EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

Workshop on the use of NGS technologies for plant pest diagnostics

Hotel Excelsior, Bari
2017-11-22/23

Istituto per la Protezione Sostenibile delle Piante
Consiglio Nazionale delle Ricerche

Workshop 2017

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Wednesday, November 22

08:00 **Registration**

08:30 **Welcome**
Mr Boscia (CNR Institute, IT)

SESSION 1: **Next Generation Sequencing: an introduction; Chair: Ms Ravnikar; Co-Chair: Mr Ziebell**

08:45 **Understanding the basics of NGS**
Mr Van der Lee (WUR, NL)

09:45 **Focus on the COST Action 'Application of NGS for the study and diagnosis of plant viral diseases in agriculture'**
Mr Massart (ULG, BE)
'*Defogging NGS and plant virus diagnostic: the role of a collaborative network (COST Action FA1407)*'

10:30 *Coffee break + poster session*

11:00 **Focus on the Euphresco project 'The application of Next-Generation Sequencing technology for the detection and diagnosis of non-culturable organisms: viruses and viroids'**
11.00-11.20 Mr Ziebell (JKI, DE)
11.20-11.40 Mr Rott (CFIA, CA)
'EUPHRESCO P-172: Next Generation Sequencing Proficiency testing of virus infected grapevine and fruit trees'

11:40 **Focus on other initiatives**
11.40-12.00 Ms Moreira (IPPC)
'Next Generation Sequencing in the context of the International Plant Protection Convention (IPPC)'
12.00-12.20 Ms Lebas and Ms Souza-Richards (MPI, NZ)
'The Impact of NGS in New Zealand'
12.20-12.40 Mr Rodoni (DPI, AU)
12.40-13.00 Mr Al Rwahnih (UC-Davis, US)
'High Throughput Sequencing as a tool for viral pathogen diagnosis and expedited release of quarantined propagative plant material'

13:00 *Lunch*

Programme

Workshop on the use of NGS technologies for plant pest diagnostics

SESSION 2: Next Generation Sequencing in practice; Chair: Mr Rott; Co-chair: Mr van der Vlugt

14:00	**Use of NGS in diagnostics** *14.00-14.15* Mr Kreuze (CIP, PE) *'Validating siRNA sequencing and assembly to replace classical virus indexing in root and tuber crops'* *14.15-14.30* Mr Candresse (INRA, FR) *'NGS-Based Viral Diagnostics, Lets Confront Some Difficulties'* *14.30-14.45* Mr Schump (Agroscope, CH) *'Deep sequencing for Quality Control of Real-time PCR diagnostic used for potato certification'* *14.45-15.00* Mr Van der Lee (WUR, NL) *15.00-15.15* Mr Westenberg (NVWA, NL) *'Comparison of NGS pipelines and traditional diagnostics in annual Daucus carota surveys for the detection of Ca. Liberibacter solanacearum and Ca. Phytoplasma solani'* *15.15-15.30* Mr Saldarelli (IPSP CNR, Bari, IT) '*NGS technology at CNR-IPSP: from de novo virus discovery to the sanitary status of plants for planting'* *15.30-15.45* Mr Boonham (Fera, GB) *'Maize lethal necrosis in East Africa: tracking an emerging disease using NGS'* *15.45-16.00* Ms Ravnikar (NIB, SI) *16.00-16.20* Discussions/questions
16.20	*Coffee break + poster session*
16.40	**Discussion on use of controls, quality assurance aspects**
17.45	**Introduction to hands-on sessions**
18.15	*Close of the first day (Free evening)*

Programme

Workshop on the use of NGS technologies for plant pest diagnostics

Thursday, November 23

SESSION 3 : Hands on NGS

9:00	**Hands on for NGS detection (3 groups in parallel)**
10:30	*Coffee break + poster session*
11.00	**Hands on for NGS detection (to continue)**
13:00	*Lunch*

SESSION 4:Harmonized guidelines on the use of NGS in diagnostics; Chair: Ms Petter; Co-chair: Mr Giovani

14:00	**Workflow after pest finding using NGS** Mr Wetzel, DLR, DE *'A framework for the evaluation of biosecurity, commercial, regulatory, and scientific impacts of plant viruses and viroids identified by NGS technologies'*
14.30	**Introduction to EPPO Standards**
14.45	**Brainstorming session on the preparation of an EPPO Standard on NGS**
16.15	*Coffee break + poster session*
16.45	**Feedback from the 7 groups (8 mn per group)**
17.45	**Conclusions and recommendations**

After the Workshop a guided tour of Bari monuments will be organized from 18.30 to 19.55. This will be followed by the Workshop dinner at the restaurant (Piccinni 28) for those who have reserved it.

Workshop on the use of NGS technologies for plant pest diagnostics, Bari (IT), 2017-11-22/23

ABSTRACTS ORAL COMMUNICATIONS

SESSION 1
Next Generation Sequencing: an introduction
Chair: Ms Ravnikar Co-Chair: Mr Ziebell

Understanding the basics of NGS

What's in the pipeline?

Theo van der Lee
Wageningen Plant Research, Wageningen, The Netherlands

Plant diagnostics have gained great momentum with the incorporation of "next-generation" RNA/DNA sequencing (NGS). NGS, with its greater parallelization, lower price tags and higher throughput has many possibilities. The advantages of using NGS to diagnose diseases, are manifold: (1) NGS-based genetic test yields the same or more information as multiple Sanger-based tests, but faster and at a significantly lower price. (2) NGS can interrogate many targets within a single run while providing a resolution of all the individual nucleotides in the genome. This makes it possible to effectively search for known contaminants in seeds and starting material. (3) NGS allows to validate the results by testing for self-consistency (4) NGS allows SNP identification compared to the targets which makes this method suitable for 'track and trace' (5) NGS also allows the detection of yet unknown contaminants based on homology. This is fundamentally different from previous diagnostics which were often tested for specific known pathogens and pests. Leveraging the power of NGS diagnostics requires knowing when and how to use it and there are also significant informatics challenges in NGS, in terms of data storage, data processing and data interpretation. Meeting these challenges requires setting up comprehensive standards in NGS analysis, increasing communication and data sharing between laboratories. This presentation will describe the different NGS procedures and the sequence analysis strategies: what's in a pipeline?

Focus on the COST Action 'Application of Next Generation Sequencing for the study and diagnosis of plant viral diseases in agriculture'

Defogging NGS and plant virus diagnostic: the role of a collaborative network (COST Action FA1407)

Sebastien Massart
University of Liège - Gembloux Agro-Bio Tech

NGS technologies have revolutionized the plant virology research. Now, they are progressively impacting the diagnostics of plant viruses. They might therefore influence current phytosanitary regulations and plant trade in the near future. To handle such complex issues and to tackle technical, scientific, regulatory and commercial challenges, a network of more than 140 scientists has been built within the COST Action DIVAS (www.cost-divas.eu) financed by the European Commission. This presentation will describe these challenges and the insights gained through this collaborative network.

Focus on the Euphresco project 'The application of NGS technology for the detection and diagnosis of non-culturable organisms: viruses and viroids'

The application of NGS technology for the detection and diagnosis of non-culturable organisms: viruses and viroids (NGS-detect). EUPHRESCO Project 2015-F172

Heiko Ziebell

Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut

The existing molecular methods (such as PCR, real-time PCR, Sanger sequencing) for the detection and the identification of non-culturable organisms and particularly plant pathogenic viruses and viroids do not always lead to the correct identification of the pathogen especially in case of multiple infection. Furthermore, they require prior knowledge about the pathogen(s) tested for (e.g. sequence information) and therefore one can only detect what has been tested for. Deep sequencing (next generation sequencing, NGS) is a new powerful technology. NGS leads to rapid and reliable holistic virus identification without a priory knowledge of the pathogen's sequence, which is needed for the development of innovative, knowledge-based solutions for plant production.

