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Joint Research Centre (JRC)

The European Commission's

Research-Based Policy Support Organisation

Validation of novel methods and technologies

Marco Mazzara

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Roadmap

Novel validation approaches

- Fuzzy logic
- Modularity

Novel analytical methods

- DualChips
- pJANUS

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Fuzzy logic

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Fuzzy-logic based procedures / software AMPE

AMPE (Analytical Method Performance Evaluation) is a tool to evaluate the performance of analytical methods (Acutis *et al.*, 2007, J. AOAC Int.)

1432 ACUTIS ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 90, NO. 5, 2007

TECHNICAL COMMUNICATIONS

Analytical Method Performance Evaluation (AMPE)—A Software Tool for Analytical Method Validation

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A Windows-based software tool [Analytical Method Performance Evaluation (AMPE)] was developed to support the validation of analytical methods. The software implements standard statistical approaches commonly adopted in validation studies to estimate analytical method performance (limits of detection and quantitation, accuracy, specificity, working range, and linearity of responses) according to ISO 5725. In addition, AMPE proposes the application of innovative and unique approaches for the assessment of analytical method performance. Specifically, AMPE proposes the use of difference-based indexes to quantify the agreement between measurements and reference values, the use of pattern indexes to quantify methods bias with respect to specific external variables, and the application of fuzzy logic to aggregate into synthetic indicators the information collected independently via the different performance statistics traditionally estimated in validation studies. Aggregated measures are particularly useful for methods comparison, when more than one method is available for a specific analysis and it may be of interest to identify the best performing one taking into account, simultaneously, the information available from different performance statistics. Illustrative examples of the type of outputs expected from AMPE-based validation sessions are given. The extensive data handling capabilities and the wide range of statistics supplied in the software package makes AMPE suitable for specific needs that may arise in different validation studies. The installation package, complete with a fully documented help file, is distributed free of charge to interested users along with input files exemplary of the type of entry data required to run validation data analyses.

Method validation is the process of assessing whether an analytical method is acceptable for its intended purpose (1). In general, a validation study demonstrating the suitability of a novel method for a specific purpose should be carried out before routine adoption of the new methodology. Formal validation is often the conclusion of a long and costly process, involving the development and optimization of a novel method. In most cases, formal validation requires the assessment of the performance of the proposed method by means of an interlaboratory study, also known as collaborative study or ring trial [see alternative approaches from Thompson et al. (2) for single-laboratory validations]. Validation trials aim at characterizing method performance, assess the potential for errors, and provide the necessary information to evaluate whether a method can be used to assess compliance with specific requirements. For instance, validated methods for the detection of genetically modified organisms (GMO) are requested by European Union (EU) legislation (Regulation EC 1829/2003) in order to authorize GMO food and feed products on the European market.

Since the 1960s (for example Barnett and Youden; 3), decisions on method performances are based on statistical tests of significance (such as the Student's *t*-test, the *F*-test, and regression analysis). In general, these statistical tests are meant to estimate the size of the deviation occurring between results obtained with a test method of interest and appropriate comparative data, thus providing an indication of the magnitude of the error associated with a given method. However, additional studies (for example Westgard and Hunt; 4) have drawn attention to the limitations of such

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Procedures based on the principles of ISO 5725 (1994) plus complementary, innovative procedures based on integrated approaches (fuzzy-logic principle) capable of summarizing the information contained in multiple validation metrics

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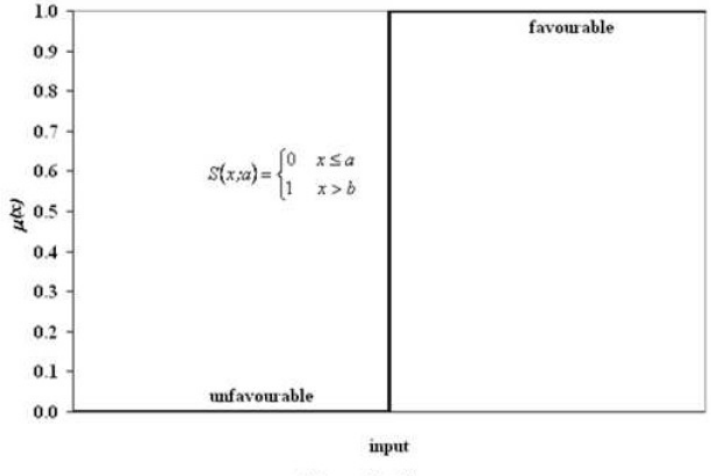
Fuzzy-logic principle

