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Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

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REVIEW OF DOCUMENT UPOV/INF/17 "GUIDELINES FOR DNA-PROFILING: MOLECULAR MARKER SELECTION AND DATABASE CONSTRUCTION ('BMT GUIDELINES')"

Document prepared by the Office of the Union

EXECUTIVE SUMMARY

1. The purpose of this document is to present background information concerning the review of document UPOV/INF/17 "Guidelines for DNA-Profiling: Molecular Marker Selection and Database Construction ('BMT Guidelines')".

2. The BMT is invited to consider document UPOV/INF/17/2 Draft 2 as a basis for a revision of document UPOV/INF/17.

3. The following abbreviations are used in this document:

BMT:	Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular
TC:	Technical Committee

4. The structure of this document is as follows:

BACKGROUND

5. The background to this matter is provided in document BMT/17/10 and BMT/17/10 Add. "Review of document UPOV/INF/17 "Guidelines for DNA-Profiling: Molecular marker selection and database construction ("BMT Guidelines")".

Developments in the BMT, at its seventeenth session

6. The Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT), at its seventeenth session, held in Montevideo, Uruguay, from September 10 to 13, 2018, considered documents BMT/17/10, BMT/17/10 Add. 'Review of document UPOV/INF/17 "Guidelines for DNA-profiling: Molecular Marker Selection and Database Construction ('BMT Guidelines')" and UPOV/INF/17/2 Draft 2 "Guidelines for DNA-Profiling: Molecular marker selection and database construction ("BMT Guidelines")" (see document BMT/17/25 "Report", paragraphs 15 to 50).

Section A. Introduction

7. The BMT agreed to amend the first sentence of the text of the Introduction to read as follows:

> "The purpose of this document (BMT Guidelines) is to provide guidance on harmonized ~~for developing harmonized methodologies~~ principles for the use of DNA based markers with the aim of generating high quality molecular data for a range of applications."

Section B. General Principles

8. The BMT agreed to revise document UPOV/INF/17 on the basis of the joint comments from the European Union, France and the Netherlands.

Section 1. Selection of a Molecular Marker Methodology

9. The BMT agreed to delete Section 1.

Section 2. Selection of Molecular Markers

10. The BMT agreed to amend the title of Section 2 to read "1. Phase 1: Selection of Molecular Markers" and renumber the section accordingly.

Section 2.1 (a)

11. The BMT agreed that the text proposed by the European Union, France and the Netherlands should be abbreviated to refer only to the need to achieve a balance between the number of markers and the resolution or discriminative power according to the objective and taking into account the error-rate. It was agreed that the figure should be omitted.

Section 2.1 (c)

12. The BMT agreed to amend Section 2.1 (c) to read as follows:

> "Coverage of the genome and the linkage should reflect the objectives. Knowing the position of the selected markers on the genome (i.e. map position) is not essential but enables the selection of markers that may be linked together to be avoided."

New Section 1.1 (d)

13. The BMT agreed to add new Section 1.1 (d). The BMT also agreed that the European Union, France and the Netherlands should revise their proposal to list the possible sources without assessment of their suitability, because this would be influenced by the circumstances.

New Sections 1.1 (f) to (k)

14. The BMT agreed to add new Sections 1.1 (f) to (k) and to move new Section 1.1 (h) "Avoidance of linkage disequilibrium" next to new Section 1.1 (c).

Section 2.2 Criteria for specific types of molecular markers

15. The BMT agreed to delete the Section 2.2.

New Sections 1.2 and 1.3

16. The BMT agreed not to include new Sections 1.2 and 1.3 proposed by the European Union, France and the Netherlands.

New Section 2

17. The BMT agreed to add a new Section 2 “Phase 2: Selection of the Detection Method” without the following text “As a prerequisite, whatever the source of material, the method for sampling and DNA extraction should be standardized and documented”.

New Section 2.1

18. The BMT agreed to add a new Section 2.1 “Genotyping methods - general criteria” with the following subsection 2.1.1. With regards to the subsection 2.1.1, the BMT agreed to avoid classifying the criteria as “Mandatory criteria” or “Optional criteria” and to delete “(e) Applicable for both diploid species and polyploidy species”. The BMT also agreed to include a new item “sustainability of databases” to subsection 2.1.1. The BMT agreed not to include a new subsection 2.1.2, concerning improvements in technology.

New Section 2.2

19. The BMT agreed that the European Union, France and the Netherlands should combine the proposed elements in new Section 2.1.

Section 3. Access to the Technology

20. The BMT agreed to renumber Section 3 to Section 2.3.

New Section 2.4

21. The BMT agreed that the European Union, France and the Netherlands should shorten the proposed text and present it in a preamble at the beginning of the document.

Section 4. Material to be Analyzed

22. The BMT agreed to move current texts and subsections in Section 4 to a new Section 5.2 “Requirements of the plant material”.

Section 4.4

23. The BMT agreed with the text proposed by the European Union, France and the Netherlands in Section 4.4, except that the third sentence should be replaced by a reference to document TGP/5: Section 1 concerning transfer of material.

Section 5. Standardization of Analytical Protocols

24. The BMT agreed to delete current Section 5 and replace with a new Section 4 “Phase 4: Harmonization and Validation of the Marker Set and Method”.

Section 5.1

25. The BMT agreed to delete current Section 5.1 and replace with a new Section 4.1 “Harmonisation and validation – general criteria”. The BMT also agreed that the European Union, France and the Netherlands should revise proposed texts under the new Section 4.1 to clarify that usage of validated methods will lead to harmonized results.

