Coping with the growing number of GMOs - towards a universal detection strategy

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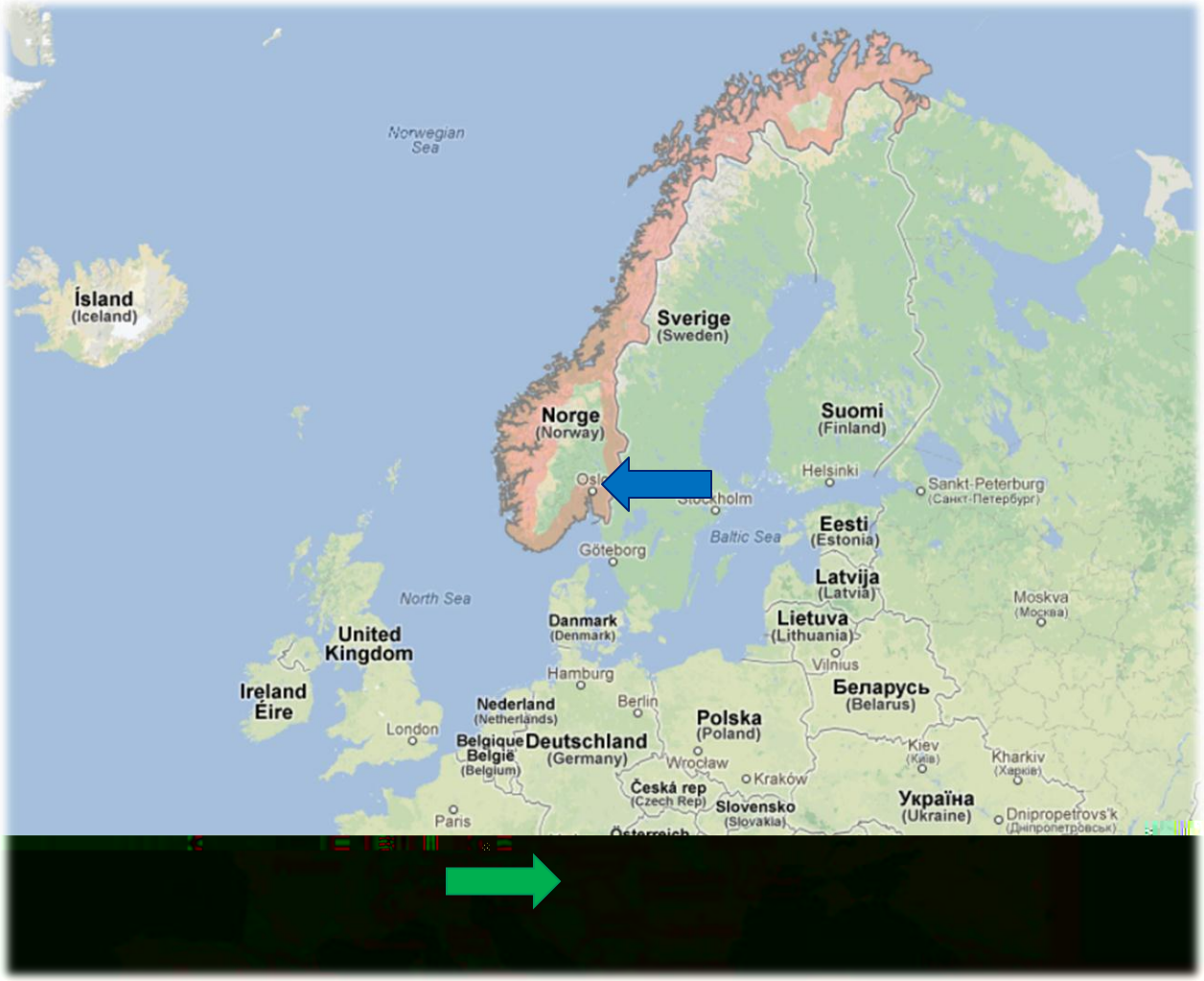
Norway



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Challenges

- Increasing no's of GMOs with increasing no's of:
  - Developers: countries, universities and companies
  - Field trials organized by the developers
  - Crops subjected to genetic modifications
  - Sequenced/isolated putatively suitable elements
    - Promoters, genes, terminators + complete gene cassettes
    - Haplo-/genotypes of these elements, including synthetic
- Lack of corresponding:
  - Transparency: developments, field trials, sequences
  - Method and detection technology developments

Detection strategies (DNA-based)

- Amplification methods:
  - Simplex PCR/isothermal
  - Oligoplex and multiplex PCR/isothermal
- Detection/identification principles
  - Non-specific fluorophore (e.g. SYBR green)
  - Agarose gel electrophoresis
  - Capillary gel electrophoresis
  - Bead-/flow cytometry (e.g. Luminex)
  - Hybridisation probe (e.g. TaqMan)
  - Macro- and microarrays
  - DNA-sequencing (Sanger and Next Generation)