The overall goal of EUPHRESCO project NGS-detect is the development and adaptation of standardised NGS technologies for the detection and identification of viruses and viroids. This includes the development of standardised methods for nucleic acid preparations that are cheap, reliable and applicable to a broad range of plant material (e.g. leaves, stems, roots, fruits, etc.) and the preparation of the nucleic acids extracts for NGS (library preparation). In addition, common bioinformatic pipelines will be adapted or developed to allow reliable and quick analysis of data. Furthermore, the developed protocols will be validated by inter-laboratory comparisons and compared with standard diagnostic techniques. Preliminary results are being presented here.

EUPHRESCO P-172: NGS Proficiency testing of virus infected grapevine and fruit trees.

Michael Rott
Canadian Food Inspection Agency

During the initial meeting of participants of EUPHRESCO P-172, a decision was made to undertake a proficiency trial for the detection of viruses infecting grapevine and fruit trees using next generation sequencing. Several laboratories agreed to take part in the trial and a summary of the progress made to date will be presented.

Focus on initiatives on the use of NGS

Next Generation Sequencing in the context of the International Plant Protection Convention (IPPC)

Adriana G. Moreira
IPPC Standard Setting Officer

The International Plant Protection Convention (IPPC) is an international plant health agreement that aims to protect cultivated, wild plants and plant products by preventing the introduction and spread of pests. International Standards for Phytosanitary Measures (ISPMs) are internationally agreed upon phytosanitary measures that have been adopted by the Commission on Phytosanitary Measures (CPM), which is the governing body of the IPPC. Currently, there are 41 adopted ISPMs and 56 annexes (including 24 diagnostic protocols (DPs) for specific pests).

The ability to detect a plant pest varies with the quality and specificity of the detection tools. Next generation sequence (NGS) technologies have provided a very powerful alternative for detection and identification of organisms without a priori knowledge. However, these detections and identifications may not be associated with evidence of living pests or damage to the plant/plant products by these organisms, i.e. these technologies bring the risk of false positives that may lead to assumptions on the pathogenicity (ability to infect). Therefore, the use of highly sensitive technologies, such as NGS, for the detection and identification of plant pests and its implications is becoming a point of concern for the IPPC community.

In the IPPC adopted ISPMs portfolio, NGS technologies and the interpretation of results have strong links with ISPM 2 (Framework for pest risk analysis), ISPM 6 (Guidelines for surveillance), ISPM 8 (Determination of pest status in an area), ISPM 11 (Pest risk analysis for quarantine pests), ISPM 17 (Pest reporting), and ISPM 27 (Diagnostic protocols for regulated pests). The IPPC Technical Panel on Diagnostic Protocols (TPDP), which manages the development of the IPPC DPs annexes to ISPM 27, has discussed the use of NGS technologies as a diagnostic tool for phytosanitary purposes. The TPDP acknowledged that the proper interpretation of results is the biggest challenge in the phytosanitary context. These technologies may currently be used for screening consignments, but not to form the basis for final decisions (e.g. destruction or rejection of consignments). The TPDP also noted that not all organisms associated with plants are pests; some may be mutualists providing benefit to the host plant or commensal agents; guidance for authors of IPPC DPs on criteria for inclusion of an NGS method in IPPC protocols are being developed.

The IPPC Standards Committee (SC) noted the TPDP recommendations and stressed that the issue is broader than diagnosis – it is also relevant for pest risk analysis and surveillance and further work is needed on NGS technologies before they can be considered as the sole method for pest detection. The CPM Bureau is planning to hold a side event on NGS technologies in the phytosanitary context during the Thirteenth Session of the CPM (CPM-13, 2018). The IPPC Secretariat welcomes suggestions for this side event. The IPPC Secretariat is also interested in the outputs from this workshop in order to help inform and provide guidance for the global phytosanitary community on ways to move forward on interpreting NGS technologies results in the phytosanitary context.

The Impact of NGS in New Zealand

Bénédicte Lebas
Plant Health and Environment Laboratory, Ministry for Primary Industries, New Zealand

Rose Souza-Richards,
Germplasm Imports, Import & Export Plants, Ministry for Primary Industries, New Zealand

The Plant Health and Environment Laboratory of the Ministry for Primary Industries, New Zealand has been using next-generation sequencing for plant pest diagnostics since 2011. Examples where this technology has been successfully applied to entomology, virology, mycology and bacteriology will be presented.

The use of high-throughput sequencing for diagnostic purposes have generated questions within an ever-changing regulatory framework. Assurances that the quality of genomic data generated by this technique is critical for supporting NPPOs in decision making for regulatory purposes.

The application of NGS technology for the detection and diagnosis of non-culturable organisms – an Australian perspective.

Brendan Rodoni, Roberto Barrero, Lisa Ward, Fiona Constable, Wycliff Kinoti, Rachel Mann, Mark Whattam

AgriBio, La Trobe University, Agriculture Victoria Research, Australia

Next Generation Sequencing (NGS) is a powerful research tool for the detection and characterisation of plant viruses and viroids. NGS is being used more frequently to support plant health diagnostic laboratories in Australia. Key findings from these activities will be presented and challenges with respect to methodology and interpretation of sequence data generated will be discussed.

High Throughput Sequencing as a tool for viral pathogen diagnosis and expedited release of quarantined propagative plant material

Maher Al Rwahnih
University of California-Davis

High Throughput Sequencing (HTS), also known as next generation sequencing, provides a rapid and robust alternative approach for the identification of plant viral pathogens. Current applications of HTS include studying diseases of unknown etiology, resequencing known viruses, and determining host pathogen interactions. HTS is also used as a diagnostic tool in post entry quarantine and in the identification of known viruses. Recent studies have found HTS to be superior to conventional methods for the detection of viruses of economic significance in grapevine and fruit trees. As such, Foundation Plant Services has a new, improved import permit that allows provisional release of propagative plant material that has been HTS screened for pathogens. When HTS is used in place of the current industry methods, growers of certified and registered material will be able to initiate propagative increase and virus elimination programs with most new accessions years earlier. While HTS remains a powerful new technology with significant benefits, there are technical challenges associated with HTS. Establishing biological significance for viruses identified via HTS analysis remains an important consideration. In addition, the identification of pathogens via HTS is only as good as the databases that are used. Finally, efforts are underway to standardize HTS methodology across laboratories.

SESSION 2
Next Generation Sequencing in practice
Chair: Mr Rott Co-chair: Mr van der Vlugt

Use of NGS in diagnostics

Validating siRNA sequencing and assembly to replace classical virus indexing in root and tuber crops

Jan Kreuze
International Potato Center, Lima, Peru

The international potato center has been using NGS based detection of viruses from small RNAs since 2008 for research and surveillance purposes. We are now generating validation data for the method to replace classical, ELISA, PCR, NASH and indicator host range based routine indexing of potato and sweetpotato which are under ISO17025 accreditation. Other CGIAR genebanks have started to test the technology also for indexing cassava and yams. I will present some of our results and experiences with small RNA based virus detection to date.

NGS-Based Viral Diagnostics, Lets Confront Some Difficulties

Thierry Candresse
INRA / Université de Bordeaux, UMR 1332 Biologie du Fruit et Pathologie

In the past few years, the rapid development of high-throughput sequencing technologies (otherwise known has next generation sequencing, NGS) has impacted many research areas. In virology and, in particular, in plant virology, NGS coupled with developments in bioinformatics have dramatically changed the way virus discovery, etiology efforts or viral population analyses are performed. Among the advantages of such approaches is that they offer for the first time the theoretical possibility to perform the complete viral indexing of a plant sample without the need for any prior knowledge [1]. As a consequence of this technological progress, many new viruses have been discovered recently from a very wide range of crops. Still, NGS-based viral indexing has yet to be applied on any significant scale in routine or in official diagnostics. The price of NGS, which is still diminishing, is not a limitation for high value plant samples such as mother plants, and already compares favorably with the cost of an extensive indexing performed with classical techniques. But other pitfalls and difficulties have yet to be overcome, such as the need for validation, the need for an understanding of the differences in sensitivity or reproducibility with existing, validated techniques, the need of strategies to deal with false positives or false negatives or the need to develop standards and implement quality controls. The ability to detect novel agents for which no or very limited biological information is available also raises a number of questions, in particular on how to deal at an official level with such a discovery.

