19 March 2019

DNA sequencing quality check: information required in GMO applications

Nikoletta Papadopoulou GMO Unit

Patricia Romero APDESK Unit

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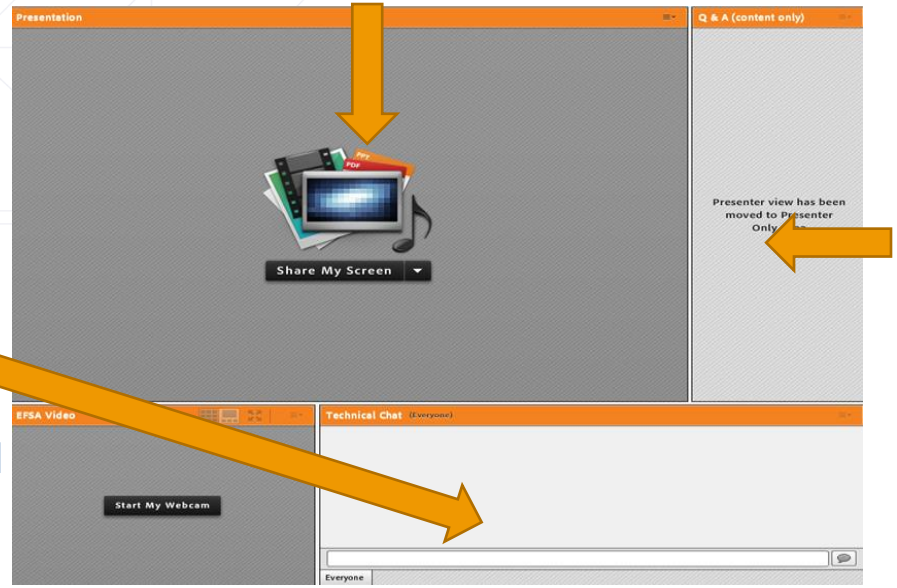
Webinar guide for attendees

- This webinar is being recorded!
- The webinar is in English and questions should be submitted in English through the platform (see hereunder).
- You are automatically connected to the audio broadcast. One-way audio (listen only mode).

Presentation window



Chat box: For technical issues related questions

Q&A box: For any questions related to the topic

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Agenda

AGENDA

TIME	ITEM
15:30 – 15:35	Objective and outline of the webinar, introduction of the two presenters
15:35-15:45	DNA sequencing requirements and recommendations
15:45-15:55	Frequently Asked Questions
15:55-16:05	Live Q&A
16:05-16:10	Closure of the webinar and take home messages

Technical Note

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Technical Note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants

EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel), Josep Casacuberta, Fabien Nogué, Hanspeter Naegeli, Andrew Nicholas Birch, Adinda De Schrijver ... See all authors

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Correspondence: gmo@efsa.europa.eu

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Question number: EFSA-Q-2017-00706
GMO Panel members:. Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.
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Purpose of the Technical Note

- To provide quality parameters and requirements, or discuss considerations, when DNA sequencing is used for parts of the molecular characterisation
- To harmonise the submission of data when DNA sequencing is used, and reflect the scientific progress on the sequencing technology
- To support MS risk assessors and harmonise the risk assessment of GMOs

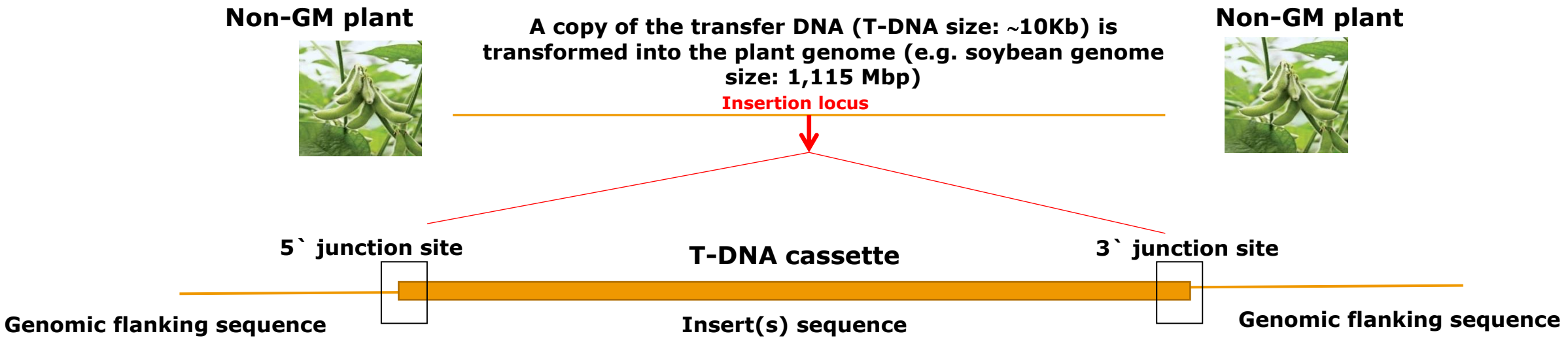
Molecular Characterisation (MC) for GM Plants