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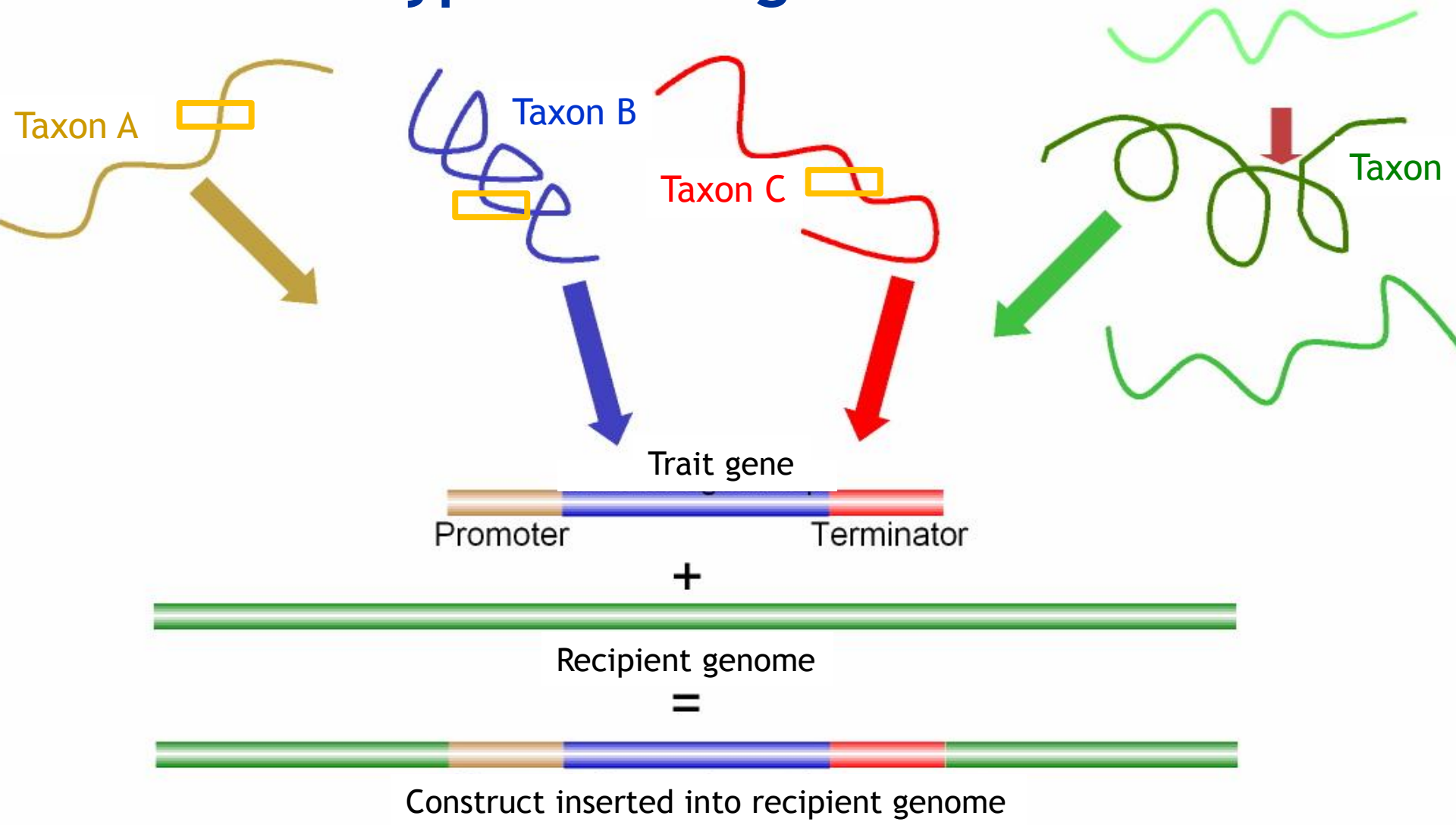
Typical transgenic scenario



Availability of sequence information:

Insert Sequence Knowledge (ISK)-classes:

- Fully characterized GMOs (ISK-class 1)
- Transformed with known constructs: unknown where/if rearranged (ISK-class 2)
- Transformed with constructs divergent from those of ISK-class 1 GMOs but containing genetic elements present also in ISK-class 1 GMOs (ISK-class 3)
- Transformed with genetic elements not used in GMOs of other ISK-classes (ISK-class 4)

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PCR method reliability

• Complete sequence data and/or event specific method available	ISK 1	Event specific	• 100% correct identification (ID) • Optimal for quantitation (QN)
• Construct in GMO detectable with available construct specific method	ISK 2	Construct specific	• Certain to be GMO • Not reliable for ID • Quite reliable for QN
• Inserted element(s) detectable with existing element specific method	ISK 3	Element specific	• Screening targets • May come from non-GM • Suitable for semi-QN
• GMOs, referenced in public domain (no method/sequence data)	ISK 4a	No PCR	• Need for alternatives to PCR • Sequence contig analysis…
• All GMOs, including those not referenced in public domain	ISK 4b		

Number of GMOs

Taxon specific

Detect (and quantify?) all events?

