[PleaseReview document review. Review title: CPM recommendation: Next Generation Sequencing technologies as a diagnostic tool for phytosanitary purposes. Document title: Draft\_CPMRecomendation\_NGS\_2018-05-11.docx]

[1]**CPM recommendation: Next Generation Sequencing technologies as a diagnostic tool for phytosanitary purposes**

[2]**Status box**

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| [3]This is not an official part of the CPM Recommendation and it will be modified by the IPPC Secretariat after adoption. | |
| [4]**Date of this document** | [5]2018-04-10 |
| [6]**Document category** | [7]Draft CPM recommendation |
| [8]**Current document stage** | [9]*To* consultation (2018-05) |
| [10]**Major stages** | [11]2018-03 Topic proposed to the IPPC work programme for a CPM Recommendation by Australia, EPPO and New Zealand  [12]2018-04 CPM-13 added the topic to the IPPC work programme for a CPM Recommendation  [13]2018-05 adjustments made following CPM-13  [14]2018-05 submitted to consultation period (15 May – 15 August 2018) |
| [15]**Notes** | [16]To be edited after the consultation period.  [17]This is a draft document and it will be presented for consultation period in English only |

[18]Background

[19]In December 2017, the CPM Bureau considered a paper prepared by the Standards Committee (SC) which reflected discussion by the IPPC Technical Panel on Diagnostic Protocols (TPDP) on opportunities and challenges in relation to the use of Next Generation Sequencing (NGS) technologies as a diagnostic tool for phytosanitary purposes. The Bureau was asked to agree that the background paper be presented to CPM-13 with a request that the CPM note the challenges associated with the use of NGS technologies and that further work is needed on NGS technologies for pest detection and identification.

[20]The CPM Bureau agreed that since this was an emerging issue that would be of interest to contracting parties, a CPM Recommendation should be drafted to provide policy advice and guidance to Contracting parties and Regional Plant Protection Organizations (RPPOs) on the use of NGS technologies as a diagnostic tool for phytosanitary purposes.

[21]At the Thirteenth session of the CPM, a draft CPM recommendation was presented by Australia, European and Mediterranean Plant Protection Organization (EPPO) and New Zealand, and the CPM-13 (2018) agreed to include the topic to the IPPC work programme for a CPM Recommendation on “Next Generation Sequencing technologies as a diagnostic tool for phytosanitary purposes”.

[22]What is NGS and how is it different to other testing methods?

[23]Next Generation Sequencing (NGS) or high throughput sequencing or deep sequencing technologies allow the sequencing of the whole genome and can be used for all types of organisms. NGS technologies can be used for targeted detection of regulated pests and also allow the detection of unknown organisms (i.e. without a priori knowledge). Indeed, application of these technologies has recently resulted in the discovery of previously undetected microorganisms, in particular viruses where the use of the technology is more advanced than for other pathogens (examples provided in this document are for viruses and viroids). Researchers and diagnosticians using NGS technologies will continue to identify and describe new taxa from among the large volume of as yet undiscovered organisms. These technologies therefore enable a new and comprehensive approach to the detection and characterization of pests in a biological sample.

[24]Phytosanitary testing for viruses and viroids in plants and plant products moved around the globe currently relies on a combination of specific (molecular and serological) and generic (visual, electron microscopy and biological indicators or bioassays) approaches. Whilst these methods are currently the best available they have a number of inherent weaknesses. The specific tests usually require a priori knowledge of the viral pathogens and each test needs to be developed and validated (including validation of the test for different pest/host combinations), making resource demands on national plant protection organisations (NPPO). The host range of many pathogens is not well defined and exotic viruses and viroids may not be detected in new pest-host combinations. Whilst bioassays have traditionally been used to detect unknown viruses, further molecular or serological testing is usually required to confirm the identity of the causal agent when disease symptoms are observed. Bioassays are heavily reliant on environmental conditions for symptom expression and often produce ambiguous results as false positives and false negatives. The time taken for bioassays means that plants spend extended periods of time in post entry quarantine stations, significantly adding to costs and delays for importers. A further drawback with bioassays is that strains may not be detected if they are asymptomatic on the indicator host.

[25]Due to the limitations with traditional diagnostic methods, new robust, reliable and cost-effective methods are required to rapidly and reliably screen plants and plant products for viruses and viroids. Studies conducted so far have demonstrated NGS to be equivalent to or better than biological indexing assays in detecting viruses and viroids of agronomic significance (Rott *et al*. 2017; Rwahnih *et al*., 2015; Mackie *et al*.., 2017; Barrero *et al*., 2017). Most importantly, the studies are demonstrating that NGS is able to produce results significantly quicker than bioassays.

[26]Regulatory and scientific challenges

[27]Research findings based on NGS technologies have significant implications within a phytosanitary framework. For example, there is a risk that plant material may be restricted in movement due to the perceived presence of a (previously unknown) microorganism (e.g. virus) that does not have the potential to be pathogenic to its host(s). Not all organisms associated with plants are pests; some may be mutualists providing benefit to the host plant or commensal agents. There is also the issue, as with other indirect methods, that NGS technologies will detect non-viable organisms.

