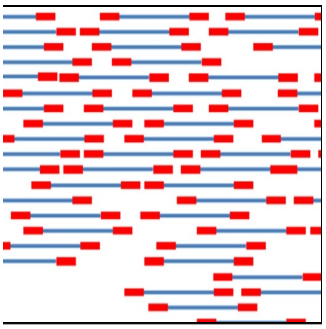
Euphresco

Success story

October 2018

Validation of high-throughput sequencing technologies pipeline for virology

Correct detection and identification of pests are essential to support inspection activities conducted by National Plant Protection Organisations (NPPOs) in the framework of their official mandate, and to evaluate the efficacy of measures taken to protect horticultural and agricultural production and international trade from quarantine viruses and viroids. Traditional approaches based on serological, molecular or biological methods require prior knowledge of the target that should be detected: genome sequences are needed for the development of specific (RT-)PCR primers, or viruses should be purified for the development of virus-specific antisera. High-throughput sequencing (HTS) allows the detection and identification of known pathogens as well as pests for which we do not have prior knowledge. HTS has therefore the potential to become an important tool in diagnostic activities.



However, so far no standardized protocols for extraction, enrichment and sample preparation using HTS exist and many different methods are available; furthermore, the sequencing output that is generated needs rare bioinformatic knowledge in order to correctly analyze and interpret the data. The NGS-detect project was funded to address these challenges.

The Canadian Food Inspection Agency organised a proficiency test to evaluate the ability to correctly identify viruses and viroids in samples consisting of fruit tree or grapevine material. Eleven laboratories participated in this test and used their 'in-house' work-flow for sample preparation, enrichment, sequencing and for the initial bioinformatic analyses. All data sets were re-analysed using VirTool (www.virtool.ca) for comparison. The results show that with increasing reads output, the numbers of false negative results decreased. However, background sequences (such as laboratory contaminations and sequencer contaminations) can increase the number of false positive results.

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Therefore, it was recommended not only to look at the numbers of reads assigned to an identified object, but also to look at the read coverage for correct decisions.

Currently, a test performance study (TPS) is being carried-out in order to validate one entire HTS workflow. Two sets of samples were sent out to eight participating laboratories together with consumables for total RNA extraction and ribo-depletion. It was possible to find one commercial supplier that will sequence all samples. All sequencing data will be analyzed initially by each laboratory to test their proficiency in pathogen identification but will also be analyzed using the same pipeline in VirTool. The results gained in the TPS will help to identify the obstacles that might occur using HTS in a diagnostic setting.

The project outputs will provide valuable information to support the development of guidelines on the use of HTS including minimum quality criteria that will be incorporated into future EPPO diagnostic protocols.

Project ID: The application of Next-Generation Sequencing technology for the detection and diagnosis of non-culturable organisms: viruses and viroids (NGS-detect)