NATIONAL INSTITUTE OF BIOLOGY

New approaches of GMO detection and quantification

Dr. Jana Žel

REGULAR TESTING SCHEME

Screening

Negative

NO GMO

Positive

Identification

GMO in sample

Authorized, LLP?

Yes

No

Illegal

Rejection

Assay individual ingredient

Quantification

< 0.9% (or 0.1%)

No need for labelling

≥ 0.9% (or 0.1%)

Labelling

DETECTION

- Screening and qualitative aspects
- Quantitative aspects

VALIDATION OF METHODS-EVENT SPECIFIC

- Submission and validation of GMO detection methods are an integral part of the EU regulatory approval process for GMOs *(EC) No. 1829/2003.*
- EU-RL GMFF assisted by the National Reference Laboratories.

VALIDATION OF SCREENING METHODS

At the 16th Meeting of the committee of competent authorities dealing with directive 2001/18/EC in Brussels on 23rd November 2009 point 6: Screening methods for GMOs it is written:

- This item relates to a recent Nature article which documented how a well recognised method used to screen GM 1507 maize failed to perform well owing to the presence of SNPs (Single Nucleotide Polymorphism) *(NIB publication).* This has implications for screening for GMO presence. A number of CAs emphasised the importance and the need to develop harmonised and validated screening methods (incorporating different markers i.e. T-nos in addition to 35S) such that it is possible to detect all GMOs including newly developed GMOs. Continual evaluation of screening methods was also deemed necessary. One CA stated that it was important to maintain flexibility of methodology so that screening could respond to rapid changes in the technology.

- COM confirmed that DG JRC has been discussing screening methods with Member States laboratories but so far DG JRC has not formally validated screening methods and has been focusing on validation of event-specific methods (since the requirement in the legislation is for the applicant to provide event-specific detection methods). The legal mandate for the validation of screening methods would need to be confirmed but COM confirmed that DG JRC can also work on the validation of screening methods. There will be continued co-ordination between the different services of the COM (namely DG Env, DG Sanco and JRC) on this issue.

MATRIX APROACH

	GMO	P-35s	T-nos	pat	bar	CTP2-CP4EPSPS
1	89034	1	1	0	0	0
2	176 (Bt 176) (b)	1	0	0	1	0
3	Bt11	1	1	1	0	0
4	Event 98140	1	0	0	0	0
5	GA21	0	1	0	0	0
6	MIR604	0	1	0	0	0
7	Mon810	1	0	0	0	0
8	Mon863	1	1	0	0	0
9	NK603	1	1	0	0	1
10	T25	1	0	1	0	0
11	TC1507	1	0	1	0	0

GMOseek matrix

- A “Matrix table” containing data on 341 GM events and 247 different genetic elements, partially counter checked by the EU-RL, was developed within the GMOseek project. The project completed in 2011. The matrix is at this moment updated on a voluntary basis, but this update is not guaranteed to be done further.

Acceptable: http://www.cra.wallonie.be/en/19/the-projects/296 or. http://www.gmoseek.com/.

BLOCK, Annette, ŽEL, Jana, GRUDEN, Kristina, MORISSET, Dany. BMC bioinformatics, 2013, vol. 14, str. [1-14], 256. http://www.biomedcentral.com/1471-2105/14/256, doi: 10.1186/1471-2105-14-256.

GMOSeek_Matrix-Program_version_211112 - Microsoft Excel

Home | Insert | Page Layout | Formulas | Data | Review | View

Cut | Copy | Format Painter | Paste | Clipboard

Arial | 10 | Font

Wrap Text | Merge & Center | Alignment

General | Number

Normal 2 | Normal | Bad | Good | Neutral | Calculation | Check Cell | Explanatory ... | Conditional Formatting | Format as Table | Styles