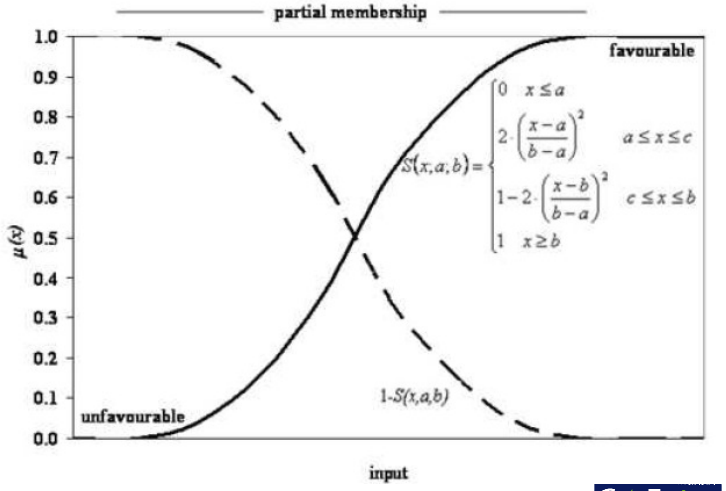
Crisp-based thresholds

- thresholds suitable for all methods and conditions are difficult to define (e.g. ENGL performance criteria)
- if the thresholds are severe no method is acceptable, if the threshold are not stringent all methods are good



Fuzzy-based thresholds

- Fuzzy logic uses membership functions from 0 (no membership) to 1 (full membership), intermediate values representing partial membership



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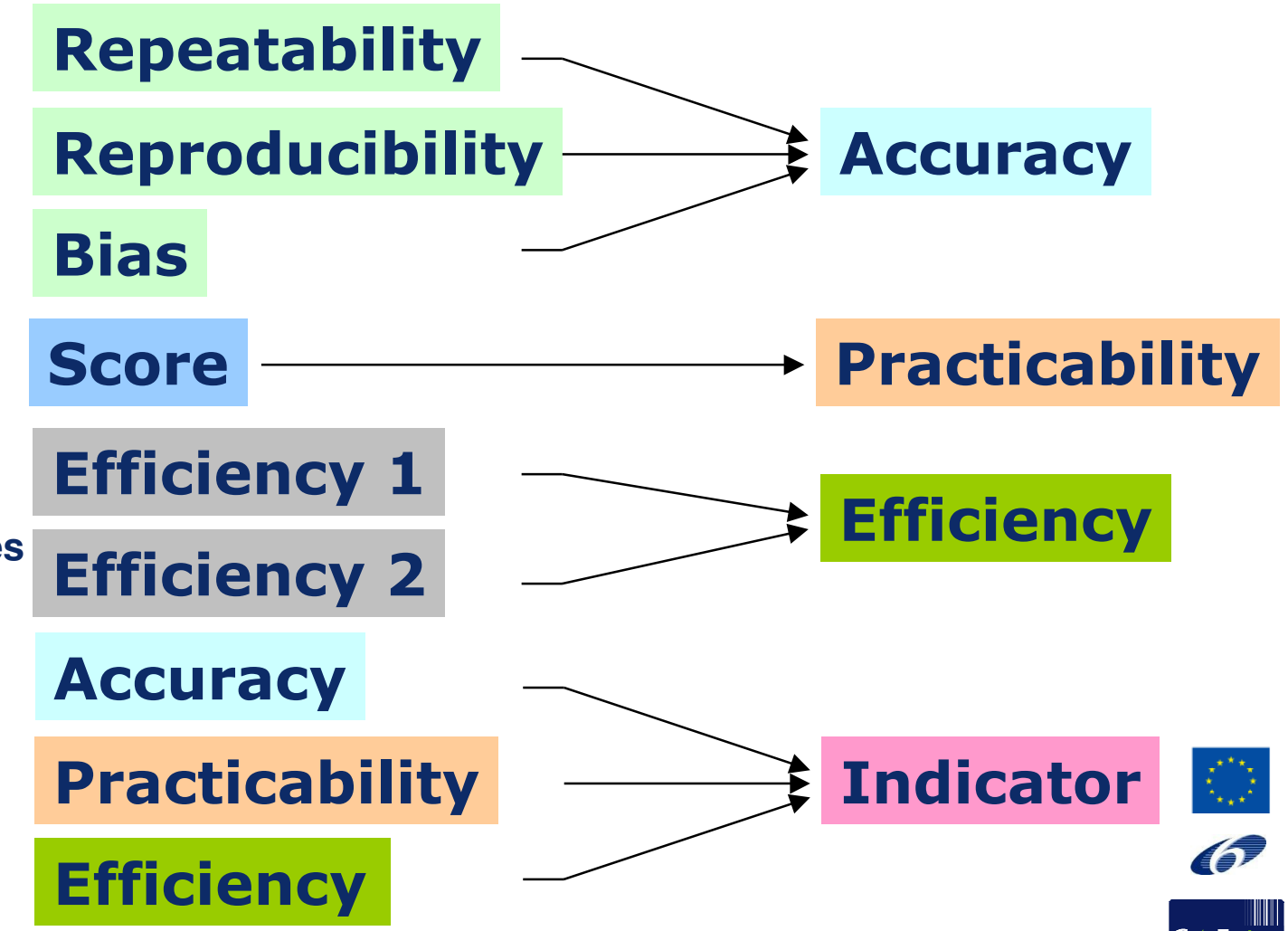
Fuzzy-logic based indicators: single target, multiple metrics