- Limit to dominant/declared ingredient taxa
  - Or all detected taxa?
- Interested only in authorized/unauthorized?
- Feasibility decreasing as no. of GMOs increase
  - Workload and costs may become prohibitive

- Proposed (temporary) solutions:
  - Multiplexing, incl. arrays and pre-spotted plates
  - Matrix approach targeting common elements (screen)

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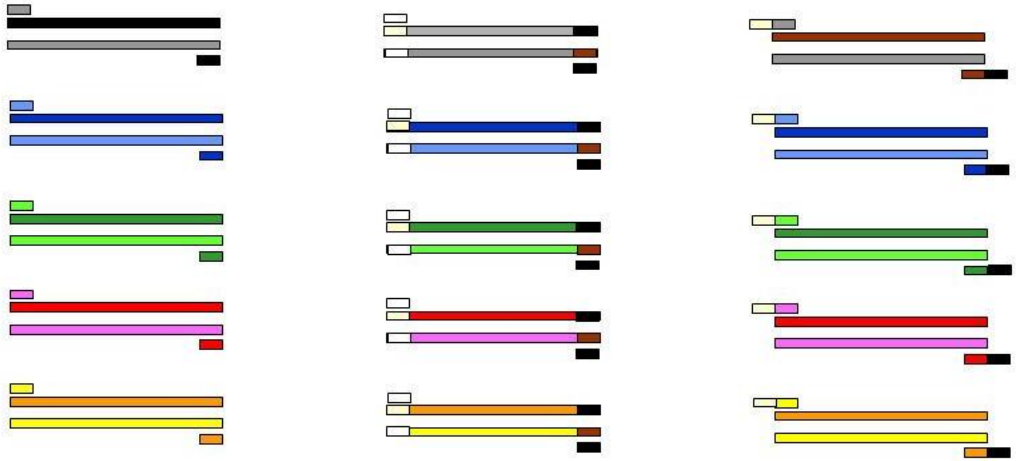
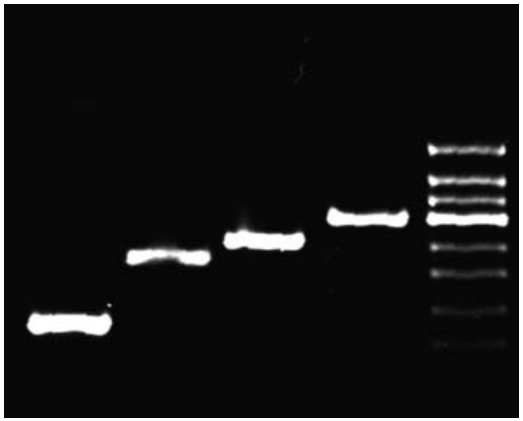
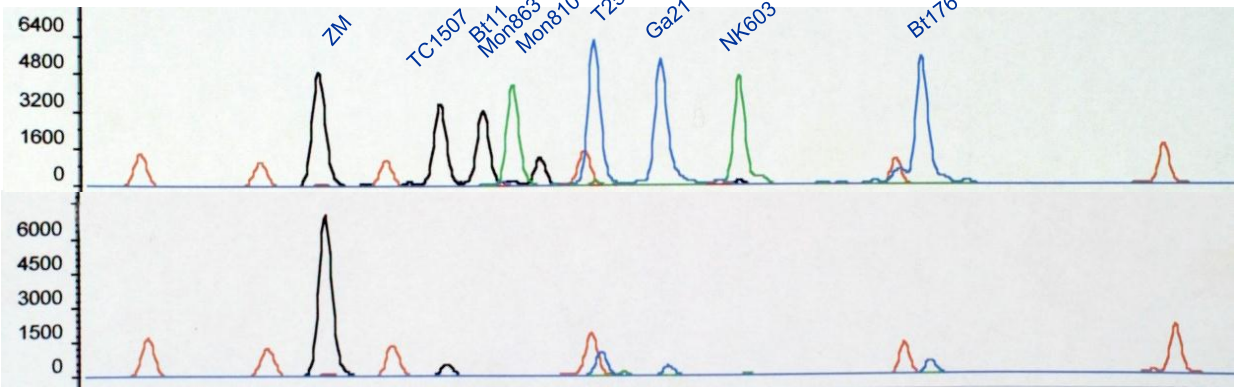
Multiplex amplification and detection







GMO screening microarray

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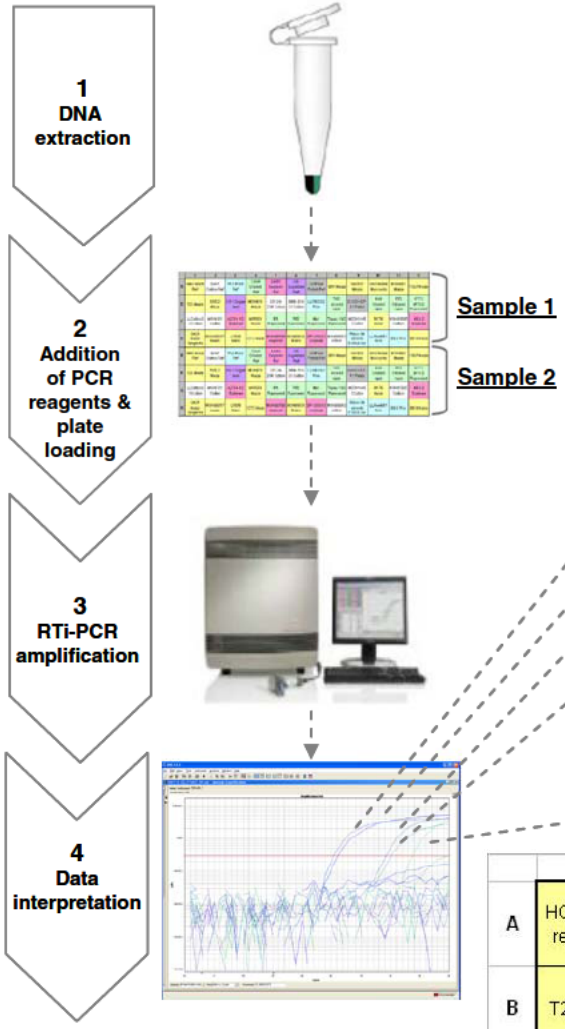
Multiple parallel analyses