Insert | Delete | Format | Cells

AutoSum | Fill | Clear | Sort & Filter | Find & Select | Editing

JB346

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT
1	Event	Unique ID	Crop	Stacked / Not Stacked	Authorized (A), Not Authorized (NA) or Low Level Presence (LLP)- EU Regulations	PROMOTERS	P-35s	P-35S/ACT8	dP-35s	P-2xOCS.35s	P-4AS1 (P-4xAS1.35s)	P-5126del	P-ALS (ZM)	P-ALS (A.th.)	P-CBI	P-E8	P-FMV (P-CmoVb., P-eFMV)	P-FMVTSF1	tsf1	act8	P-HelSsu	P-Kti3	P-PCA65	P-PCDK	P-PEPC	P-Ptac	P-SsuAra	P-ArabSSu1	P-SCP1 (P-35S/Rsyn7 Syn II) / TMV	P-TA29	P-mac	P-mas	P-ManS (A. tumefaciens)	P-napin (seed-specific promoter)	P-NOS	2xP-nos	P-SAMS	P-tact	P-actin2	P-OCS	P.ubiZM1(2)	P-ubi (th)	P-MTL (P-MT)	P-Glb1	P-Ozein	P-Tap (P-TaPer, Peroxidase Triticum aestivum)
95	MIR162 x MIR604 x GA21	SYN-IR162-4 x SYN-IR	Maize	S	NA	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
96	MIR162 x TC1507	SYN-IR162-4 x DAS-0	Maize	S	NA	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
97	MIR162 x TC1507 x GA21	SYN-IR162-4 x DAS-0	Maize	S	NA	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
98	MIR604	SYN-IR604-5	Maize	NS	A	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
99	MIR604 x GA21	SYN-IR604-5 x MON-	Maize	S	NA	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
100	MIR604 x NK603	SYN-IR604-5 x MON-	Maize	S	NA	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
101	MIR604 x TC1507	SYN-IR604-5 x DAS-0	Maize	S	NA	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
102	Mon80100	MON-80100	Maize	NS	NA	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	Mon802	MON-80200-7	Maize	NS	NA	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	Mon809	PH-MON809-2	Maize	NS	NA	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	Mon810	MON-00810-6	Maize	NS	A	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	Mon832		Maize	NS	NA	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107	Mon863	MON-00863-5	Maize	NS	A	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
108	MON863xMON810	MON-00863-5xMON-	Maize	S	A	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109	MON863xNK603	MON-00863-5xMON-	Maize	S	A	3	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
110	MON863xNK603xMON810	MON-00863-5xMON-	Maize	S	A	3	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
111	MS3	ACS-ZM001-9	Maize	NS	NA	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	MS6	ACS-ZM005-4	Maize	NS	NA	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
113	NK603	MON-00603-6	Maize	NS	A	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
114	NK603 x T25	MON-00603-6 x ACS-	Maize	S	NA	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
115	T14	ACS-ZM002-1,	Maize	NS	NA	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	T25	ACS-ZM003-2	Maize	NS	A	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
117	TC1507	DAS-01507-1	Maize	NS	A	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
118	TC1507 x 59122 x MON810 x MIR604 x NK603	DAS-01507-1 x DAS-5	Maize	S	NA	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1
119	TC1507 x 59122 x MON810	DAS-01507-1 x DAS-5	Maize	S	NA	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
120	TC1507 x 59122 x MON810 x NK603	DAS-01507-1 x DAS-5	Maize	S	NA	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
121	TC1507 x 59122 x 88017	DAS-01507-1 x DAS-5	Maize	S	NA	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
122	TC1507 x 88017	DAS-01507-1 x MON-(	Maize	S	NA	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
123	TC1507 x GA21	DAS-01507-1 x MON-(	Maize	S	NA	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
124	TC1507 x MIR604 x NK603	DAS-01507-1 x SYN-IR	Maize	S	NA	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
125	TC1507 x Mon810	DAS-01507-1 x MON-(	Maize	S	NA	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
126	TC1507 x Mon810 x NK603	DAS-01507-1 x MON-(	Maize	S	NA	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
127	1507x59122	DAS-01507-1 x DAS-5	Maize	S	A	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
128	1507xNK603	DAS-01507-1xMON00	Maize	S	A	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
129	59122	DAS-59122-7	Maize	NS	A	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
130	59122x1507xNk603x	DAS-59122-7xDAS015	Maize	S	A	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
131	59122 x GA21	DAS-59122-7 x MON-	Maize	S	NA	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
132	59122xNK603	DAS-59122-7xMON00	Maize	S	A	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
133	59122 x MIR604	DAS-59122-7 x SYN-I	Maize	S	NA	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
134	59122 x MIR604 x GA21	DAS-59122-7 x SYN-I	Maize	S	NA	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1