Two-stage, fuzzy-based expert systems allow:

- aggregate similar validation metrics into modules ranging from 0 (best) to 1 (worst)
- aggregate modules into a global indicator of method validity ranging from 0 (best) to 1 (worst)



Bellocchi *et al.*, 2008, Food Anal. Methods

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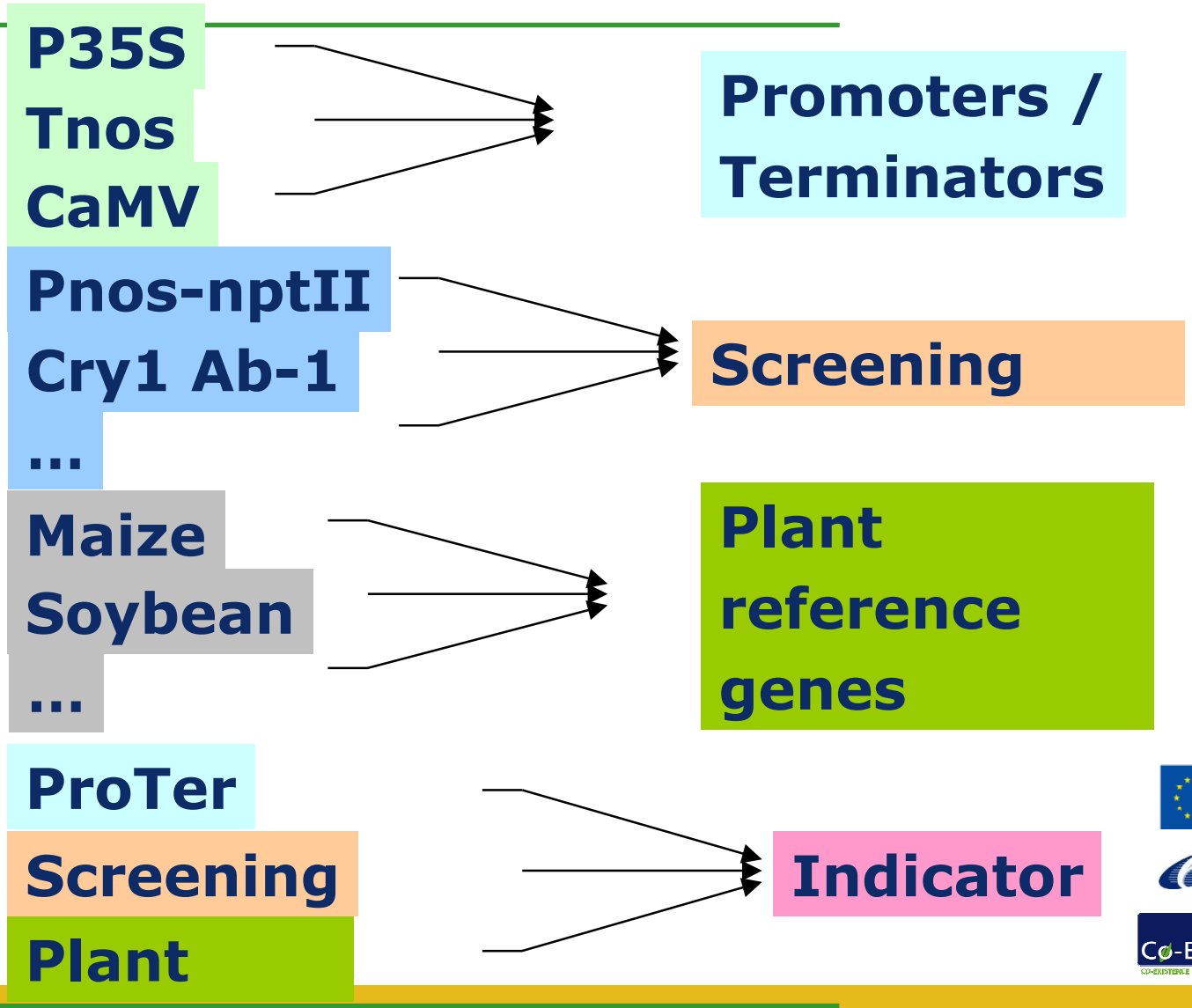
Fuzzy-logic based indicators: multiple targets, single metric

Two-stage, fuzzy-based expert systems allow:

- aggregate accuracy rates of similar GM targets into modules ranging from 0 (best) to 1 (worst)
- aggregate modules into a global indicator of method validity ranging from 0 (best) to 1 (worst)



- Leimanis, 2008, Eur. Food Res. Technol.
- Bellocchi *et al.*, under revision, Transgenic Res.

