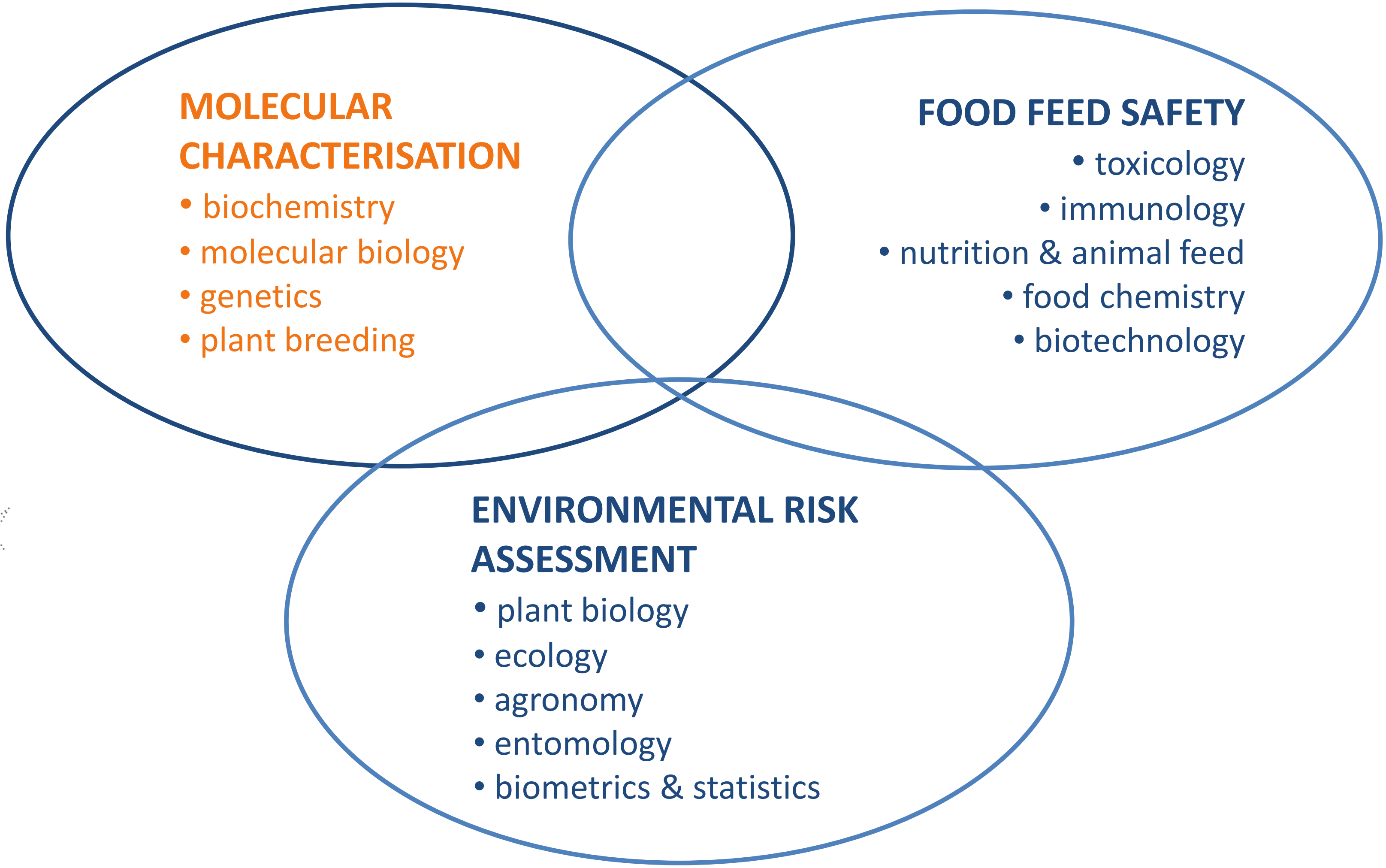
The use of Sequencing technology in GMO risk assessment