GMOSeek | Matrix | Liste Event | Legal use | Authors | Info - Tools

Ready

85%

EUginius GMO database

- http://www.wageningenur.nl/en/project/EUginius-GMO-database.htm

- Aim is to develop the European GMO EUginius database. This database contributes to the enforcement of EU legislation on Genetically Modified Organisms (GMOs).
- The focus is on worldwide non-authorized GMOs that are not yet safe for humans, animals and the environment. The database is designed as a user-friendly, web-based European database. EUginius has links to the related Chinese initiative GMDD and is complementary to European initiatives such as the database of the EC-JRC in Ispra.
- The EUginius database is completed mid 2012 and accessible via the Internet. Confidential information is protected through login-construction and will be visible only to authorized users.

INFORMATION ON DATA

- International cooperation

SCREENING PHASE - coverage

- PENTAPLEX (P-35s,T-nos, pat, bar, CTP2-CP4EPSPS)
- five GMevents are not targeted from all EU approved ones, including LLP (3 soybeans (2 of them LLP), 1 maize (LLP), 1 cotton).
- Therefore we need to test additionaly this event specific ones in first phase.

DEVELOPMENT AND VALIDATION OF NEW SCREENING METHODS

- ENGL Advisory Group Method Selection for Validation
- European Committee for Standardization (CEN), TC275/WG11 – Preparation of document for matrix approach

GMOval project

http://www.gmoval.com/

GMOval project (cont. of GMOseek)

New screening methods - validation

- bar/pat TaqMan® duplex,
- the tE9 TaqMan® qPCR and a corresponding TaqMan® assay for pea (to control the presence of the natural donor organism of the tE9 terminator),
- the SYBR®Green Cry3Bb qPCR assay

- Financed by: Food Standards Agency (NIB heading).

TOOLS TO HELP IN GM ANALYSES

GMOseek SOFTWARE

- Matrixes are too complex to be handled manualy.
- Therefore development of software.
- Publicly available: http://www.gmoseek.com/.

- Cooperation of NIB and IJS - Morisset D., Kralj Novak P., Zupanič D., Gruden K., Lavrač N., Žel J. (2013). GMOseek: a user friendly tool for optimized GMO testing. (in preparation)
- Financed by: Food Standard Agency (FSA, UK) and the Federal Office of Consumer Protection and Food Safety (BVL, Germany) within the European excellence in food safety research programming, SAFEFOODERA.

GMOseek SAFEFOODERA

GMOseek SOFTWARE

Matrix used by GMOseek software

- Excell table in .tab format
- Adaptable to each laboratory needs

GMOseek SOFTWARE

Different purposes:

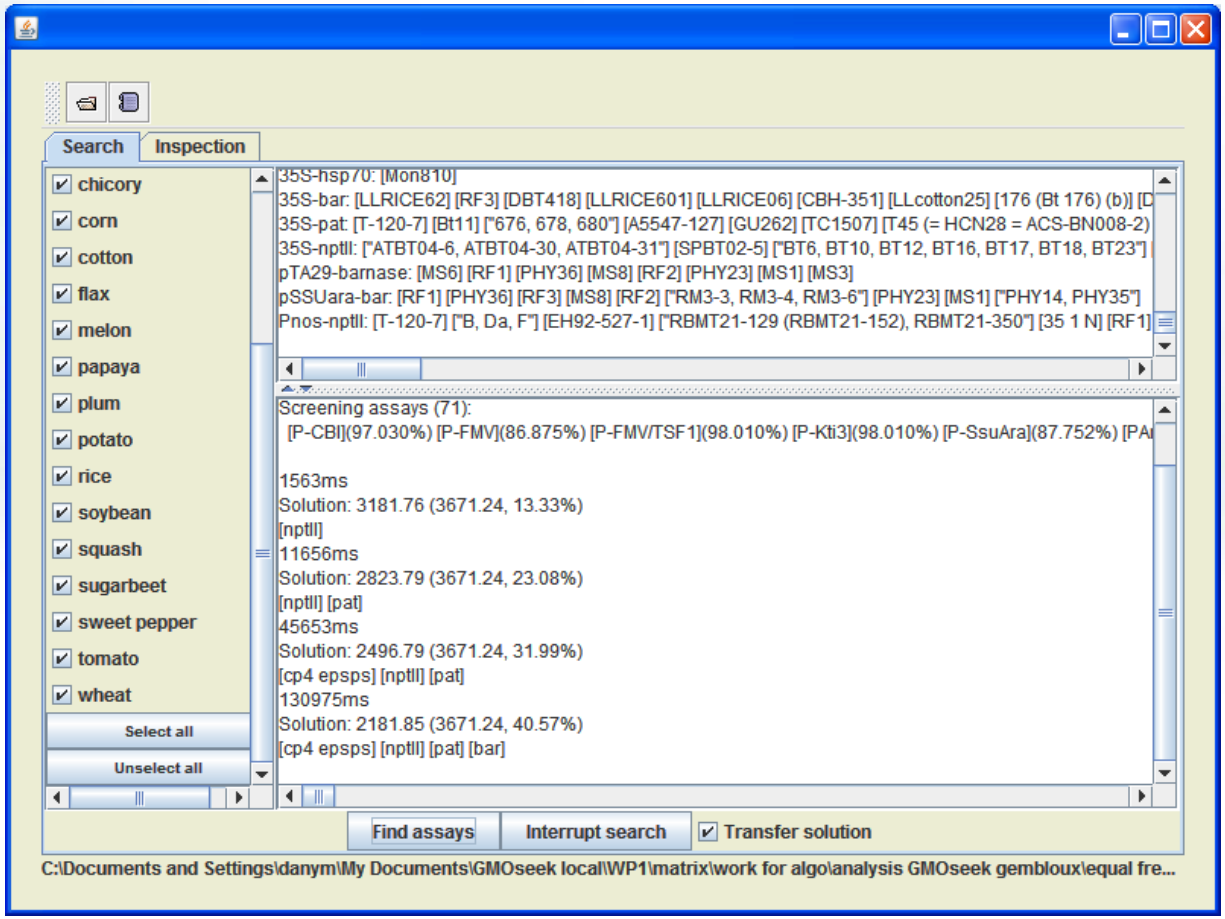
- Proposal of smart selection of screening elements to be used on samples to have as high coverage as possible
  - Highest GMO coverage
  - Cost efficient GMO detection
  - Discovery of new useful methods

GMOseek SOFTWARE

Different purposes:

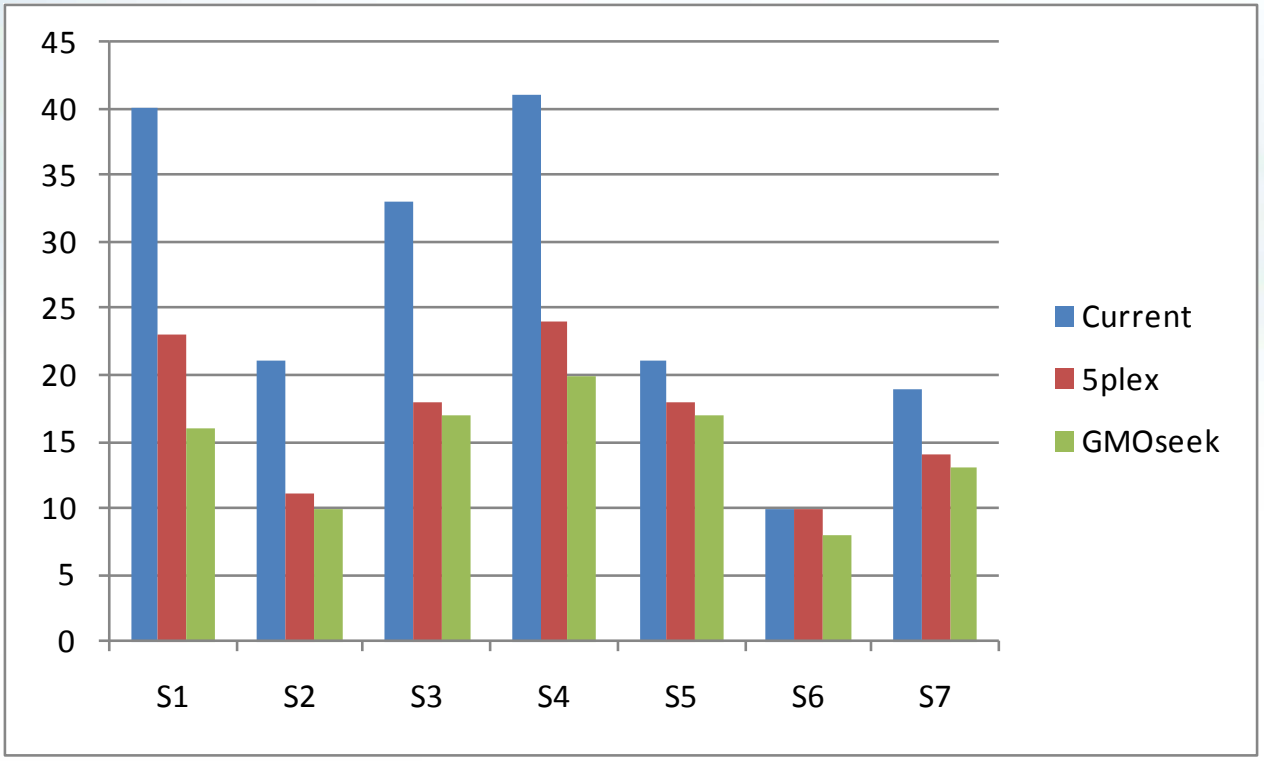
- Decision support system for daily use in labs (NIB already uses it daily). Help to decide which GMOs to test after screening phase