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Modularity

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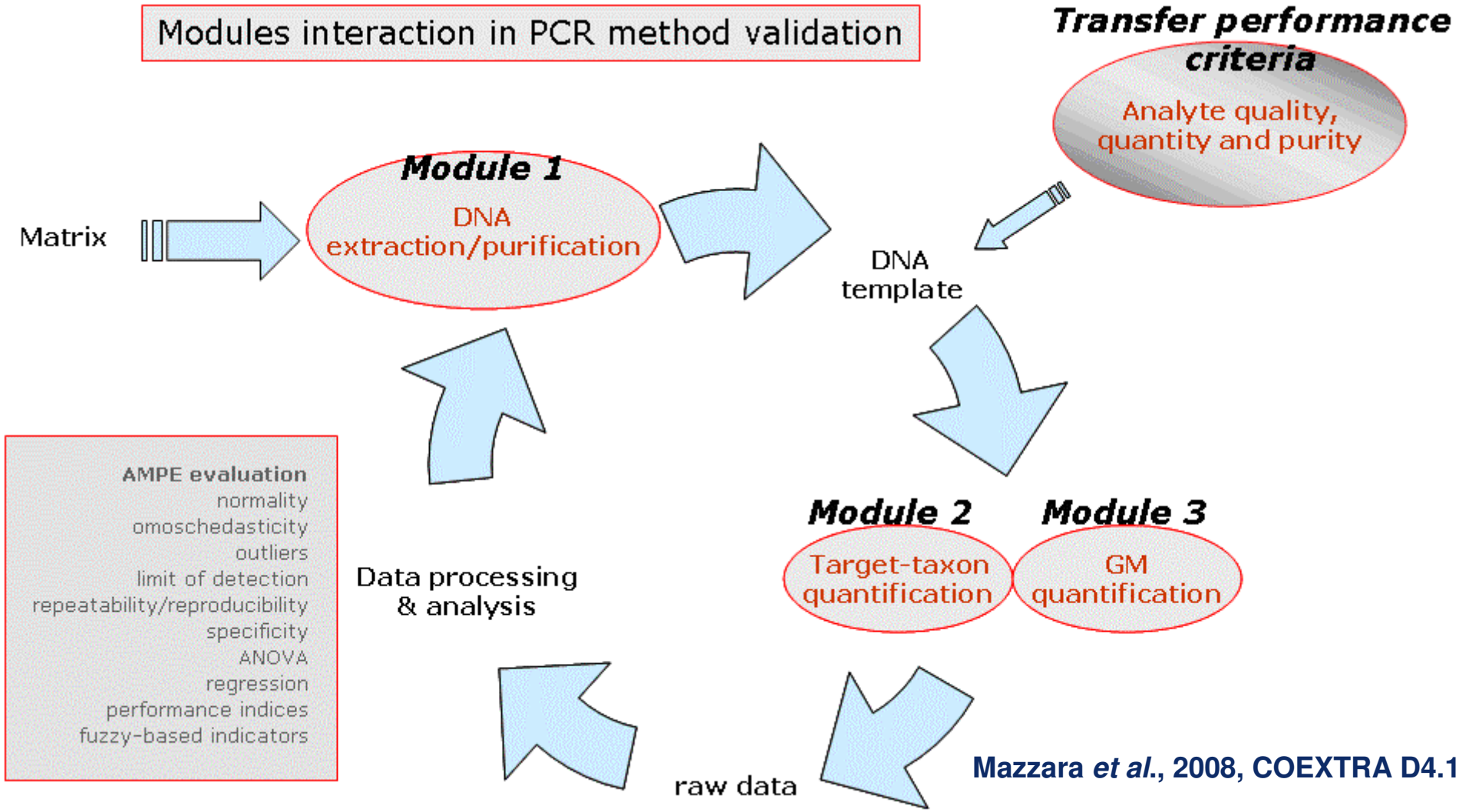
Modularity: holistic approach