Given the huge potential advantages NGS-based indexing can bring, there is little doubt that with time they will be integrated in routine or official diagnostic protocols. Yet, similar to the situation of PCR and RT-PCR in the early 1990's, specific individual or collaborative efforts are now needed to ensure that NGS-based indexing makes as efficiently as possible the transition from research labs to diagnostic labs. Using some recent results obtained on stone fruits, grapevine and a few other crops, some of the difficulties that can be encountered when applying NGS-based indexing in a diagnostic context will be presented and discussed.

Deep sequencing for Quality Control of Real-time PCR diagnostic used for potato certification

Olivier Schumpp[1], Laurent Farinelli [2], Victor Golyaev[1], Nicolas Gonzalez[2], Patricia Otten[2], Mikhail Pooggin[3], Jean-Sébastien Reynard[1]
[1]Agroscope, CP 1012, CH-1260 Nyon, Switzerland; [2] Fasteris, CH-1228 Plan-les-Ouates, Switzerland, [3]INRA, UMR BGPI, 34398 Montpellier, France

Certification of virus-free plants is the most efficient strategy to protect cultivated plants from viral diseases. Switzerland currently uses real time quantitative RT-PCR to certify dormant seed potato tubers for the absence of six regulated RNA viruses.

However, PCR amplification is affected by mutations in virus genomes. These mutations may lead to primer/target virus mismatches possibly altering diagnostic sensitivity and leading to false negative results. Such RT-PCR failures in the detection of one or more viruses may have serious economic impact on crop production.

We developed a quality control method based on RNA sequencing using Illumina platform to secure the diagnostic procedure from PCR failures because of primer/target virus mismatches. The procedure also ensure a qualitative survey of non-regulated viruses in potatoes. Data produced in 2015, 2016 and 2017 show that the presence of one or two infected dormant tubers in a single sRNA library produced from ca. 18 000 tuber samples is sufficient for virus detection with reasonably good coverage of the virus genomes. We compared several mapping strategies for detection of single nucleotide variations in virus genome sequence and tested the influence of mismatches in primer sequence on sensitivity of real time RT-PCR.

In search for the causal agent of faba bean gall disease in herbarium samples.

Theo van der Lee
Wageningen Plant Research, Wageningen, The Netherlands

One of the extra features of NGS is that it also allows the detection of yet unknown contaminants based on even remote homology. We applied this strategy to increase our understanding and search for the causal agent for various plant diseases. This presentation will describe a recent case of the use of NGS on faba bean gall disease. We compared symptomatic and asymptomatic leaf material and identified a contaminant specific for the diseased material. Although, validating that the implied organism indeed causes the disease requires the completion Koch's postulates, NGS can help to design research strategies for isolation and management strategies.

Comparison of NGS pipelines and traditional diagnostics in annual *Daucus carota* surveys for the detection of *Ca.* Liberibacter solanacearum and *Ca.* Phytoplasma solani

Marcel Westenberg, B.T.L.H. van de Vossenberg, M. Botermans, L. Tjou-Tam-Sin, A. Roenhorst, M. Bergsma-Vlami
Dutch National Plant Protection Organization

Next Generation Sequencing (NGS) is a generic method for generating large amounts of sequence data that has many different applications in research and diagnostics. To determine if NGS can be used structurally in the NRC diagnostic process we compared traditional molecular diagnostic tests and several NGS pipelines using samples from an annual phytosanitary survey on carrot (Daucus carota). In this survey, the presence of Ca. Liberibacter solanacearum (CaLsol) and Ca. Phytoplasma solani (CaPsol) in symptomatic carrot material are determined. Since the start of the survey (2011), both quarantine organisms have not been detected and Ca. Phytoplasma asteris (CaPast) was found to be the suspected causal agent of the symptoms in the majority of cases. Methods in the traditional diagnostic process encompass conventional and real-time PCR, and Sanger Sequencing. These were compared to two NGS strategies focusing on 1. Running costs, 2. Hands-on time, 3.Turn-over time, and 4. Diagnostic power (i.e. detection of putative causal agents for the symptoms observed). NGS strategies used either DNA or RNA to generate Illumina NextSeq data which were used as input for the individual pipelines. Both pipelines consist of a reference based workflow using selected single copy orthologues shared by CaLsol, CaPsol and CaPast, and a explorative de novo assembly workflow. The first track allows specific and sensitive detection and identification of CaLsol, CaPsol and CaPast, whereas the latter allows detection of species present in the diagnostic sample. Using CaPast to compare the two diagnostic processes, identical qualitative results were obtained. The turn-over time for both diagnostic processes were similar, but running costs for traditional diagnostics were lower. However, NGS pipelines outperformed in terms of hands-on time and diagnostic power of the analyses with the detection of, among others, several carrot viruses.

NGS technology at CNR-IPSP: from de novo virus discovery to the sanitary status of plants for planting

Pasquale Saldarelli
CNR - Institute for Sustainable Plant Protection

NGS was welcomed as a “gold rush” at CNR-IPSP for the detection of virus and virus-like diseases of Mediterranean woody and horticultural crops, particularly grapevine, citrus, stone fruits and also minor fruits (quince, mulberry, fig). A major input to the adoption of this techniques relied on the propagation practices of particularly woody species, which multiply infections and spread viruses and viroids. The Institute now possesses skilled personnel and hardware facilities able to perform current NGS protocols in Plant Virology and to respond to a continuously evolving technology. Technologies followed the achievements on RNA silencing for the analysis of small RNA libraries as well as addressed mRNAs- and double stranded RNA-based libraries. Results of the last nine-year activities consisting in the discovery of several new viruses and the use of NGS data in the description of plant-virus/viroids interactions will be presented, coming to the recent application of the technology on commercial grapevine cultivars. The advantages of using NGS technology in the production of certified plant propagation material and the proposal of a future “vine metagenome passport” accompanying mother plants in commercial exchanges will be discussed.

Maize lethal necrosis in East Africa: tracking an emerging disease using NGS

Neil Boonham, Ian Adams, Adrian Fox and Julian Smith
Fera, Sand Hutton, York; Newcastle University, Newcastle upon Tyne, UK

Maize lethal necrosis is a disease caused by a synergistic interaction between Maize chlorotic mottle virus (MCMoV) and a potyvirus, in the recent outbreak in East Africa this is commonly Sugarcane mosaic virus (ScMV). Whilst not a new disease, the 2012 outbreak in Kenya was not readily diagnosed and NGS was used to identify the causal agents (Adams et al., 2012). Based on the initial diagnosis work on the Kenya strains, real-time PCR diagnostics were developed for MCMV. These are now in routine use by Kenyan Plant Health Inspectorate Services in the certification of maize seed. Subsequently we have continued sequencing samples from new locations (Adams et al., 2014; Mahuku et al., 2015) and samples with unexpected diagnostic results and have revealed information on the epidemiology of the disease as well as confusion over field diagnosis of this and other diseases of maize. We have also explored the use of nanopore sequencing using the MinIon (Oxford Nanopore) which may prove useful in resource poor settings where conventional NGS approaches are not available.