Well	Method	Detector	Reporter	Ct
1	HMG	FAM-TAMRA	FAM	26.097576
2	Cotton	FAM-TAMRA	FAM	Undetermined
3	Rice	FAM-TAMRA	FAM	Undetermined
4	OSR	FAM-TAMRA	FAM	Undetermined
5	Soya	FAM-TAMRA	FAM	25.456295
6	SB	FAM-TAMRA	FAM	Undetermined
7	Potato	FAM-TAMRA	FAM	Undetermined
8	Bt11	FAM-TAMRA	FAM	Undetermined
9	NK603	FAM-TAMRA	FAM	35.07207
10	GA21	FAM-TAMRA	FAM	Undetermined
11	MON863	FAM-TAMRA	FAM	Undetermined
12	1507	FAM-TAMRA	FAM	Undetermined
13	T25	FAM-TAMRA	FAM	Undetermined
14	59122	FAM-TAMRA	FAM	Undetermined
15	H7-1	FAM-TAMRA	FAM	Undetermined
16	MON810	FAM-TAMRA	FAM	34.13422
17	281	FAM-TAMRA	FAM	Undetermined
18	3006	FAM-TAMRA	FAM	Undetermined
19	LL62	FAM-TAMRA	FAM	Undetermined
20	T45	FAM-TAMRA	FAM	Undetermined
21	EH-92	FAM-TAMRA	FAM	Undetermined
22	Ms8	FAM-TAMRA	FAM	Undetermined
23	Rf3	FAM-TAMRA	FAM	Undetermined
24	GT73	FAM-TAMRA	FAM	Undetermined
25	LL25	FAM-TAMRA	FAM	Undetermined
26	531	FAM-TAMRA	FAM	Undetermined
27	A2704	FAM-TAMRA	FAM	Undetermined
28	MIR604	FAM-TAMRA	FAM	Undetermined
29	Rf1	FAM-TAMRA	FAM	Undetermined
30	Rf2	FAM-TAMRA	FAM	Undetermined
31	Ms1	FAM-TAMRA	FAM	Undetermined
32	Topas	FAM-TAMRA	FAM	Undetermined
33	1445	FAM-TAMRA	FAM	Undetermined
34	Bt176	FAM-TAMRA	FAM	Undetermined
35	15985	FAM-TAMRA	FAM	Undetermined
36	40-3-2	FAM-TAMRA	FAM	38.601147
37	GA21	FAM-TAMRA	FAM	Undetermined
38	88017	FAM-TAMRA	FAM	Undetermined
39	LY038	FAM-TAMRA	FAM	Undetermined
40	3272	FAM-TAMRA	FAM	Undetermined

From:
Querci et al. (2010)
Anal. Bioanal. Chem. 396: 1991-2002

	1	2	3	4	5	6	7	8	9	10	11	12
A	HGM Maize reference	SAH7 Cotton reference	PLD Rice reference	CruA Oilseed reference	Lectin Soybean reference	GS Sugar beet reference	UGPase Potato reference	Bt11 Maize	NK603 Maize	GA21Maize Monsanto	MON863 Maize	1507 Maize
B	T25 Maize	59122 Maize	H7-1 Sugar beet	MON810 Maize	281-24-236 Cotton	3006-210-23 Cotton	LLRICE62 Rice	T45 oilseed rape	EH92-527-1 Potato	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 (RT63) Rapeseed
C	LLCotton25 Cotton	MON 531 Cotton	A2704-12 Soybean	MIR604 Maize	Rf1 Rapeseed	Rf2 Rapeseed	Ms1 Rapeseed	Topas 19/2 Rapeseed	MON1445 Cotton	Bt176 Maize	MON15985 Cotton	40-3-2 Soybean
D	GA21 Maize Syngenta	MON88017 maize	LY038 Maize	3272 Maize	MON89788 soybean	MON89034 Maize	DP-356043 soybean	MON88913 cotton	Rice GM events P35S::bar	LLRice601 Rice	Bt63 Rice	Bt10 Maize

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Reduce number of tests required by smart screening approach?