Mazzara *et al.*, 2008, COEXTRA D4.1

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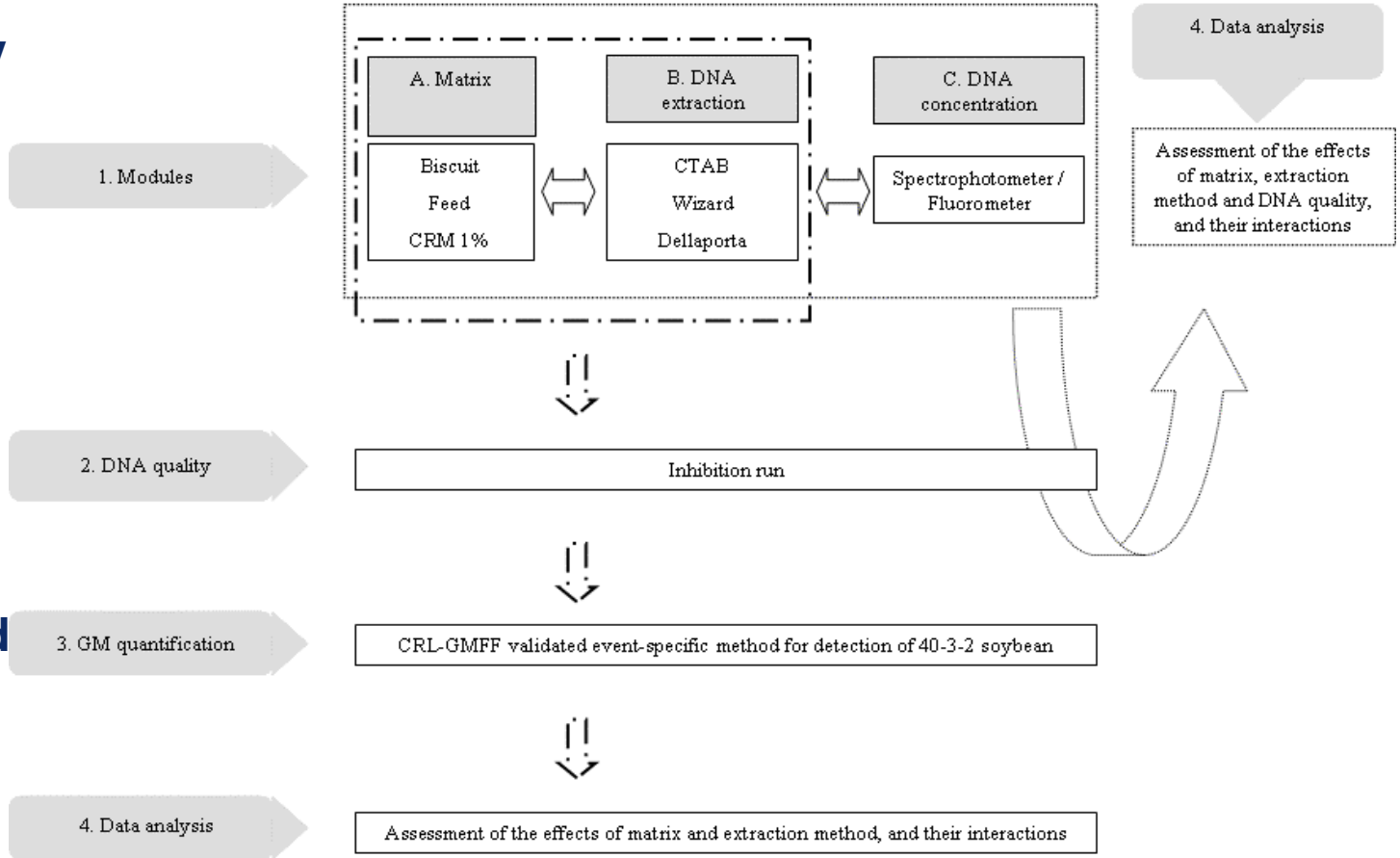
Modularity: experimental design

- **Four-stage modularity testing:**
  - **– Module 1: array of DNA extraction methods from alternative matrices**
  - **– Transfer performance criteria: inhibition test**
  - **– Modules 2-3: RT-PCR target-taxon and GM DNA quantification**
  - **– Data processing: ANOVA, fuzzy-logic based indicator**



Bellocchi *et al.*, under development, COEXTRA D4.1 annex

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DualChip GMO validation

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DualChip GMO validation / 1

Target elements detected on the DualChip GMO microarray assay

GMO screening targets	- CaMV 35S promoter (P35S) - Nopaline synthase terminator (Tnos) - Phosphinothricin N-acetyltransferase (Pat) - Cry1Ab delta-endotoxin (Cry1Ab) - 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) - The junction between the Nopaline synthase promoter and the neomycin phosphotransferase II gene (Pnos-nptI
Species targets	- Invertase (Maize) - Cruciferin (Rapeseed) - Lectin (Soybean) - rBCL (plant universal)
Control target	- Cauliflower Mosaic Virus (CaMV)

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DualChip GMO validation / 2

Modules, targets, and relative weights

Module	Target element	Weight of targets into the module*	Weight of modules into the indicator
ProTer (Promoters and Terminators)	*P35S*	0.40	0.4
	Tnos	0.40	
	CaMV	0.20	
Screening (Inserted genes)	*Pnos-nptII*	0.14	0.3
	PAT	0.14	
	cry1Ab-1	0.14	
	cry1Ab-2	0.14	
	cry1Ab-3	0.14	
	EPSPS-1	0.14	
	EPSPS-2	0.14	
Plant (Species reference genes)	*Maize*	0.30	0.3
	Soybean	0.30	
	Rapeseed	0.30	
	Plant	0.10	

* Weights were re-arranged when one or more elements were missing in the analysis.