Section 5.2

26. The BMT agreed to delete current Section 5.2 and replace with a new Section 4.2 “Performance criteria”. With regards to the proposed texts under the new Section 4.1, the BMT agreed to list the criteria without the additional explanatory information.

Section 5.3

27. The BMT agreed to delete current Section 5.3 and replace with a new Section 3 “Phase 3: Evaluation of the Selected Marker Set and Detection Method (fit for purpose validation of the marker set and technological validation of the method)”. With regards to the proposed subsection 3.1.1 under the new Section 3, the BMT agreed that the European Union, France and the Netherlands should revise the texts in order to explain the need to use a suitable set of varieties to develop marker sets and a further set of varieties to evaluate the marker set. With regards to the proposed subsection 3.1.2, the BMT agreed that the European Union, France and the Netherlands should review the text.

New Section 4.3

28. The BMT agreed to add a new section 4.3 “Consistence criteria - harmonization of markers and methods in different laboratories Performance criteria”. The BMT also agreed that the European Union, France and the Netherlands should review this new section in order to avoid duplication with previous sections.

Section 6. Databases

29. The BMT agreed to introduce a new Section 6. “Data exchange” after Section 5 “Databases”. With regards to the texts proposed by the European Union, France and the Netherlands, the BMT agreed that the European Union, France and the Netherlands should remove the wording “shared databases” from their revised proposal on databases and should provide the full names for “VCF” and “BCF” in the list of acronyms.

New Section 5.3

30. The BMT agreed that the European Union, France and the Netherlands should avoid a recommendation for “open-source tools” in Section 5.3 (d), replace the word “cultivar” with “variety” and provide the meaning of “bam” and “CRAM” in the list of acronyms.

Section 6.1

31. The BMT agreed to renumber Section 6.1. as new Section 5.4. With the proposed text, the BMT agreed that the European Union, France and the Netherlands should delete the link to the standard and review whether it should be indicated as a preferred method.

Section 6.2

32. The BMT agreed to renumber Section 6.2. as Section 5.5 and to add the following sentences to the end of the current texts “For variants obtained from sequencing data, storing VCF files in a relational or no SQL database is recommended. In this case, each database record for a variant has a defined genome version, chromosome, position, reference allele”.

Section 6.3

33. The BMT agreed to renumber Section 6.3. as Section 5.6.

Section 6.3.1 (b)

34. The BMT agreed to amend the title of Section 6.3.1 (b) to read “Reference genome position / Locus code:” with the following texts “Preferably, a genome assembly version, chromosome and position should be provided if a reference genome is available for the species concerned, e.g. SL2.50ch05:63309763 for tomato Solanum lycopersicum assembly version 2.50 on chromosome 5 position 63309763. If no reference genome is available or the location is unknown, a name or code of the locus for the species concerned can be used, e.g. gwm 149, A2, etc.”

Section 6.3.1 (c)

35. The BMT agreed to amend the title of Section 6.3.1 (c) to read “Genotype” with the following texts “For SNP genotypes, the allele composition of the SNP or MNP should be given, e.g. A/T or A/A. For other techniques, genotype indicates the name or code of the allele of a given locus for the species concerned, e.g. 1, 123, etc.” The BMT agreed that the European Union, France and the Netherlands should provide the meaning of “MNP” in the list of acronyms.

Section 6.3.1 (d)

36. The BMT agreed to amend the title of Section (d) to read “Allele depths / Data value:” with the following texts “For SNPs obtained from next generation sequencing data this should indicate the depth of coverage for alleles e.g. 10/20 for an A/T allele in which the A is covered by 10 reads and the T by 20. Otherwise, indicates a data value for a given sample on a given locus-allele, e.g. 0 (absence), 1 (presence), 0.25 (frequency) etc.”.

New section 6. “Phase 4: Database Management”

37. The BMT agreed not to include the proposal to add the new Section 6.

Section 7. Summary

38. The BMT agreed that the summary would need to be revised in accordance with the changes to the structure and content of the document.

New section C “DEFINITIONS”

39. The BMT agreed not to add a new section C “DEFINITIONS”.

GLOSSARY

40. The BMT agreed that the glossary should become a list of acronyms providing the meanings of abbreviations but should not provide explanations of any terms.

41. The BMT agreed to propose to the Technical Committee (TC) that the EU, France, Netherlands to prepare a new draft of INF/17 for consideration of the eighteenth session of the BMT.

Developments in the TC, at its fifty-fourth session

42. The TC, at its fifty-fourth session, held in Geneva, on October 29 and 30, 2018, agreed with the proposal by the BMT, at its seventeenth session, for the European Union, France and the Netherlands to prepare a new draft of document UPOV/INF/17 “Guidelines for DNA-Profiling: Molecular marker selection and database construction (‘BMT Guidelines’)” (document UPOV/INF/17/2 Draft 2) for consideration at the eighteenth session of the BMT, to be held in Hangzhou, China, from October 16 to 18, 2019 (see document TC/54/31 “Report”, paragraph 264).

NEW DRAFT OF DOCUMENT UPOV/INF/17

43. Document UPOV/INF/17/2 Draft 2 has been prepared by experts from the European Union, France and the Netherlands.

44. The BMT is invited to consider document UPOV/INF/17/2 Draft 2 as a basis for a revision of document UPOV/INF/17.

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