Detection of Plant Viruses by Next Generation Sequencing

Maja Ravnikar, Nataša Mehle, Ion Gutierrez Aguirre, Anja Pecman, Ian Adams, Adrian Fox, Neil Boonham, Denis Kutnjak

National Institute of Biology, SI (maja.ravnikar@nib.si);

NGS was introduced at NIB as offical diagnostic process for symptomatic samples for which identification by classical screening methods failed. Different metodological approcahes was tested in viral nucleic acid extraction step as well as in data analysis. The comparison of different viral nucleic acid inputs (sequencing of small RNA (sRNA) and sequencing of ribosomal RNA (rRNA) depleted total RNA) for detection and identification of several plant viruses, which differ in genome organization, and viroids from both known families, showed that both approaches can be used for detection and identification of a wide array of known plant viruses/viroids in the tested samples. An user-friendly bioinformatic pipeline in CLC Genomics Workbench for identification of viruses from small RNA deep sequencing data was developed at NIB. With NGS analyses of ornamental and vegetable samples, we discovered known and new plant viruses in Slovenia. Tomato was shown to be a new host of Henbane mosaic virus, which cause severe symptoms. NGS allows also more effective determination of unknown (still to be described) viral nucleotide sequences.

In the presentation, we will outline the significance and new challenges after the introduction of NGS in the diagnosis of plant viruses.

SESSION 4
Harmonized guidelines on the use of NGS in diagnostics
Chair: Ms Petter Co-chair: Mr Giovanni

Framework for the evaluation of biosecurity, commercial, regulatory and scientific impacts of plant viruses and viroids identified by NGS technologies.

Thierry Wetzel, Sebastien Massart, Thierry Candresse, José Gil, Christophe Lacomme, Lukas Predajna, Maja Ravnikar, Jean-Sébastien Reynard, Artemis Rombou, Pasquale Saldarelli, Dijana Škorić, Eeva Vainio, Jari P.T. Valkonen, Hervé Vanderschuren, Christina Varveri
DLR Rheinpfalz, Institute of Plant Protection

Recent advances in high-throughput sequencing technologies and bioinformatics have generated new opportunities for the discovery and diagnostics of viroids and viral pathogens, and previously uncharacterized human, animal and plant viruses are described monthly in the scientific literature. Plant virology has undoubtedly benefited from these viral discoveries, but at the same time it encounters an important bottleneck: the biological characterization of the newly discovered viruses and the analysis of their impact at biosecurity, commercial, regulatory and scientific levels. Considering existing frameworks for known viruses, our opinion paper proposes a scaled and progressive scientific framework for an efficient biological characterization and risk evaluation when a new plant virus is detected by next generation sequencing (NGS) technologies. We present case studies illustrating the need for such a framework, and discuss the proposed scenarios.

Introduction to EPPO Standards

Françoise Petter, Madeleine McMullen& Baldissera Giovani
EPPO

EPPO is one of the Regional Plant Protection Organizations recognized under the International Plant Protection Convention. One of its functions is to develop Regional Standards. EPPO has a long standing and active program for Standard setting in several areas, including diagnostics. At the request of its members, EPPO started a program to prepare diagnostic Standards for regulated pests in 1998, in order to achieve a harmonized approach to detection and identification for regulated pests. The work is conducted by the Panel on Diagnostics and Quality Assurance in collaboration with specialized Panels (Diagnostics in Bacteriology, Entomology, Mycology, Nematology, and Virology and Phytoplasmology). Panels are composed of specialists from member countries, proposed by their respective NPPOs. As of November 2017, 131 Pest-specific diagnostic protocols and horizontal diagnostic Standards have been developed. Pest-specific protocols are written according to a “common format and content”. Horizontal Standards do not have a pre-defined format. The first drafts of Standards are prepared by an assigned expert author(s) or by a drafting team and are reviewed by the relevant Panels and the Working Party on Phytosanitary Regulations.

Diagnostic Standards are approved following the regular EPPO Standards approval procedure and revisions of pest specific Diagnostic Protocols are approved following a fast track procedure. Both procedures involve a formal consultation of all EPPO Member countries.

ABSTRACTS POSTERS

Spread of Citrus *Tristeza* Virus in Greece and Characterisation of Identified Isolates with Classical and Modern Tecnologies (NGS)

Beri D, Kektsidou O & Varveri C (PDF of poster available)

Virtool: Software for Detection of Plant Virus Using Next Generation Sequencing

Boyes I & Rott M (PDF of poster available)

Fast Dissection of Viral Infection by Minion Sequencer

Calassanzio M, Prodi A & Ratti C (PDF of poster available)

MinION consists of a chip with incorporated nanopores that measure the changes in electrical conductivity generated by the passage of DNA strands through a biological pore. The current in the nanopore is measured by a sensor several thousand times per second, and the data streams are passed to a microchip called the Application-Specific Integrated Circuit (ASIC). Data processing is carried out by the MinKNOW software, which deals with data acquisition and analysis. With MinION genomic fragments can be sequenced up to 50kb with single-strand read accuracy better than 92%. The aim of this work was to use the MinION device to identify all viruses infecting plant samples and filamentous fungi. DNA libraries were generated from dsRNA, total RNA or TNA from purified virions from tobacco, rose, tomato, ficus, cabbage or F. culmorum strains and subject to analysis in pools of 10-15 samples. The reads generated by short runs (2 hours) using the developed protocols range from 100 to 4000 bp in size with the main distribution around 400 bp. BLAST analysis of the assembled contigs provided sufficient information to identify various mycoviruses and phytoviruses in the analyzed samples. Nanopore DNA strand sequencing has proved to satisfy our purpose offering a universal device that could allow the rapidly identification of known and unknown pathogens that could substitute complex and time consuming traditional approaches.

Next Generation Sequencing for virus discovery and large-scale virus screening in the cassava collection at CIAT's genebank

Carvajal-Yepes M, Cuervo M, Olaya1 C, Martínez A, Lozano I, Niño D, Aranzales E, Debouck D, Wenzl P, Cuellar W & Tohme J (PDF of poster available)

Analyzing the virome of plants using next generation sequencing (NGS) has accelerated the identification of viruses associated with disease that could not be isolated otherwise. Deep sequencing of small interfering RNAs (siRNAs) is used to diagnose and identify viruses in different crops showing disease symptoms, such as cassava, bean, rice and papaya. By using this approach, novel viruses infecting cassava fields in Colombia, were discovered and reported from plants with Cassava Frogskin disease (CFSD) symptoms (Carvajal-Yepes et al. 2014). CFSD is a quarantine disease that causes significant yield losses. The Genetic Resource Program (GRP) at the International Center for Tropical Agriculture (CIAT) conserves the world's largest and most diverse collection of cassava (Manihot esculenta). The health certification of the cassava collection material is done by the Germplasm Health Laboratory (GHL), ensuring the safety distribution of these materials. Following discoveries in cassava with NGS, in addition to screening other viruses such as: Cassava virus X (CsXV), Cassava common mosaic virus (CsCMV) and Cassava vein mosaic virus (CVMV), it was necessary to implement and standardize methods for the detection of Cassava torrado-like virus (CsTLV), Cassava polero-like virus (CsPLV), Cassava frogskin-associated virus (CsFSaV) and Cassava new alphaflexivirus (CsNAV) in the large-scale screening workflow. Up to date, 57% of the in vitro cassava collection has been evaluated. The use of NGS for the discovery of important quarantine pathogens is foreseen and will contribute to the safety distribution of germplasm around the world."

NGS technologies for plant pests diagnostics in Romania: where we are and what we aim?

Ciceoi R & Bădulescu L (PDF of poster available)

Summary: In Romania, given to some EU projects, as DISCO, th use of NGS for production of natural bioactive compounds from plants have been discussed (http://disco-fp7.eu/). For plant protection and plant pest diagnostics, few initiatives have been taken. An overview of the curent status of these actions is presented.

