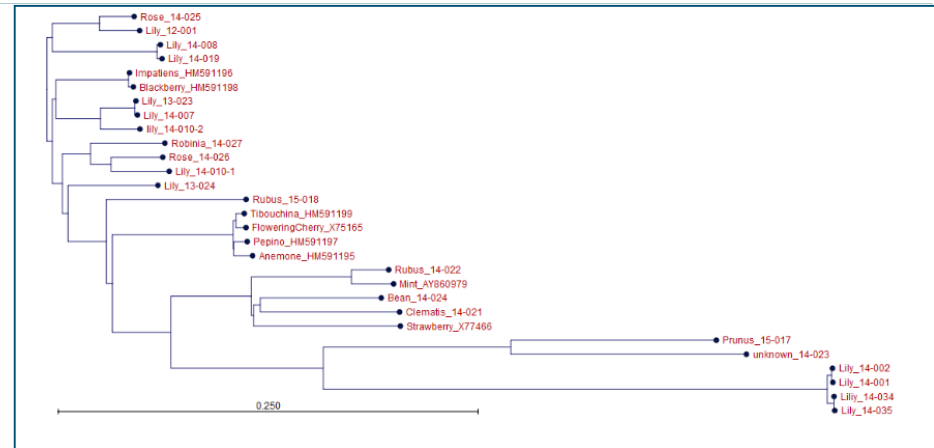
Results

21 new SLRSV sequences

- 12 from Lily

- 9 from virus collection

- Very variable nt-sequences
- 62-99% identity in cp region)



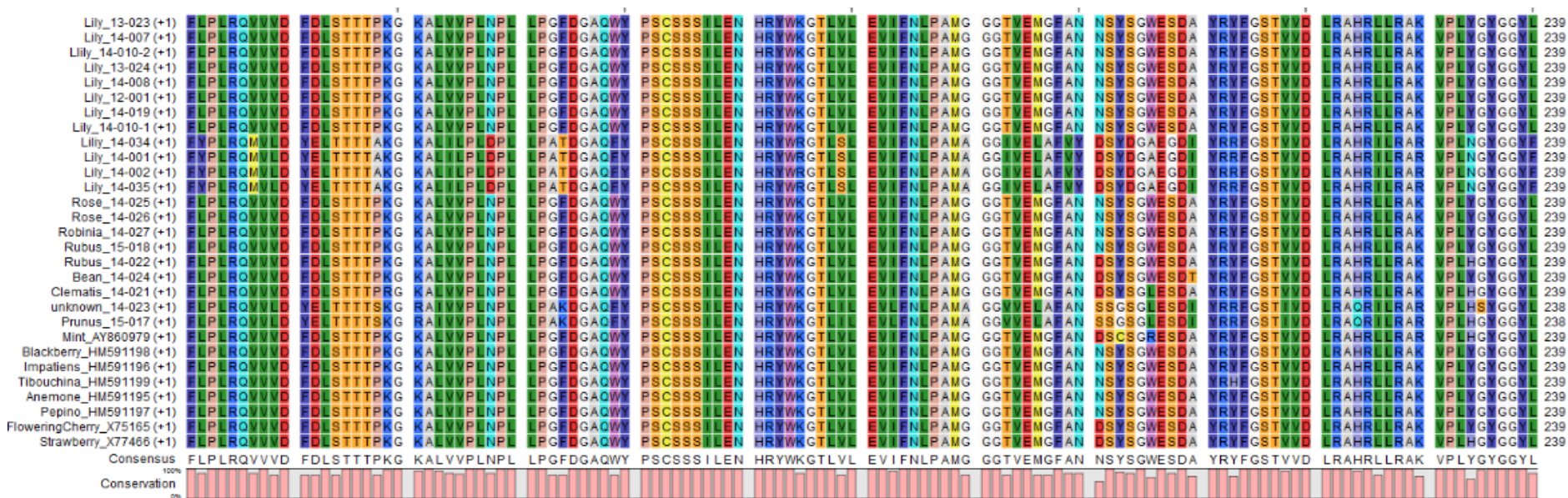
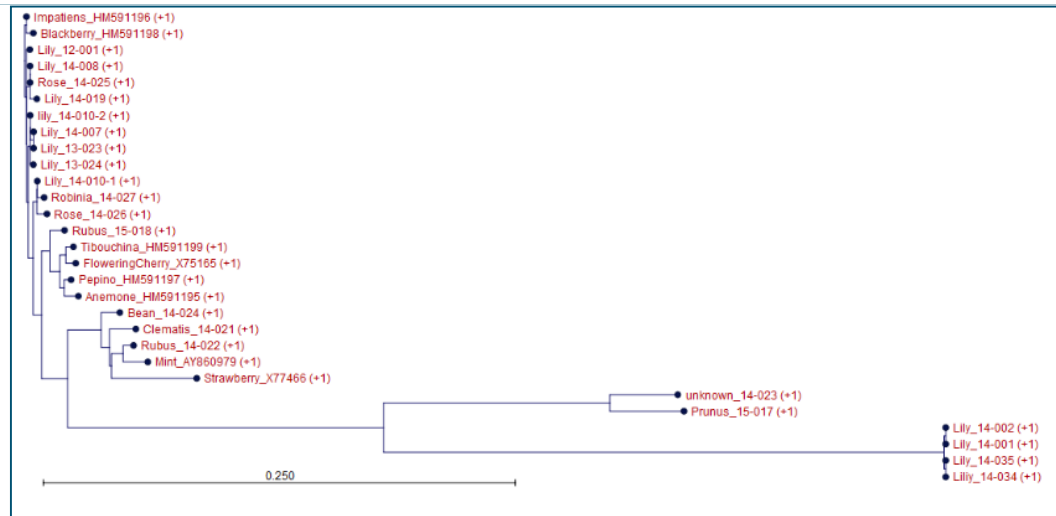
Results

aa alignment

■ Much higher identity

- 2 groups

- >93 & 63-72% identity



Next step

- Develop specific (real time-)RT-PCR test for routine screening by the Dutch Flower Bulb Inspection Service (BKD)
 - Select the best region
 - Cover all known sequences

Advantages of NGS in Virology

- Fast
 - Virus sequence
 - Help to develop purification strategy
 - Virus identification
 - Insight in population variance
 - Epidemiology studies
 - Disease management
- Result: (PCR) detection based on several isolate sequences

Acknowledgements

- Dutch Flower Bulb Inspection Service (BKD):

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- Marcel Westerberg



Netherlands Food and Consumer
Product Safety Authority
Ministry of Economic Affairs

- NL funding: PPS diagnostics



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For quality of life