66th Advisory Forum meeting,
EFSA, Parma - IT,
5-6 December 2017

efsa
European Food Safety Authority

DATA SETS in GMO APPLICATIONS



MOLECULAR CHARACTERISATION

- biochemistry
- molecular biology
- genetics
- plant breeding

FOOD FEED SAFETY

- toxicology
- immunology
- nutrition & animal feed
- food chemistry
- biotechnology

ENVIRONMENTAL RISK ASSESSMENT

- plant biology
- ecology
- agronomy
- entomology
- biometrics & statistics

MOLECULAR CHARACTERISATION DATA IN GMO APPICATIONS

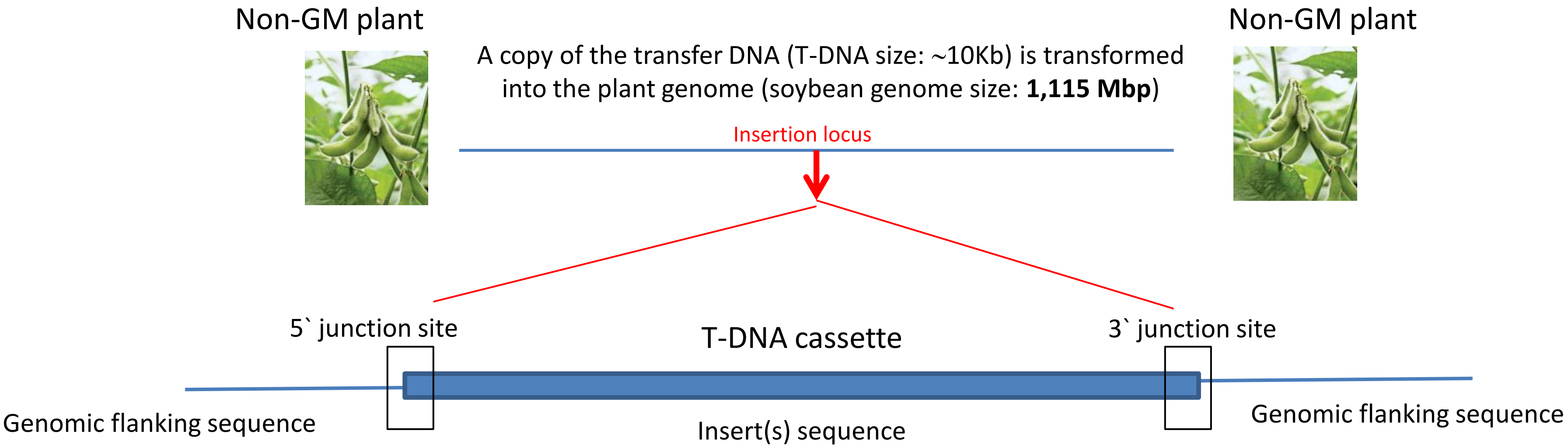
GMO applications include ***confidential*** *sequence information for the:*

I. **Characterisation of the T-DNA insertion site in the plant genome and its junction sites**
   a. Generational stability and integrity of a T-DNA
   b. Determination of copy number

NGS by Junction Read Analysis

II. **The confirmation of the DNA insert (s) sequences**

NGS



Non-GM plant

A copy of the transfer DNA (T-DNA size: ~10Kb) is transformed into the plant genome (soybean genome size: **1,115 Mbp**)