Unravelling the Little Cherry disease complex at European scale to improve transnational diagnostics and management of the disease

Kris De Jonghe (PDF of poster available)

LChV1 and LChV2 have been identified as the causal agents of the little cherry disease (Eastwell and Li, 1994; Jelkmann & Eastwell, 2011). During surveys, various organs from symptomatic trees (root, leaves, bark) are sampled in order to confirm the presence of one of these two viruses through current molecular detection technologies. Nevertheless, none of these two viruses have been detected from 102 out of 321 trees (31.8 %) presenting little cherry disease symptoms during the surveys made by ILVO and partners during a national survey project. Three main hypotheses can explain the absence of detection from these assumed infected trees, based on symptom formation: (1) presence of another known or unknown virus causing the disease, (2) divergent strain of the LChV1 or LChV2 not recognized by the molecular tools, or (3) level of viruses below the limit of detection. The last hypothesis has been discarded as the trees showed diseases and were sampled at several organs, including symptomatic ones, following best practices and therefore limiting the risk of missing the virus. The objective of the study is therefore to elucidate the two main hypotheses by applying high throughput sequencing (also called Next Generation Sequencing or NGS) technologies. NGS technologies became available at the onset of the 21st century. They provide a highly efficient, rapid, and low cost DNA sequencing platform beyond the reach of the standard and traditional DNA sequencing technologies developed in the late 1970s. NGS technologies have therefore the ability to detect and characterize by a holistic approach the presence of any virus in a plant sample, including distant variants of known viruses or of unknown or novel agents infecting any plant sample and potentially even in vectors or various substrates. It should be stressed that no other technique currently has the potential to deliver such broad-spectrum diagnostics. Ten samples (trees) are included in this etiology effort. Whenever possible, RNA extracted from various plant organs are pooled in a single sample for each tree. The study is carried out within the framework of the EUPHRESCO EURAVELCH project and will continue for the coming 2 years. The poster will present the first preliminary results.

Benefits of Integrative Methods on Analysis of Low Coverage NGS Data

Dudic D (PDF of poster available)

Introduction: With repetitive nature of some genomes, a high number of multi-mapped reads and high duplication rate need to be addressed. This issue is even more critical for low-coverage NGS data, when the need to get as much as possible information arises. We tested different bioinformatics tools on low-coverage maize total transcriptome data, setting an optimal integrative approach to achieve the lowest duplicate reads rates with the highest percentage of reads mapped to exon regions. Material and Methods: 151bp pair end RNA-seq low coverage data for one maize inbred line adequately preprocessed according to exhaustive QC evaluation; Three splice aware mapping tools: STAR, Subread, Tophat (equalized parameters set, insert size=130, standard deviation=50, and maximum intron size=100.000) and three assembly tools: Oases, TransAbySS, Trinity (kmer sizes 15-31, minimum contig length 30) were tested.

Data processing was conducted in four ways: mapping (M), assembling followed by mapping (AM), assembling with repetitive sequences followed by mapping (ARM), and mapping followed by assembling followed by mapping (MAM).

Results: Mapping tools results varied: for mapping 95.4-99.1%, for duplicate rates 54.5-57.2%, for unique reads 19.4-23.4%, for reads assigned to exons 46.6-68.6%/introns 7.6-22%, with STAR giving the best results. Assembly tools revealed total lengths from 6157777 to 15565235 bp, average contig length from 50.3 to 221.6, with Trinity

for kmer 25 giving the best results. Integrative methods processing outputs: for mapping 98.9-99.7%, for duplicate rates 3.3-15.4%, for unique reads 75.9-91%, for reads assigned to exons 46.6-66.4%/introns 18.9-38%, with ARM giving the best results.
Conclusions: For low coverage NGS data derived from repetitive genomes, we advise using integrative methods like AM, ARM and MAM, depending on level of repetitiveness of sequenced data.

Full Genome characterisation of 10 Tospoviruses By Next Generation Sequencing

Dullemans AM, van Bekkum PJ, Roenhorst AJW, Kormelink R & van der Vlugt RAA (PDF of poster available)

BACKGROUND and OBJECTIVES
The genus Tospovirus currently contains 11 officially recognized species (ICTV, 2015) however, more tentative species have been described. For most tospoviruses no specific antisera are available and only partial sequence data have been described. This hampers reliable detection of these agronomical important viruses. To facilitate the development of diagnostics and ensure the future availability of reference isolates the full RNA genomes of 10 different tospoviruses were determined, their sequences and biological data included in Q-bank and the individual isolates included in the physical plant virus collection at Wageningen UR.
MATERIALS and METHODS
Isolates of the different tospoviruses were obtained from plant virus collections from the Dutch NPPO and Wageningen UR and inoculated on indicator plants. Total RNA extracts were used for Illumina RiboZero 125 base paired-ends library preparations with individual MIDs and subsequently run in batch on a HiSeq 2500. After MID splitting individual datasets were fed into custom designed workflows within CLC Genomics Workbench (Qiagen, Denmark). These workflows comprised 'De novo' and reference-assemblies with and without substraction of plant-related reads. Resulting contigs were analysed by BlastN and BlastX against the NCBI database to identify tospovirus related sequences. Primer walking and 5'- and 3'-RACE and Sanger sequencing was used to determine the complete genomes.
RESULTS
Most assemblies of the individual tospovirus datasets resulted in near full length RNA segments (L, M and S). A few RNAs were split in two contigs most likely by poor assembly of the hairpin regions. Primer walking and conventional Sanger sequencing resulted in linking contigs enabling assemblies of full length RNA sequences. Full sequences and additional information on the tospoviruses will be made publically available through Q-bank (www.q-bank.eu/virus).
CONCLUSIONS
NGS proofed to be a quick and relatively easy method to determine the complete sequences of a significant number of tospoviruses simultaneously. These sequences can now be used for the development of diagnostics and the identification of viral sequences obtained from plant samples through the virus ID function in Q-bank/virus.
REFERENCES
ICTV 2015, Master species list V4: http://talk.ictvonline.org/files/ictv_documents/m/msl/5208.aspx

High-throughput sequencing for discovery & genome assembly of plant viruses at the DSMZ Plant Virus Department

Knierim D, Margaria P, Menzel W & Winter S (PDF of poster available)

Effect of template enrichment for RNA-Seq library preparation: case study of multiple viral infection in red clover

Koloniuk I, Fránová J, Sarkisova T, Přibylová, Petrzik K, Lenz O & Špak J (PDF of poster available)

Viruses and viroids of grapevines revealed by NGS in Czech Republic

Kominek P, Komínková M, Eichmeie A & Baránek M (PDF of poster available)

Using next generation sequencing, whole virome of grapevines selected from the territory of the Czech Republic was evaluated. Using this approach, Grapevine rupestris vein feathering virus and Grapevine yellow speckle viroid 1 were recorded in the Czech Republic for the first time.

Identification of Nectarine stem pitting-associated virus infecting *Prunus persica* by Next Generation Sequencing in Hungary

Krizbai L, Kriston E, Kreuze J & Melika G (PDF of poster available)

Detection of plant viruses by next-generation sequencing (NGS)

Mehle N, Kutnjak D, Pecman A & Ravnikar M (PDF of poster available)

Diagnosis of Viruses and Viroids via Next Generation Sequencing in Local Grapevine, Turkey

Önder S, Gökçe ZNÖ & Serçe ÇU (PDF of poster available)