- Many GMOs transformed with similar constructs
  - Or at least with some of the same elements
- May also exploit other information
  - E.g. origin of sample (country, region)

- Several related concepts published
  - Each screening test must be validated for every GMO
  - With only theoretical data, risk of false negatives

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Species	GMO	Auth. status	Screen A	Screen B	Screen C	Screen D	Screen E
Cotton	Cotton A	A	+	-	-	-	-
	Cotton B	A	-	+	-	-	-
	Cotton C	U	+	+	-	-	-
Maize	Maize A	A	+	-	-	-	-
	Maize B	A	+	+	-	-	-
	Maize C	A	+	-	+	-	-
	Maize D	A	+	-	+	+	-
	Maize E	A	-	+	-	-	+
	Maize F	A	+	-	-	+	-
	Maize G	U	+	-	-	+	-
	Maize H	U	+	+	-	-	-
	Maize I	U	+	-	-	+	+
Potato	Potato A	A	+	+	-	-	-
	Potato B	A	+	+	-	+	-
	Potato C	U	+	+	+	-	-
Rapeseed	Rapeseed A	A	+	-	+	+	+
	Rapeseed B	A	+	+	-	-	-
	Rapeseed C	A	+	+	-	+	-
	Rapeseed D	U	+	+	+	-	-
	Rapeseed E	U	-	+	-	+	-
Rice	Rice A	U	+	-	-	+	-
	Rice B	U	-	+	-	+	-
	Rice C	U	+	-	+	-	+
	Rice D	U	+	+	-	-	-
Soybean	Soybean A	A	+	+	-	+	-
	Soybean B	A	-	+	-	+	-
	Soybean C	A	-	-	+	-	+
	Soybean D	U	+	-	-	-	+
	Soybean E	U	-	+	-	+	-
	Soybean F	U	+	-	+	-	-
Sugarbeet	Sugarbeet A	A	+	+	-	-	-
	Sugarbeet B	U	+	-	+	-	-

From:
Holst-Jensen et al. (2013)
Chapter 20 in (Bertehau Y. ed.)
Genetically modified and non-genetically modified food supply chains:
Co-existence and traceability. Wiley-Blackwell. ISBN 978-1-4443-3778-5

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Matrix approach applied

Species	GMO	Auth. status	Screen A	Screen B	Screen C	Screen D	Screen E	
Cotton		Not tested						
Maize	Maize A	A	+	-	-	-	-	Possible < 0.9%
	Maize B	A	+	+	-	-	-	Possible < 0.9%
	Maize C	A	+	-	+	-	-	Figure
	Maize D	A	+	-	+	+	-	Not possible
	Maize E	A	-	+	-	-	+	Not possible
	Maize F	A	+	-	-	+	-	Not possible
	Maize G	U	+	-	-	+	-	Not possible
	Maize H	U	+	+	-	-	-	Possible Event specific test
	Maize I	U	+	-	-	+	+	Not possible
Potato		Not tested						
Rapeseed		Negative in test						
Rice		Not tested						
Soybean	Soybean A	A	+	+	-	+	-	Not possible
	Soybean B	A	-	+	-	+	-	Not possible
	Soybean C	A	-	-	+	-	+	Not possible
	Soybean D	U	+	-	-	-	+	Not possible
	Soybean E	U	-	+	-	+	-	Not possible
	Soybean F	U	+	-	+	-	-	Not possible
Sugarbeet		Not tested						
SAMPLE	Test result		Positive 0.5% of maize	Positive 0.3% of maize	Negative	Negative	Negative	Negative

Example: Maize product with some soybean

From:
Holst-Jensen et al. (2013)

Species	GMO	Auth. status	Screen A	Screen B	Screen C	Screen D	Screen E		QN test ?	Event specific test?
Cotton		Negative in test								
Maize	Maize A	A	+	-	-	-	-	Possible	Yes! <0.3%	
	Maize B	A	+	+	-	-	-	Possible	Yes! <0.3%	
	Maize C	A	+	-	+	-	-	Not possible		
	Maize D	A	+	-	+	+	-	Not possible		
	Maize E	A	-	+	-	-	+	Possible	No	
	Maize F	A	+	-	-	+	-	Possible	No	
	Maize G	U	+	-	-	+	-	Possible		Yes! Negative
	Maize H	U	+	+	-	-	-	Possible		Yes! Negative
	Maize I	U	+	-	-	+	+	Possible		Yes! Positive
Potato		Negative in test								
Rapeseed	Rapeseed A	A	+	-	+	+	+	Not possible		
	Rapeseed B	A	+	+	-	-	-	Possible	No	
	Rapeseed C	A	+	+	-	+	-	Possible	No	
	Rapeseed D	U	+	+	+	-	-	Not possible		
	Rapeseed E	U	-	+	-	+	-	Possible		No
Rice		Negative in test								
Soybean	Soybean A	A	+	+	-	+	-	Possible	Yes! <0.2%	
	Soybean B	A	-	+	-	+	-	Possible	No	
	Soybean C	A	-	-	+	-	+	Not possible		
	Soybean D	U	+	-	-	-	+	Possible		Yes! Negative
	Soybean E	U	-	+	-	+	-	Possible		Yes! Negative
	Soybean F	U	+	-	+	-	-	Not possible		
Sugarbeet	Sugarbeet A	A	+	+	-	-	-	Possible	No	
	Sugarbeet B	U	+	-	+	-	-	Not possible		
SAMPLE	Test result		Positive	Positive	Negative	Positive	Positive			
Ingredient quantities:	maize (very high)	>	soybean (medium)	>	rapeseed (low)	>	sugarbeet (very low)			