GMO applications include confidential sequence information:

I. for the characterisation of the insert(s) and flanking regions

II. for the determination of the number of all detectable inserts

III. for the genetic stability, and integrity



Two main technologies used for RA of GM Plants

- Sanger sequencing is used:
  a. for the characterisation of the insert(s) and flanking regions
  b. for the demonstration of genetic stability across generations

- Next generation sequencing (NGS) can be used:
  a. for the characterisation of the insert(s) and flanking regions
  b. for the determination of the number of all detectable inserts
  c. for the genetic stability and integrity

Material and Sample preparation

- Information on the sample information should include:
  - Source of material (GM plant, tissues)
  - Breeding tree
  - Number of individuals used for the material collection
  - Report on sample preparation (DNA extraction protocol(s), overall strategy)
  - It is strongly recommended to use the same DNA extraction protocol for sample preparation, if multiple DNA extractions are needed

General requirements for DNA sequencing

Guideline for the submission of DNA sequences derived from genetically modified organisms and associated annotations within the framework of Directive 2001/18/EC and Regulation (EC) No 1829/2003

European Union Reference Laboratory for Genetically Modified Food and Feed

(EURL GMFF)

2016
with explanations of April 2017

For Sanger sequencing:

- Two independent PCR reactions
- Bi-directional sequencing
  - The raw sequence of each nucleotide should be covered 4 times

Information required for NGS experiments

To assess the quality of the NGS methodology and generated datasets the following information will be considered, relevant to RA considered:

- Library preparation and sequencing strategy
- Quality of datasets
- Coverage (average read depth)
- Description of bioinformatics (flowchart of analysis)

Sequencing of the insert(s) and flanking regions

- Specific considerations when addressed by Sanger sequencing
  - *Information on any manual editing performed (base–calling, trimming); to be reported and justified.*
  - *Data format requirements for final event sequence*

- Specific considerations as outlined for NGS
  - *Average read depth description when using WGS for insert identification*
  - *Minimum read depth for insert description*
- A combination of approaches may be needed in cases where the configuration of the inserted sequences is more challenging, e.g. longer reads such as PacBio, sequencing of cloned genomic fragments or PCR amplicons (including by Sanger)

Determining the number of all detectable inserts

- To identify junction sites: detect junction reads partially matching both insert and host genome (chimeric)

- Information on genome coverage (specific for Junction Sequence Analysis)
  - Depending on the genome
  - Depending on the sequencing technology used

- Justification by the applicant

Genetic Stability

- **Single events:** Sequencing may be used to demonstrate that the insertion site(s) and the structure of the insert(s) is maintained over multiple generations.

- **Stacks:** Regulation (EU) No 503/2013 requires the sequence of the events in the stack to be determined and compared to that of the single event. This can be achieved by Sanger or NGS.

Data Format Requirements

- **Final sequence in ASCII text files:** must be annotated according to INSDC Feature Table Definition document.
  - Including specific descriptors and features

- **Specific requirements for Sanger experiments:**
  - ABI or FASTQ format
  - All sequences must be aligned to generate a consensus sequence or final sequence.
  - Alignment in CLUSTAL or FASTA format

- **Specific requirements for NGS experiments:**
  - Raw NGS reads in FASTQ format
  - Aligned mapped sequences provided in SAM, BAM or CRAM format

Submission harmonization

Supporting Information

Filename	Description
efs25345-sup-0001-Annex-01.pdf PDF document, 548.4 KB	List of information to be submitted to EFSA for each GM event, according to the Technical Note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants
efs25345-sup-0002-Annex-02.pdf PDF document, 226.9 KB	Instructions to organise the sequencing information to be submitted to EFSA in accordance with the Technical Note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants
efs25345-sup-0003-SequencingInfo.zip Zip archive, 5 KB	Sequencing Info

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