

The GMO Global Matrix project



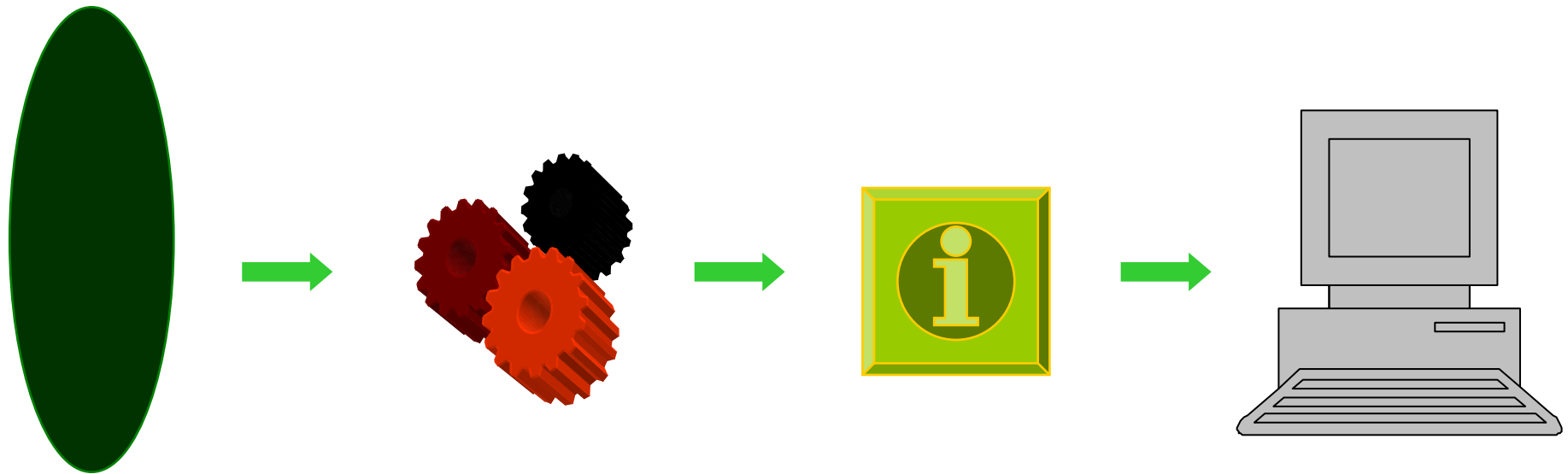
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GMO analysis



Sample

Homogenization

Analysis

Decision

MATRIX APPROACH

CONTENTS

- General principles in matrix-based screening approaches
- Example : COSYPS analysis (incl. Decision Support System DSS)
- Next steps for Global Matrix project

What is a “GMO Matrix” (for screening purposes)

- 1) A description of a set of GMOs in form of a table (Excel) table
- 2) Each GMO is described as a combination of genetic elements which are the targets for the screening (such as the p35S, tNOS, CryIAb,)
- 3) A (mathematical) “matrix” form wherein the relation between targets and GMO is represented
 - the **(molecular) targets** are listed as **columns**
 - the **GMO** are represented as **rows**

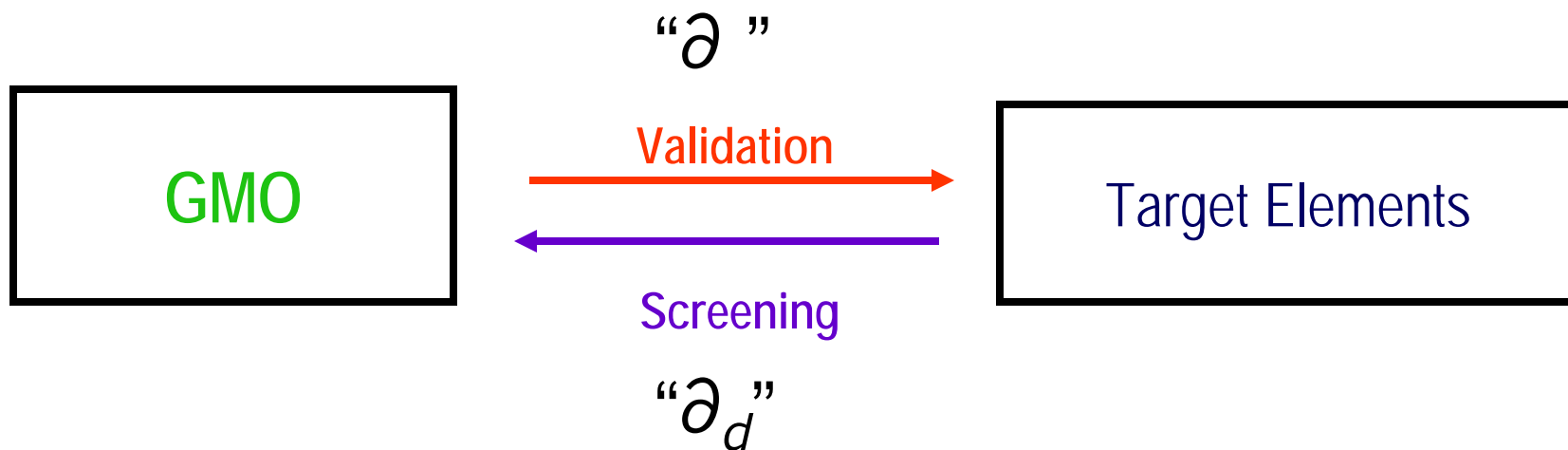
In the GMO Universe, a table is a “practical” GMO Matrix

	Target 1	Target 2	Target 3	Target 4	Target 5	Target 6
GMO-1	+	-	-	-	-	-
GMO-2	+	+	+	+	-	-
GMO-3	-	+	+		+	+
GMO-4	-	-	-	-	-	-
GMO-5	-	-	-	+	-	-