Example: Feed product with uncertain composition

From:
Holst-Jensen et al. (2013)

Species	GMO	Auth. status	Screen A	Screen B	Screen C	Screen D	Screen E		QN test ?	Event specific test?
Cotton		Negative in test								
Maize	Maize A	A	+	-	-	-	-	Possible	Yes! <0.3%	
	Maize B	A	+	+	-	-	-	Possible	Yes! <0.3%	
	Maize C	A	+	-	+	-	-	Not possible		
	Maize D	A	+	-	+	+	-	Not possible		
	Maize E	A	-	+	-	-	+	Possible	No	
	Maize F	A	+	-	-	+	-	Possible	No	
	Maize G	U	+	-	-	+	-	Possible		Yes! Negative
	Maize H	U	+	+	-	-	-	Possible		Yes! Negative
	Maize I	U	+	-	-	+	+	Possible		Yes! Positive
Potato		Negative in test								
Rapeseed	Rapeseed A	A	+	-	+	+	+	Not possible		
	Rapeseed B	A	+	+	-	-	-	Possible	No	
	Rapeseed C	A	+	+	-	+	-	Possible	No	
	Rapeseed D	U	+	+	+	-	-	Not possible		
	Rapeseed E	U	-	+	-	+	-	Possible		No
Rice		Negative in test								
Soybean	Soybean A	A	+	+	-	+	-	Possible	Yes! <0.2%	
	Soybean B	A	-	+	-	+	-	Possible	No	
	Soybean C	A	-	-	+	-	+	Not possible		
	Soybean D	U	+	-	-	-	+	Possible		Yes! Negative
	Soybean E	U	-	+	-	+	-	Possible		Yes! Negative
	Soybean F	U	+	-	+	-	-	Not possible		
Sugarbeet	Sugarbeet A	A	+	+	-	-	-	Possible	No	
	Sugarbeet B	U	+	-	+	-	-	Not possible		
SAMPLE	Test result		Positive	Positive	Negative	Positive	Positive			

Ingredient quantities: maize (very high) > soybean (medium) > rapeseed (low) > sugarbeet (very low)

Example: Feed product with uncertain composition

From:
Holst-Jensen et al. (2013)

Species	GMO	Auth. status	Screen A	Screen B	Screen C	Screen D	Screen E		QN test ?	Event specific test?
Cotton		Negative in test								
Maize	Maize A	A	+	-	-	-	-	Possible	Yes! <0.3%	
	Maize B	A	+	+	-	-	-	Possible	Yes! <0.3%	
	Maize C	A	+	-	+	-	-	Not possible		
	Maize D	A	+	-	+	+	-	Not possible		
	Maize E	A	-	+	-	-	+	Possible	No	
	Maize F	A	+	-	-	+	-	Possible	No	
	Maize G	U	+	-	-	+	-	Possible		Yes! Negative
	Maize H	U	+	+	-	-	-	Possible		Yes! Negative
	Maize I	U	+	-	-	+	+	Possible		Yes! Positive
Potato		Negative in test								
Rapeseed	Rapeseed A	A	+	-	+	+	+	Not possible		
	Rapeseed B	A	+	+	-	-	-	Possible	No	
	Rapeseed C	A	+	+	-	+	-	Possible	No	
	Rapeseed D	U	+	+	+	-	-	Not possible		
	Rapeseed E	U	-	+	-	+	-	Possible		No
Rice		Negative in test								
Soybean	Soybean A	A	+	+	-	+	-	Possible	Yes! <0.2%	
	Soybean B	A	-	+	-	+	-	Possible	No	
	Soybean C	A	-	-	+	-	+	Not possible		
	Soybean D	U	+	-	-	-	+	Possible		Yes! Negative
	Soybean E	U	-	+	-	+	-	Possible		Yes! Negative
	Soybean F	U	+	-	+	-	-	Not possible		
Sugarbeet	Sugarbeet A	A	+	+	-	-	-	Possible	No	
	Sugarbeet B	U	+	-	+	-	-	Not possible		
SAMPLE	Test result		Positive	Positive	Negative	Positive	Positive			
Ingredient quantities:	maize (very high)	>	soybean (medium)	>	rapeseed (low)	>	sugarbeet (very low)			

Example: Feed product with uncertain composition

From:
Holst-Jensen et al. (2013)

High throughput sequencing

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Information and method availability

- Reported GMOs
  - LMO registry of BCH under CBD http://bch.cbd.int/database/organisms/
  - Competent authorities, approvals and applications
  - Literature and internet
- Methods reported in literature + databases, e.g.:
  - EU-RL GMFF gmo-crl.jrc.ec.europa.eu/gmomethods/
  - GMDD http://gmdd.shgmo.org/
- Sequence information:
  - EMBL/NCBI GenBank/DDBJ
  - Patent applications
  - Literature (and internet)

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Unharmonized world

- Reporting on GMOs
  - Who reports to LMO registry of BCH, and what?
  - Nations transparent? Language can be a challenge
  - Property rights protection, intended secrecy
- Methods reported in literature + databases
  - Uncertain validation status, access ref.materials
  - Compatibility/complementarity to existing methods
- Sequence information:
  - Access to databases?
  - Quality/reliability, updating, new input to database?
  - Confidential info. for detection, not for competition

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ID	Description	
442 record(s) found		
	2007	1 record
15395	Tomato 1345-4 (delayed ripening)	
	2008	55 records
	2009	6 records
	2010	
	2011	
	2012	
	2013	

Characteristics of the transformation process

Techniques used for the modification

- Agrobacterium-mediated DNA transfer

Introduced or modified genetic elements

Some of these genetic elements may be present as fragments or truncated forms. Please see notes below, where applicable.