Non-GM plant

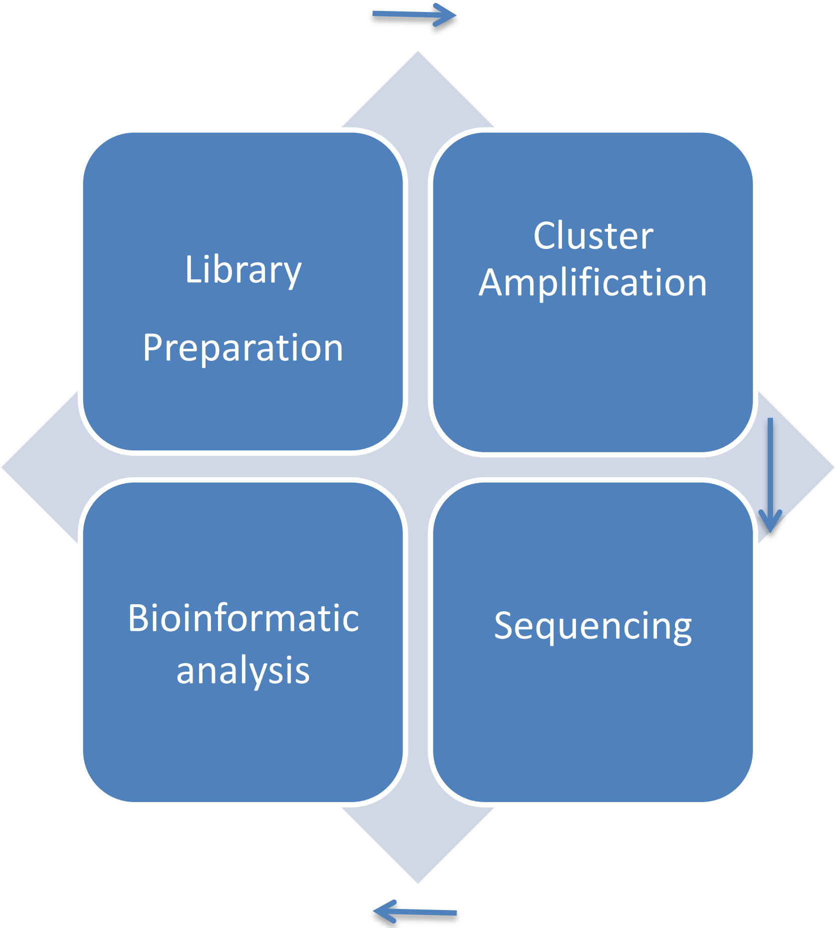
NEXT GENERATION SEQUENCING (NGS) OVERVIEW

Preparation of NGS library from gDNA

Hybridisation and cluster amplification

Labelling and Sequencing (*n* cycles)

Read alignment to reference genome with bioinformatics software



NGS QUALITY PARAMETERS FOR JUNCTION READ ANALYSIS

I. Sequence data and quality

II. Read depth

III. Read alignment to the plant reference genome

IV. Description of data and processing

NGS QUALITY PARAMETERS FOR JUNCTION READ ANALYSIS

I. Sequence data and quality

e.g. Library preparation methods

Sequencing platform information

Paired or single-end read sequencing

Quality statistics of reads (FASTQC)

NGS QUALITY PARAMETERS FOR JUNCTION READ ANALYSIS

I. Sequence data and quality

II. **Read depth**

e.g. Number of reads from each experiment

Calculation of the number of reads required to cover the genome to a specified depth

Criteria used to trim or discard reads

List of the number of reads that were discarded

NGS QUALITY PARAMETERS FOR JUNCTION READ ANALYSIS

I. **Sequence data and quality**

II. **Read depth**

III. **Read alignment to the plant reference genome**

e.g. Genome coverage, Mapping parameters, average gene coverage

- The degree of validation of sequence coverage is greatly dependent on:

  - the organism in question

  - the availability of genomic resources

NGS QUALITY PARAMETERS FOR JUNCTION READ ANALYSIS

I. Sequence data and quality

II. Read depth

III. Read alignment to the plant reference genome

IV. **Bioinformatics analysis**

e.g. Description of bioinformatics analysis; step-by step

Parameters and versions of software used

Flow chart of analysis process (incl. filtering process)

Justification of discarded reads

NGS for confirmation of the insert sequence

- When NGS can be used for the confirmation of full insert(s) sequences, different set of criteria need to be considered to avoid sequencing errors

Full insert sequence

NEW EC MANDATE ON DNA SEQUENCING GUIDANCE

- **EC Mandate** Technical note to applicants on, and checking of, the quality of the methodology, analysis and reporting covering full sequencing and insertion site analysis of GM event, and generational stability and integrity