'+' : present

'-' : absent

Matrix-based screening approach



Description of the relationships is represented by the "Matrix" format

GMO Screening approach:

- analyte: **DNA**
- unit of measurement: **Haploid Genome Equivalent**
- detection technology: **PCR**
- reference material: **genomic DNA (plasmids)**
- critical parameters: ISO-standards (Guidelines of the European Network of GMO Laboratories (ENGL) also applied)

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The COSYPS example

- **C**ombinatory **S**Ybergreen **P**cr **S**creening
- developed at Scientific Institute of Public Health IPH (B) 2005-2009
- patented in 2007
- collaborative trial in 2009

COSYPS development (2005-2009)


Strategy:

1. GMO universe definition in a matrix-format (June 2005, constant update)
2. Definition of screening elements
3. Unix Dbase of relevant recombinant DNA sequences
4. Develop uniform SYBR[®]Green Q-PCR methods for screening
(primers, PCR conditions, reference materials)
5. Common validation criteria for all SYBR[®]Green Q-PCR methods
6. Development of a Mathematical Analysis tool
7. Development of integrated 96-well plate screening format and DSS

Definition of Screening elements

Linear [1 x N] matrix model for GM events

Subclasses of constituents:

- **Kingdom** (e.g. plant, animals, bacteria,...)
 - **Taxon-/Species-specific** (e.g. maize, soy, oilseed rape, ...)
 - **Construct-specific** (inserted DNA sequence)
 - **Generic recombinant** (e.g. 35S promoter, T-Nos terminator, ...)
 - **Trait-specific** (e.g. CP4-EPSPS, PAT, BAR, CryIAb,)
 - **Event-specific** (plant/insert junctions, ...)
 - **Donor species markers** (e.g. RT CaMV)
- 
- Screening markers

COSYPS Q-PCR method parameters

Standardized PCR running conditions

50 ng template DNA/reaction

260nM primer concentration (except for RBCL)

Standard PCR program

10'	95°C	1x	(hot start)
15"	95°C	40x	(denaturation)
1'	60°C		(elongation)
20'	60-95°	1x	(melting analysis)

Validation of COSYPS SYBR®Green Q-PCR methods (collaborative trial 2009)

All COSYPS methods were validated “in house” (ISO standards).

Validation parameters:

- Amplification of positive controls
- No amplification of negative controls
- One unique T_m with a clear dissociation peak
- One band on agarose gel at the expected size for positives
- Sequencing of the amplicon give the expected sequence
- No (or almost no) primer-dimer formation
- Identification of the limit of detection (LOD_6) on dilution series with 6 repeats
(LOD_6 is defined as the lowest DNA amount detectable 6/6 times)
- Robustness (by Proficiency tests)
- Repeatability (by Reference Material testing)

COSYPS Mathematical Analysis Tool: a 'prime number' molecular encoding, the Godel Prime Product (GPP) for all Q-PCR results

- When an element is present: the score is set at the corresponding prime number for that element
- When an element is absent: the score is set at « 1 » for that element

	Plant	Lect	ADH	p35S	Tnos	CP4	CryIaB	PAT	BAR	CRU	GPP
GTS40-3-2	3	5	1	13	17	19	1	1	1	1	62985
T25	3	1	7	13	1	1	1	29	1	1	7917
NK603	3	1	7	13	17	19	1	1	1	1	88179
GA21	3	1	7	1	17	1	1	1	1	1	357
Bt176	3	1	7	13	17	1	23	1	31	1	3309033
Bt11	3	1	7	13	1	1	23	29	1	1	182091
BT10	3	1	7	13	1	1	23	29	1	1	182091
MON810	3	1	7	13	17	1	23	1	1	1	106743
TC1507	3	1	7	13	1	1	23	29	1	1	182091
DAS59122	3	1	7	13	1	1	1	29	1	1	7917
MON863	3	1	7	13	17	1	1	1	1	1	4641
Topas19/2	3	1	1	13	17	1	1	29	1	11	211497
MS8/RF3	3	1	1	1	17	1	1	1	31	11	17391
MS1/RF1/RF2	3	1	1	1	17	1	1	1	31	11	17391
T45	3	1	1	13	1	1	1	29	1	11	12441
GT73	3	1	1	1	1	19	1	1	1	11	627
LL62 rice	3	1	1	13	1	1	1	1	31	1	1209
LL601	3	1	1	13	1	1	1	1	31	1	1209

Summary COSYPS decision support aspects

- Unique Prime number identifiers (GPP modulation function for identification of GMO possibly present)
- Matrix information on Presence/Absence of targets in Prime Numbers
- Decision thresholds (LOQ_6 and LOD_6)
- Integrates screening and identification data
- So far Excel-based support format (but soon DSS web application (eGMOLAN) – first prototype available)

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Conclusion

- The GMO Matrix is close to completion at EU level :
 - List of GM events and screening markers (for which info available in the EU) was integrated in the GMO matrix
 - Validation of each detection combination in the GMO matrix was completed
 - First prototype of web DSS is available
 - Production of screening plates (96 wells) is under preparation

Conclusion

- The GMO Matrix can now become global
- Extension of scope of the EU-GMO Matrix to other regions of the world (Asia, South-America, Africa)
- Further input/information welcome from non-EU countries incl. Asia
 - List of commercial GMOs (incl. “local” GMOs)
 - GMO description (incl. genetic elements for detection)
 - GMO control material (seeds, tissue, powder, DNA...)
- Collaboration on the “Global GMO Matrix” project may be laid down in a bilateral “Memorandum of Understanding (MoU)”

Thank you for your attention!