Over 65 viruses and viroids have been recorded until now in grapevine. Reliable and accurate detection all of those agents one by one via serological and/or PCR based methods in each sample is very difficult. The Next Generation Sequencing (NGS) technologies allows the recovery of hundreds of thousand sequences from total RNA of infected plants that can derive from a multiplicity of viruses and viroids present in grapevine simultaneously. NGS platforms were used to identify viruses and viroids from a grapevine variety Trakya Ilkeren showing severe mosaic symptoms in Bursa province of Turkey in 2015. Total RNA was extracted and rRNA depletion was performed by treatment of Ribo-Zero rRNA Plant Removal Kit. NEBNext? Ultra??? RNA Library Prep Kit was used for library preparation. Deep sequencing was performed using Illumina Hiseq2000 RNAseq technology with 2x150 read length and 40 million depths for each reads from Admera Health (USA) laboratories. In totally, deep sequencing analysis yielded around 150 million sequences. For bioinformatic analyses, Geneious R10 software was used. After de novo assembly, 28.863 contigs were obtained with Tadpole assembler and all obtained contigs were analyzed by blastn against NCBI viral database to detect known viruses and viroids. After then, genome mapping was performed against reference sequences one by one with all reads. At the end of the genome mapping results, reference genomes for Grapevine yellow speckle viroid-1 (GYSVd-1) (100 %), Grapevine deformation virus (GDefV) RNA2 segment (29 %), Grapevine Syrah virus-1 (GSYV-1) (20.2 %), Grapevine fanleaf virus (GFLV) RNA1 segment (4.4 %), GFLV RNA2 segment (3.9 %), GDefV RNA1 segment (3.8 %), Arabis mosaic virus (ArMV) RNA2 segment (2.7 %), Grapevine asteroid mosaic associated virus (GAMaV) (1.9 %), Grapevine rupestrist stem pitting associated virus (GRSPaV) (1.6 %) and Grapevine leafroll-associated virus-1 (GLRaV-1) (0.7 %) were recovered with indicated percentage. These results showed that recovering full genome for all of detected viruses and viroids with RNASeq is very complicated. In addition to this, to our knowledge GAMaV is the first report for Turkey grapevine production areas, which needs to be confirmed by other identification tools.

Acknowledgement: This study supported by the project TUBITAK-115O014 and COST-DIVAS of COST FA-1407 Action.

Deep Sequencing of a Local Grapevine Variety in Turkey for Diagnosis of Viruses and Viroids via Next Generation Sequencing Techniques

Önder S, Gökçe ZNÖ & Serçe ÇU (PDF of poster available)

How many, virus positive, NGS reads is enough? *Sambucus* case.

Safarova D & Navrati M (no PDF of poster available)

Next generation sequencing represents the intensive developing approach used for the both, detection of the new viruses and screening of known viruses in plant material. The high sensitivity of all platforms applied and relative

high risk of sample contamination is raising the question of the real positivity of analysed samples, i.e. detection of virus presence and existence of some border allowing discrimination of true and false positive results.
The effectivity of ssRNA viruses detection using the next generation sequencing of double-strand RNA by Illumina platform and will be shown in the real case of the wild growing elderberry trees. Strategies of (i) contigs de novo assembling using CLC Genomics Workbench and Geneious software, (ii) the reads mapping against the full genomic sequence, and the results comparison as well as confirmation (iii) by standard RT-PCR detection will be shown.
This work is supported by the MEYS CZ project COST LD15048 and COST Action FA1407-DIVAS.

Identification and characterization of Phytophthora hybrids using genotyping-by-sequencing

Van Poucke K, Haegeman A, Goedefroit T, Ruttink T & Heungens K (PDF of poster available)

About twenty years ago, several hybrids belonging to different phylogenetic clades of the oomycete Phytophthora were discovered. Species which are not occurring together naturally are brought in contact with each other as a result of intensified international plant trade .It is suspected that this leads to an increase in the number of hybridization events, which is potentially dangerous since hybrids might be more aggressive or have an expanded host range in comparison to the parental species. Early detection of new hybrids is however not always successful via traditional screening methods. Therefore, we developed a method based on genotyping-by-sequencing (GBS) to reliably characterize Phytophthora isolates and to identify potential new hybrids.
GBS is a technique to generate genome-wide sets of molecular markers. Briefly, DNA was extracted from a collection of hundreds of European isolates and digested using two restriction enzymes. Indexed adapters were ligated to the resulting fragments which were then amplified, purified and sequenced using Illumina technology (HiSeq 3000). Read data was processed using a reference-free approach to identify a global set of GBS tags across all samples. Subsequently, per sample the absence/presence profiles were scored together with SNP profiles for each GBS tag. The method was highly reproducible and generated about 15000 to 30000 tags for a non-hybrid species. When isolates share a large number of GBS tags with at least two Phytophthora species, this is indicative of a hybridization event. Using this approach, we were able to detect a previously unidentified hybrid. Moreover, many isolates showed a large number of GBS tags, which implies that possible historic hybridization events might have been more common in the genus Phytophthora than previously assumed.

Two novel virus-like sequences from grapevine

Vončina D & Alemida RPP (PDF of poster available)

The etiology of watermelon "hard fruit syndrome": an NGS approach

T Zaagueri, L Miozzi, M Mnari-Hattab, E Noris, GP Accotto & AM Vaira (PDF of poster available)

Workshop on the use of NGS technologies for plant pest diagnostics.

22nd and 23rd of November 2017

Hotel Excelsior, Via Giulio Petroni 15, 70124 Bari, Italy

Hands on session Thursday 23rd November 2017 – Bari
09.00-13.00

General introduction

During the NGS Workshop, 3 parallel hands-on NGS sessions will be organized. The topics of these sessions have been identified by the Organizing Committee and are related to the expertise declared when registering to the meeting.
Please note that the committee has finally decided to allow more participants than originally scheduled however, the number of participants to the hands-on sessions could not be increased and some participants will only be able to attend the plenary parts of the meeting.

Group 1 experts with no expertise on NGS and bioinformatic tools
This group will be asked to consider the regulatory consequences of a detection of a new virus or a new host using NGS. This is essential to be aware of the processes to be followed.

Group 2 experts with some expertise on NGS and bioinformatic tools
Participants will be provided a first overview on bioinformatic analysis of NGS sequence data for virus detection.

Group 3 experts with expertise on NGS and bioinformatic tools
This group will conduct a teamwork on Quality issues.

A description of the different group is provided in this document.

GROUP 1
Hands-onsession for experts with no expertise on NGS and bioinformatic tools

Presenters for the case studies: Mr Neil Boonham (Fera Science Limited, United Kingdom); Mr Thierry Candresse (INRA, France); Mr Michael Rott (CFIA, Canada); Mr Thierry Wetzel (DLR Rheinpfalz, Germany);

Facilitators in addition to trainers: Mr Baldissera Giovani (OEPP/EPPO); Ms Françoise Petter (OEPP/EPPO)

Objective:
The session 1 is designed for participant with no or limited experience in molecular biology and will be focused on the regulatory consequences of the discovery of a new virus or a new host in two study cases. Each study case will be presented in detailto the participant: virus discovered, family of the virus, context of detection (quarantine, certification or interception, symptoms (or not) on the plant, countries involved).

Organisation:
Five groups of 10 participants will independently work on each of these study cases. These groups will act as the plant health regulatory authorities.
The groups will need to define the most important steps and information requested for appropriate Pest Risk Assessment and in order to take a decision. Two and a half hours will be dedicated to group discussion and each group will report its conclusions during the last hour (5 minutes per case).

Indicative timing
09.00-10.30 Presentations of the case studies.
10.30-11.00 Coffee break
11.00-13.00 Work in small groups on the different case studies

GROUP 2

Hands-on session for experts with some expertise on NGS and bioinformatic tools

Session organizer: Sébastien Massart (University of Liège, Belgium)
Trainers: Ms Michela Chiumenti (CNR, Italy); Ms Dragana Dudic (Center for Data Mining and Bioinformatics, Serbia) ; Mr Sebastien Massart (University of Liège, Belgium) ; Ms Laura Miozzi (Istituto per la protezionesostenibiledellepiante, Italy) ; Ms Noa Sela (Agricultural Research Organization, Israel)

Objective:
The objective of the session is to provide to the participants a first overview on bioinformatic analysis of NGS sequence data for virus detection. The session is organized through theoretical lessons and practical applications.

Material and background requested:
The participants must bring their own personal computer and must have installed the Geneious software (information will be provided in due time for downloading and activating). The participants must have a good knowledge of molecular biology and virology.

Practical NGS data analysis:
Three sets of data will be available as fastq files. They correspond each to 50,000 sequences from smallRNA. At least one of them will be analysed in real-time by the participants on their own computer using the downloaded software with the support of the trainers.