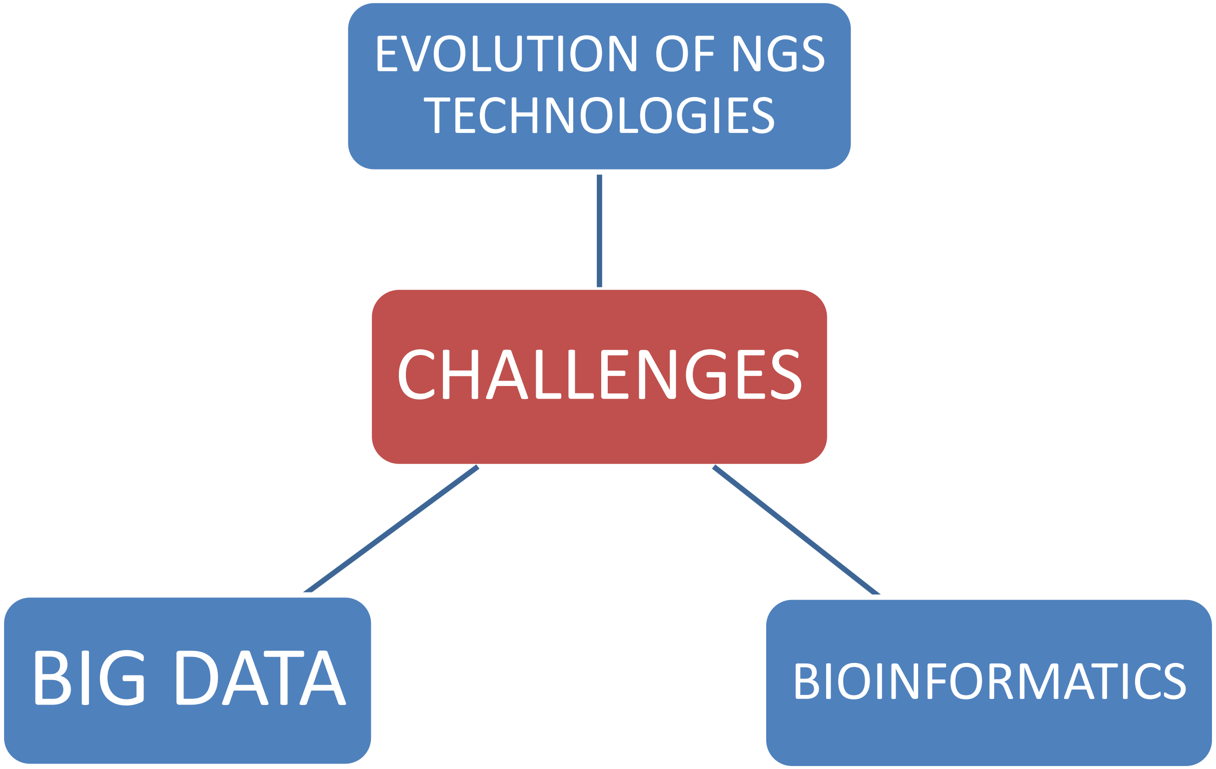
- **State of play** Ongoing set-up of an *ad hoc* Working Group (EC, JRC)
  Meetings scheduled for 1st and 11th December 2017
  More meetings foreseen in 2018, January-May.

- **Deadline** September 2018

NEW EC MANDATE ON DNA SEQUENCING GUIDANCE

- To support the MS risk assessors
- To harmonise the risk assessment of GMOs
- To harmonise the submission of data when DNA sequencing is used
- To define minimum requirements and recommendations for the sequencing of the insert(s) and flanking regions building on the current JRC guidelines
- To cover junction read analysis and generational stability and integrity when addressed by NGS
- To reflect the scientific progress of the methodology used

CHALLENGES OF NGS IN GMO APPLICATIONS



EVOLUTION OF NGS TECHNOLOGIES

CHALLENGES

BIG DATA

BIOINFORMATICS

KIC on biotechnology and molecular methodology supports the EFSA-wide challenge for the needs on NGS and other bioinformatics tools

THANK YOU FOR YOUR ATTENTION

- GMO Unit
- Molecular Characterisation WG
- GMO Panel

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THANK YOU for your Attention

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