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DualChip GMO validation / 3

Accuracy rate per target in each PCR. The detection accuracy rates reported in the table are expressed in % of total valid assays. For each set of PCR, accuracy rates of the expected positive signals are indicated in bold. Accuracy rates for non-expected signals are indicated in non-bold

module	target	PCR2a	PCR3a(1)	PCR3a(2)	PCR4a	PCR4b	PCR5a	PCR5b	PCR6a	PCR6b	PCR7a
Group 1: "ProTer"	P35S	0.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**92.50%**	**100.00%**
	Tnos	0.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**	0.00%	0.00%	0.00%	0.00%	**97.60%**
	CaMV	0.00%	0.00%	0.00%	0.00%	0.00%	**100.00%**	**100.00%**	**100.00%**	**95.00%**	0.00%
Group 2: "Screening"	Pnos-nptII	0.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**	2.40%	0.00%	0.00%	0.00%	**97.60%**
	PAT	0.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**	0.00%	0.00%	0.00%	0.00%	**100.00%**
	cry1Ab-1	0.00%	0.00%	0.00%	0.00%	0.00%	**100.00%**	**100.00%**	**100.00%**	**97.50%**	0.00%
Group 3: "Plant"	cry1Ab-2	0.00%	**100.00%**	**97.70%**	**100.00%**	**97.10%**	0.00%	0.00%	0.00%	0.00%	0.00%
	cry1Ab-3	0.00%	**100.00%**	**100.00%**	**100.00%**	**94.10%**	0.00%	0.00%	0.00%	0.00%	0.00%
	EPSPS-1	0.00%	0.00%	0.00%	0.00%	0.00%	**97.60%**	**100.00%**	**100.00%**	**87.50%**	0.00%
	EPSPS-2	0.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**	0.00%	5.10%	0.00%	0.00%	**100.00%**
	Maize	0.00%	**97.60%**	**95.30%**	**100.00%**	**100.00%**	11.90%	0.00%	0.00%	0.00%	0.00%
	Soybean	0.00%	0.00%	0.00%	0.00%	0.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**
	Rapeseed	0.00%	0.00%	0.00%	0.00%	0.00%	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**
	Plant	0.00%	**100.00%**	**96.80%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**	**100.00%**

PCR2a: 0% plant; PCR3a(1): 1% GMO, 50% plant; PCR3a(2): 0.5% GMO, 5% plant; PCR4a: 0.1% GMO, 1% plant; PCR4b: 0.045% GMO, 0.5% plant; PCR5a: 1% GMO, 50% plant; PCR5b: 0.5% GMO, 5% plant; PCR6a: 0.1% GMO, 1% plant; PCR6b: 0.045% GMO, 0.5% plant; PCR7a: 0.1% RRS, 99.9% Topasl 9/2

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DualChip GMO validation / 4

Values of modules "ProTer", "Screening", and "Plant", and of the fuzzy indicator of global validity (I_{DCGMO})

PCR event and GM percentage	ProTer	Screening	Plant	I_{DCGMO}
PCR3a 1%	0.0000	0.0000	0.0000	0.0000
PCR3b 0.5%	0.0000	0.0000	0.0000	0.0000
PCR4a 1%	0.0000	0.0000	0.0000	0.0000
PCR4b 0.045%	0.0000	0.0130	0.0000	0.0001
PCR5a 1%	0.0000	0.0000	0.0000	0.0000
PCR5b 0.5%	0.0000	0.0000	0.0000	0.0000
PCR6a 0.1%	0.0000	0.0000	0.0000	0.0000
PCR6b 0.045%	0.3333	0.5000	0.0000	0.2729
PCR7a	0.0000	0.0000	0.0000	0.0000
PCR2a	0.0000	0.0000	0.0000	0.0000

Validation report available at http://mbg.jrc.ec.europa.eu/home/documents/report-JRC-EAT.pdf

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