[28]The correct interpretation of results is one of the major challenges in using NGS. Very large and well curated databases of the whole genomes of known pests and micro-organisms are required as the reference for comparison with NGS generated sequence data. NPPOs will face the challenge to make decisions on the basis of data analysis but incomplete information about the biological significance of the finding and the ability of the micro-organism to infect. This decision-making process distances the diagnostic outcome from any analysis of pathogenicity and poses questions in deciding whether the data is linked to the actual presence of a viable biological entity that is a quarantine pest. However this same challenge is presented with current molecular sequencing methods and particularly for ‘new to science’ viruses, so this is not a new problem. Other challenges in using NGS for regulatory purposes are noted by Massart *et al*. (2017) and Martin *et al*. (2016).

[29]To give confidence to NPPOs in adopting NGS technologies for pest diagnosis, internationally harmonized approaches are required, including development of operational guidelines to reliably and repeatedly perform NGS including quality controls and validation data to interpret NGS outputs (Boonham *et al*., 2014). Validation of the technology against existing methods, which also takes into account the limits of current procedures, is also needed.

[30]Global collaboration

[31]There are a number of initiatives underway in different regions of the world that are exploring the use of NGS technologies as a diagnostic tool for phytosanitary purposes (for example in Australasia, Europe and North America). These include discussions on associated policies that may be developed. Co-ordination of outcomes from these initiatives is required to progress the timely development of internationally harmonised standards to use NGS in a regulatory setting.

[32]The CPM Recommendation (Attachment 1) on “Next Generation Sequencing technologies as a diagnostic tool for phytosanitary purposes” will provide a focus for this activity.

[33]References

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[37]Martin, R.R, Constable, F. and Tzanetakis, I.E. (2016) Quarantine Regulations and the Impact of Modern Detection Methods. Ann. Rev. Phytopath. Vol. 54:189-205 <http://www.annualreviews.org/doi/abs/10.1146/annurev-phyto-080615-100105>

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[40]<https://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-03-17-0306-RE>

[41]Rwahnih M. A., Daubert, S., Golino, D., Islas, C. and Rowhani, A. (2015) Comparison of Next-Generation Sequencing Versus Biological Indexing for the Optimal Detection of Viral Pathogens in Grapevine. Phytopathology 105:6:758-763 <https://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO-06-14-0165-R>

[42]**Attachment 1**

[43]Recommendation on: Next Generation Sequencing technologies as a diagnostic tool for phytosanitary purposes

[44]BACKGROUND

[45]The Commission on Phytosanitary Measures (CPM) recognizes that accurate and timely pest diagnosis underpins export certification, import inspections and the application of appropriate phytosanitary measures[[1]](#footnote-2). It is widely accepted that the ability to detect and identify a plant pest varies with the accuracy and reproducibility and specificity of the detection tools.

[47]Next Generation Sequencing (NGS) technologies, also known as high throughput or deep sequencing, have provided a powerful alternative to the detection and identification of organisms. However, the NGS-based diagnostic outcomes may not be associated with evidence of living pests or damage to the plant or plant products by these organisms. As such, the use of highly sensitive technologies, such as NGS for the detection and identification of plant pests should be introduced cautiously and with due consideration of the risks and consequences of applying NGS-diagnostic outcomes to regulate phytosanitary risks.

[48]ADDRESSED TO

[49]Contracting parties and regional plant protection organizations.

[50]RECOMMENDATIONS

[51]The Commission notes findings based on NGS technologies of an unknown microorganism need to be further investigated to demonstrate the potential of that microorganism to be a plant pest that would qualify as a regulated pest. The Commission notes that there are existing challenges and further work is needed on NGS technologies before they can be considered as the sole method for pest detection and identification as the basis for applying phytosanitary regulations. Findings based on NGS technologies of an unknown microorganism need to be further investigated to demonstrate the potential of that microorganism to be a plant pest and that would qualify as a regulated pest.

[52]To improve the capacity and capability of contracting parties to adopt NGS technologies, the Commission *encourages* contracting parties and regional plant protection organizations to:

1. [53]*Actively engage and support* international efforts in developing and finalising standardised operational guidelines for NGS including proper interpretation of results and agreed quality control measures to ensure NGS data outputs are robust and accurate;
2. [54]*Support* international efforts in obtaining more scientific evidence on reliability and accuracy of NGS by conducting trials comparing NGS against existing diagnostic platforms;
3. [55]*Share* knowledge and expertise amongst countries where possible and support the development of NGS training programs including delivery of best laboratory practice courses online, and co-ordinating international proficiency testing to independently assess laboratory capability;
4. [56]*Share* agreed international NGS protocols, guidelines and training material on the IPPC phytosanitary resources page once finalised;
5. [57] *Establish* plans for appropriate infrastructure and investments in Information Technology and bioinformatics, and education/trainings on bioinformatics, for the appropriate interpretation of test results and to support effective implementation of these technologies.

[58]RECOMMENDATION(S) SUPERSEDED BY THE ABOVE

[59]None.

1. [46] See also CPM recommendation R-07: The importance of pest diagnosis ([https://www.ippc.int/en/publications/84234/](https://www.ippc.int/en/publications/84234/%20)) [↑](#footnote-ref-2)