1-amino-cyclopropane-1-carboxylic acid deaminase - Pseudomonas chlororaphis — Record #15013
Changes in physiology and/or production - Ripening

Neomycin Phosphotransferase II - Escherichia coli — Record #15001
Resistance to antibiotics - Kanamycin

Notes regarding the genetic elements introduced or modified in this LMO

Delayed ripening tomatoes, created through introduction of a gene sequence encoding the enzyme 1-amino-cyclopropane -1-carboxylic acid deaminase (ACCd) that metabolizes the precursor of the fruit ripening hormone ethylene. The neomycin phosphotransferase II (npt II) confers resistance to the antibiotic kanamycin.

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	2009	**6 records**
45938	Genetically modified Escherichia coli for use as DNA 'biomarker' molecules (GMD04096)	
45939	Genetically modified Pseudomonas fluorescens for use as DNA 'biomarker' molecules (GMD04096)	
45954	Saccharomyces cerevisiae as modified by gene deletions in which the open reading frame (ORF) of a gene is replaced with a deletion cassette module (GMC05018)	
45976	Escherichia coli (non-pathogenic strains) modified with 5'methylthioadenosine nucleosidase gene from Klebsiella pneumoniae (GMD04022)	
45979	Escherichia coli (non-pathogenic strains) modified with 5'methylthioadenosine nucleosidase gene from Pseudomonas aeruginosa (GMD04022)	
100333	Sugar cane resistant to insects	

Characteristics of the transformation process

Notes regarding the genetic elements introduced or modified in this LMO

The sugar cane variety Ja.60-5 was transformed with a cry1a(b) gene to introduce resistance to insects.
No further information is available on this gene or LMO.

LMO characteristics

Common use(s)

- Food
- Feed
- Biofuel

Additional Information

Additional Information

This LMO record refers to multiple transformation events.

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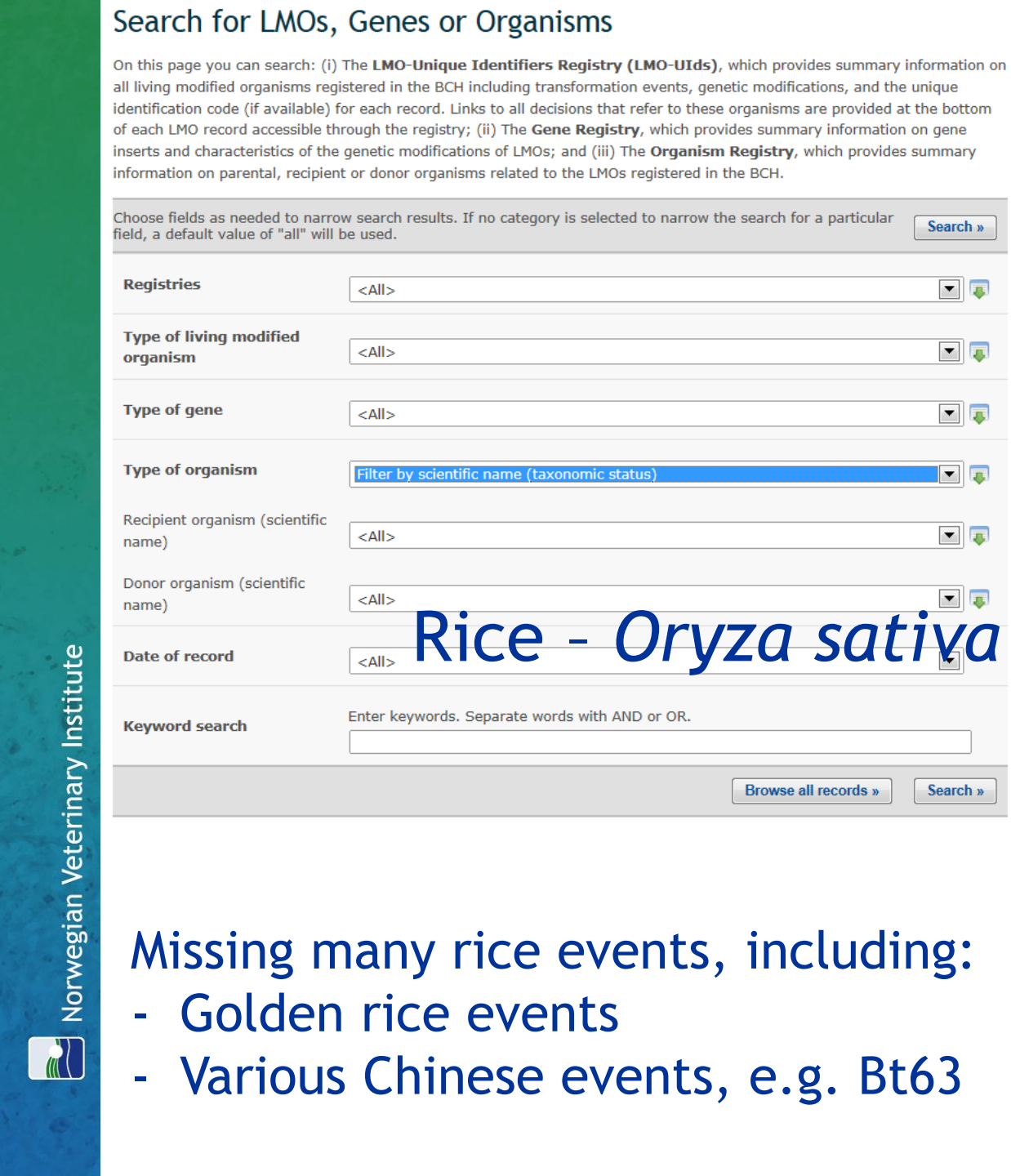