Organisation:
9:00-9:45: general introduction to NGS data analysis: steps involved, key factors, use of the software
9:45-10:30: first steps of bioinformatic analysis on the first set of data
11:00-12:15: bioinformatic analysis of the datasets
12:15-13:00: summary of the activity (virus detected by the participants), conclusion and feedback/exchange with the participants

GROUP 3
Hands-on session for experts with expertise on NGS and bioinformatic tools

Session organizer: Maja Ravnikar (National Institute of Biology, Slovenia)
Trainers: Mr Jan Kreuze (International Potato Center, Peru); Mr Denis Kutnjak (National Institute of Biology, Slovenia); Ms Maja Ravnikar (National Institute of Biology, Slovenia); Mr René Van der Vlugt (Wageningen Plant Research, Netherlands)

Objective: Teamwork on Quality standards
The sessionwill include participants with a high expertise in NGS technologies for virus diagnostic. These participants will be distributed in 5 groups. There will be 5 hot questions related to validation, quality control and quality assurance needed for diagnostic use of NGS (and the development of an EPPO standard on NGS). Each group will have 30 minutes to answer each question through exchanges and interactions. After 30 minutes, the group will address another question.

Questions to consider
Q1 What type of controls are needed to ensure the reliability of NGS results including when the laboratory is outsourcing some parts of the process?
Q2 How to validate an NGS protocol?
Q3 Which depth of sequencing do you require to achieve acceptable sensitivity?
Q4 What are the criteria to report the presence of a virus from NGS data?
Q5 What can be done to minimize cross contamination?

Technique used: World Café

Some explanation on the World Café
A World Café or Knowledge Café is a structured conversational process in which groups of people discuss a topic at several tables, with individuals switching tables periodically and getting introduced to the previous discussion at their new table by a "table host". A café ambience is created in order to facilitate conversation. In some versions, a degree of formality is retained to make sure that everyone gets a chance to speak. Alternatively, the café concept can be taken more literally with everyone potentially talking at once. As well as speaking and listening, individuals may be encouraged to write or doodle on the tablecloth so that when people change tables, they can see what previous members have written as well as hearing the table host's view of what has been happening.

Workshop on the use of NGS technologies for plant pest diagnostics.

22nd and 23rd of November 2017

Hotel Excelsior, Via Giulio Petroni 15, 70124 Bari, Italy

Background information for the brainstorming session on the EPPO Standard on NGS
23rd of November 14.30-17.45

Background on EPPO Diagnostic activities and the context

One of the main roles of the European and Mediterranean Plant Protection Organization (EPPO) is to help its member countries to prevent entry or spread of dangerous pests. In this framework, one of the activities that EPPO is conducting to support its members is to develop diagnostic standards (both pest specific and horizontal standards). EPPO is consequently following development of new technologies for diagnostics which are then discussed in EPPO diagnostic Panels.

EPPO Diagnostic activities are placed in a context of **official plant pest diagnostic**, i.e. diagnostics performed by laboratories in the framework of official controls (inspections performed for imported or exported commodities, surveillance on the territory). Detections based on NGS technologies may have significant implications and there is a need for guidelines on procedures for the use of NGS technologies for pest detection in a regulatory framework. The EPPO Council has agreed to include in the EPPO's Work programme the preparation of a Standard on NGS.

Objectives of the brainstorming session

The brainstorming session organized during the Workshop on NGS is intended to identify the needs of laboratories to determine the main scope and content of such Standard. The EPPO Secretariat has identified possible sections for such Standards which are presented below and can be used to structure the discussion.

1. Scope
2. Sampling and sample preparation (technical guidelines and quality assurance)
3. Library preparation (technical guidelines and quality assurance)
4. Sequencing (quality assurance)
5. Bioinformatics pipeline (quality assurance)
6. Validation
7. Reporting (in particular, communication to risk managers)

For each of the 7 points, the groups should discuss:

- To which level of technical details should the Standard go?
- How to organise the technical parts that are needed?

As an increasing number of laboratories operate under accreditation in the EPPO region, it is also important to discuss how ISO 17025 accreditation of NGS results can achieved?

Participants will be split in medium size groups (max 20) to promote interaction and each group will report to the Plenary (a rapporteur will need to be identified for each group). The outcomes will be further considered in the EPPO framework and the formation of a specific expert working group to develop this Standard will be considered. Potential members can be identified already during the workshop.

Leaders for the sessions will be identified in advance of the meeting

Workshop on the use of NGS technologies for plant pest diagnostics

Acknowledgements:

Trainers:

Neil Boonham
Thierry Candresse
Michela Chiumenti
Dragana Dudic
Baldissera Giovani
Jan Kreuze
Denis Kutnjak
Sebastien Massart
Laura Miozzi
Françoise Petter
Maja Ravnikar
Michael Rott
Noa Sela
René Van Der Vlugt
Thierry Wetzel

Local organizers:

Angelantonio Minafra
Michela Chiumenti
Annalisa Giampetruzzi
Francesco Palmisano
Vitantonio Pantaleo
Pasquale Saldarelli
Maria Saponari
Luciana Savino

The EPPO Secretariat would like to express its gratitude to the trainers and local organizers for their support for this Workshop

Workshop 2017

EPPO diagnostic activities:
Serving the needs of plant pest diagnostic laboratories

What is EPPO?

The European and Mediterranean Plant Protection Organization (EPPO) was created in 1951 to prevent the introduction of dangerous pests from other parts of the world, and limit their spread within Europe if they were introduced. EPPO is one of the Regional Plant Protection Organizations recognized under the International Plant Protection Convention. Today, 51 European and Mediterranean countries (including the 28 members of the European Union) are members of the Organization. Key partners of EPPO are National Plant Protection Organizations (NPPOs), i.e. the official services which are responsible for plant protection in each country. The results of EPPO's work are recommendations officially approved by EPPO's Council where all countries are represented. These recommendations are internationally considered as 'regional standards'.

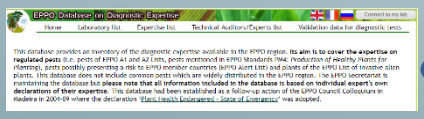
Development of Diagnostic Standards

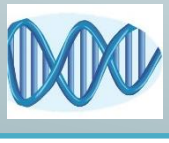
EPPO has a long standing and active program for Standard setting in several areas, including diagnostics. At the request of its members, EPPO started a program to prepare diagnostic Standards for regulated pests in 1998, in order to achieve a harmonized approach to detection and identification for regulated pests. The work is conducted by the Panel on Diagnostics and Quality Assurance in collaboration with specialized Panels (Diagnostics in Bacteriology, Entomology, Mycology, Nematology, and Virology and Phytoplasmology). Panels are composed of specialists from member countries, proposed by their respective NPPOs. As of November 2017, 131 Pest-specific diagnostic protocols and horizontal diagnostic Standards have been developed. Pest-specific protocols are written according to a "common format and content". Horizontal Standards do not have a pre-defined format. The first drafts of Standards are prepared by an assigned expert author(s) or by a drafting team and are reviewed by the relevant Panels and the Working Party on Phytosanitary Regulations. Diagnostic Standards are approved following the regular EPPO Standards approval procedure and revisions of pest specific Diagnostic Protocols are approved following a fast track procedure. Both procedures involve a formal consultation of all EPPO Member countries.

https://gd.eppo.int/standards/PM7/

Number of approved standards per discipline

Bacterium – 25 standards
Fungi – 22 standards
Insects – 39 standards
Nematodes – 12 standards
Phytoplasmas – 4 standards
Viruses – 15 standards
Horizontal standards – 8 standards





Laboratory

EPPO databases and information services

- EPPO database on diagnostic expertise (inventory of the diagnostic expertise available in the EPPO region & validation data for tests). http://dc.eppo.int/index.php
- EPPO Global Database (database on quarantine pests which provides data on: all the pests of the EPPO A1 and A2 lists and of EU Directive 2000/29; pests of the EPPO Alert List; plants of the EPPO List of invasive alien plants many other quarantine pests and invasive plants of interest to other regions of the world) https://gd.eppo.int/
- EPPO Alert List and Reporting Service (Information on emerging pests and on events of phytosanitary concern, including new diagnostic tools). https://www.eppo.int/QUARANTINE/Alert_List/alert_list.htm

EPPO programme on accreditation and quality assurance

Guidelines on quality assurance have been developed:

- PM 7/84 Basic requirements for quality management in plant pest diagnostic laboratories
- PM 7/98 Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity (revised in 2014) including specific quality management requirements for laboratories preparing for accreditation according to the ISO/ IEC Standard 17025

Other guidelines:

Guidelines for the organization of interlaboratory comparisons by plant pest diagnostic laboratories. Guidelines on the authorization of laboratories to perform diagnostic activities for regulated pests. Guidelines on the main tasks of Reference Laboratories for official plant pest diagnostics.