Smart selection of screening elements



Comparison of diferent screening elements used

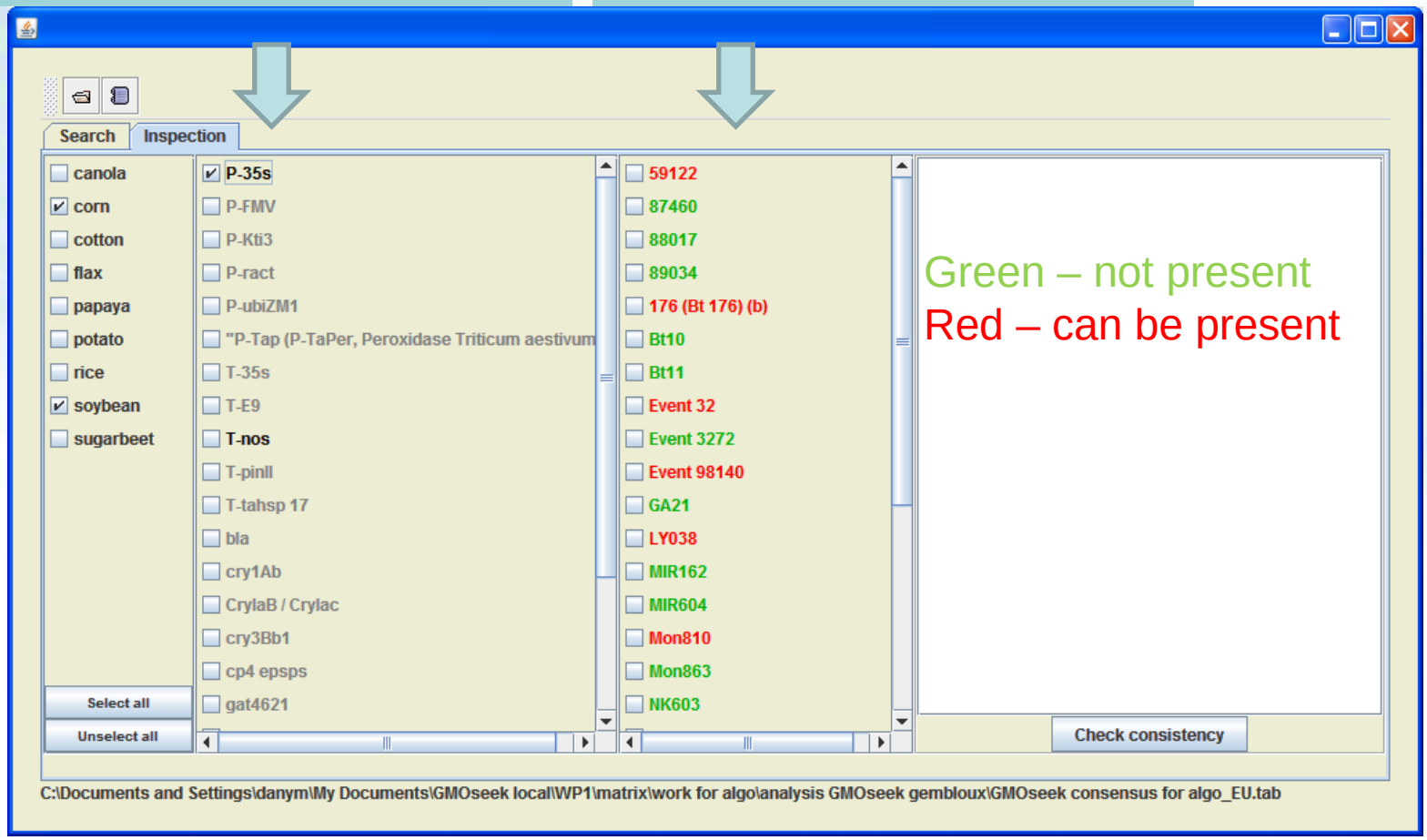
Number of screening analyses needed



Decission support

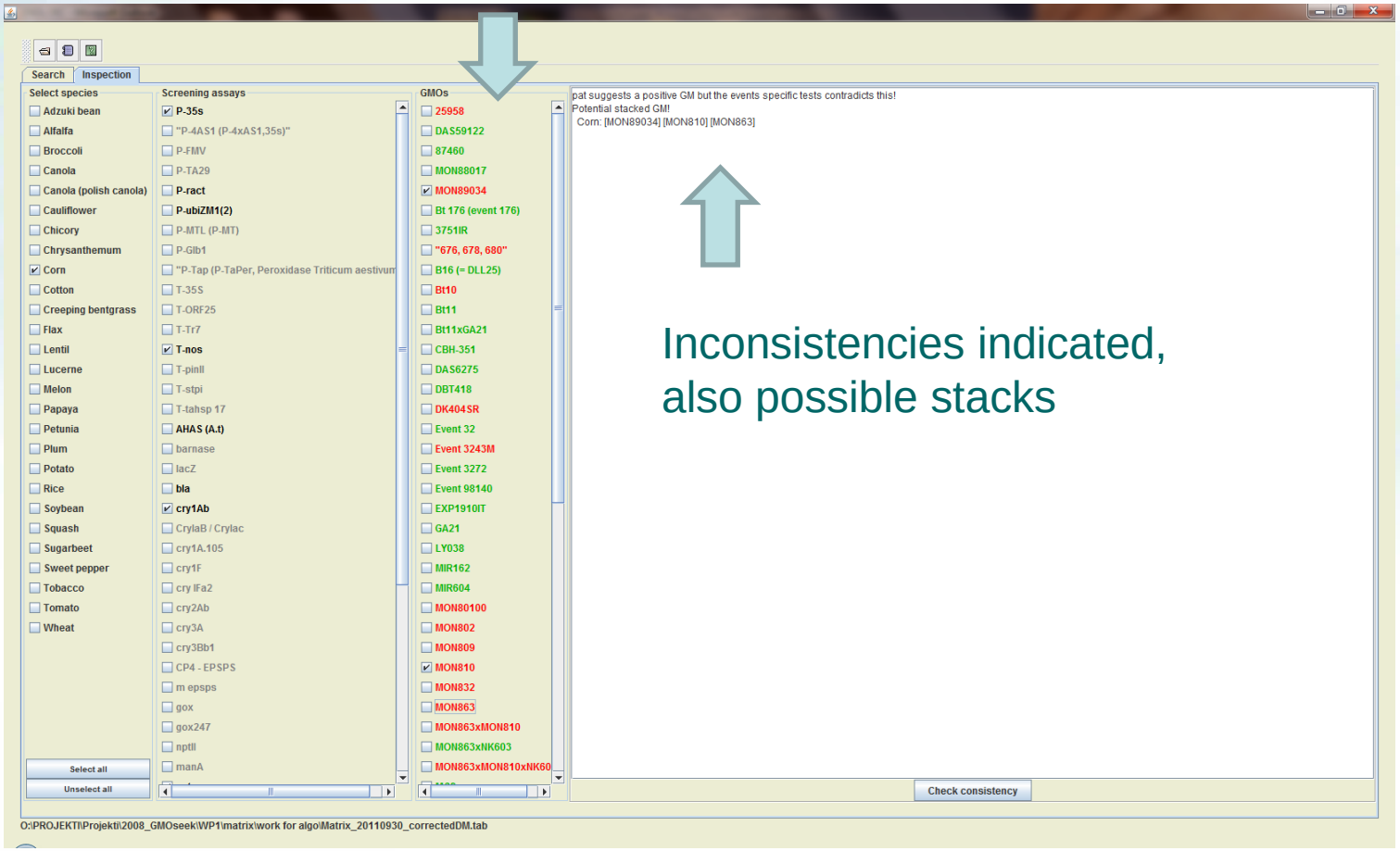
Screening results introduced

GMOs proposed to be tested



Decission support

Results of GMOs identified introduced



Inconsistencies indicated,
also possible stacks

GENEIO– in plan to be used in NIB for GMO analyses

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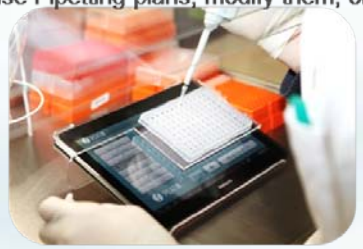

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NEW MOLECULAR TECHNOLOGIES

- Digital PCR
- New generation sequencing
- Isothermal methods (e.g. LAMP)

- DECATHLON 7FPproject – expected start 2014

ENGL ACTIVE WG

- Working group on Detection, Interpretation and Reporting on the presence of authorised versus unauthorised genetically modified materials.
- WG Method Performance Requirements
- Working Group Sample Preparation Procedure
- Advisory Group Method Selection for Validation
- WG for identification of stacked GM events

NIB member of all WG

NIB SCIENTIFIC PUBLICATIONS 2013 (GMOs)

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