Conferences and workshops on diagnostics

Since 1985, EPPO has organized a series of conferences and workshops on Diagnostics:

- Conferences on diagnostics (1985, 1994 & 2000,NL; 2009, GB; 2012, NL; 2015, FR)
- Workshops on Quality Assurance (2007, DK, 2009 GB, 2014 GB)
- Workshop for heads of laboratories (AT 2011, TN 2013, IT 2015)
- Workshop on Flexible scope (NL, 2017)

Workshop on Quality Assurance - 2014 in York (GB)

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
21 Bd Richard Lenoir, 75011 Paris - www.eppo.int

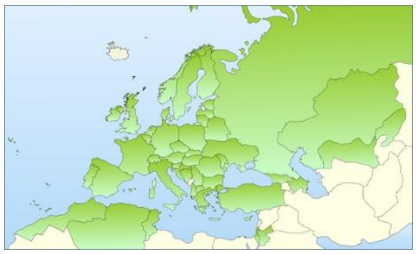
Françoise Petter (Assistant Director ; petter@eppo.int), Madeleine McMullen (Managing Editor; mm@eppo.int), Yury Shneyder (All Russian Plant Quarantine Center)

The European and Mediterranean Plant Protection Organization

What is EPPO?

EPPO is an intergovernmental organization responsible for international cooperation in plant protection in the European and Mediterranean region. In the sense of the article IX of the FAO International Plant Protection Convention (IPPC), it is the Regional Plant Protection Organization for Europe.

Founded in 1951 with 15 member governments, it now has 51 member governments including nearly every country of Western and Eastern Europe and the Mediterranean region (in green on the map).



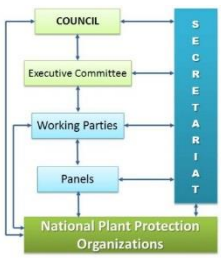
Aims of EPPO

EPPO has two main areas of activity: plant quarantine and pest control. Its main aims are:

- To protect plant health in agriculture, forestry and the uncultivated environment.
- To develop an international strategy against the introduction and spread of pests (including invasive alien plants) that damage cultivated and wild plants, in natural and agricultural ecosystems.
- To encourage harmonization of phytosanitary regulations and all other areas of official plant protection action.
- To promote the use of modern, safe, and effective pest control methods.
- To provide a documentation service on plant protection.

EPPO Structure

The Organization is administered by its Council (representatives of all member governments) and Executive Committee (7 governments elected on a rotational basis). A Secretariat of 14 persons is based in Paris. EPPO is financed directly by annual contributions from its member governments. Its official languages are English, French and for certain purposes Russian. The technical work is overseen by the Working Parties and Panels (experts are nominated by their National Plant Protection Organization).



EPPO Council Session

EPPO Standards

As a result of the work being done within the different technical bodies, EPPO makes recommendations to the National Plant Protection Organizations of its member governments. These recommendations are considered as Regional Standards in the sense of the IPPC.

Standards on plant protection products:

- Efficacy evaluation of plant protection products
- Good plant protection practice
- Environmental risk assessment of plant protection products

Standards on phytosanitary measures:

- General phytosanitary measures
- Phytosanitary procedures
- Production of healthy plants for planting
- Pest Risk Analysis (PRA)
- Safe use of biological control
- Diagnostic protocols
- Commodity-specific phytosanitary measures
- National regulatory control systems
- Phytosanitary treatments

EPPO information services

EPPO provides many information services to its members, most of them are freely available from the EPPO website:

www.eppo.int

Examples of EPPO publications and databases:

- EPPO Bulletin (official journal of the Organization)
- EPPO Global Database / PQR (the EPPO database on quarantine pests)
- Pest Risk Analyses and CAPRA software
- EPPO database on diagnostic expertise
- EPPO Reporting Service (monthly newsletter)
- Datasheets on quarantine pests and pictures
- EPPO Alert List …

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
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Anne-Sophie Roy (EPPO Information Officer, roy@eppo.int)

The European and Mediterranean Plant Protection Organization

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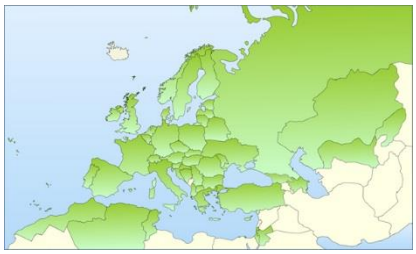
EPPO is an intergovernmental organization responsible for international cooperation in plant protection in the European and Mediterranean region. In the sense of the article IX of the FAO International Plant Protection Convention (IPPC), it is the Regional Plant Protection Organization for Europe.

Founded in 1951 with 15 member governments, it now has 51 member governments including nearly every country of Western and Eastern Europe and the Mediterranean region (in green on the map).



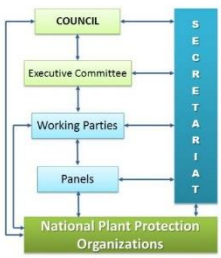
Aims of EPPO

EPPO has two main areas of activity: plant quarantine and pest control. Its main aims are:

- To protect plant health in agriculture, forestry and the uncultivated environment.
- To develop an international strategy against the introduction and spread of pests (including invasive alien plants) that damage cultivated and wild plants, in natural and agricultural ecosystems.
- To encourage harmonization of phytosanitary regulations and all other areas of official plant protection action.
- To promote the use of modern, safe, and effective pest control methods.
- To provide a documentation service on plant protection.

EPPO Structure

The Organization is administered by its Council (representatives of all member governments) and Executive Committee (7 governments elected on a rotational basis). A Secretariat of 14 persons is based in Paris. EPPO is financed directly by annual contributions from its member governments. Its official languages are English, French and for certain purposes Russian. The technical work is overseen by the Working Parties and Panels (experts are nominated by their National Plant Protection Organization).



EPPO Council Session

EPPO Standards

As a result of the work being done within the different technical bodies, EPPO makes recommendations to the National Plant Protection Organizations of its member governments. These recommendations are considered as Regional Standards in the sense of the IPPC.

Standards on plant protection products:

- Efficacy evaluation of plant protection products
- Good plant protection practice
- Environmental risk assessment of plant protection products

Standards on phytosanitary measures:

- General phytosanitary measures
- Phytosanitary procedures
- Production of healthy plants for planting
- Pest Risk Analysis (PRA)
- Safe use of biological control
- Diagnostic protocols
- Commodity-specific phytosanitary measures
- National regulatory control systems
- Phytosanitary treatments

EPPO information services

EPPO provides many information services to its members, most of them are freely available from the EPPO website:

www.eppo.int

Examples of EPPO publications and databases:

- EPPO Bulletin (official journal of the Organization)
- EPPO Global Database / PQR (the EPPO database on quarantine pests)
- Pest Risk Analyses and CAPRA software
- EPPO database on diagnostic expertise
- EPPO Reporting Service (monthly newsletter)
- Datasheets on quarantine pests and pictures
- EPPO Alert List …

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