Search for LMOs, Genes or Organisms

On this page you can search: (i) The **LMO-Unique Identifiers Registry (LMO-UIds)**, which provides summary information on all living modified organisms registered in the BCH including transformation events, genetic modifications, and the unique identification code (if available) for each record. Links to all decisions that refer to these organisms are provided at the bottom of each LMO record accessible through the registry; (ii) The **Gene Registry**, which provides summary information on gene inserts and characteristics of the genetic modifications of LMOs; and (iii) The **Organism Registry**, which provides summary information on parental, recipient or donor organisms related to the LMOs registered in the BCH.

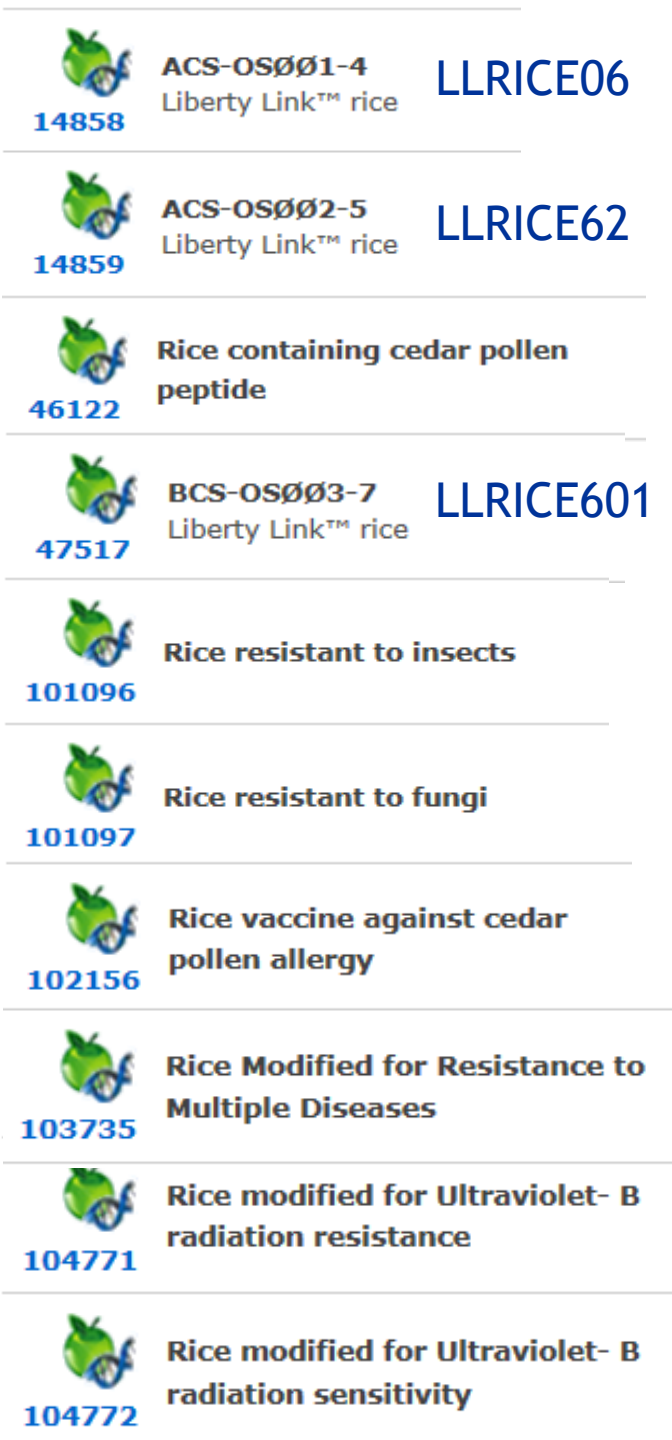
Choose fields as needed to narrow search results. If no category is selected to narrow the search for a particular field, a default value of "all" will be used. Search »

Registries	<All>
Type of living modified organism	<All>
Type of gene	<All>
Type of organism	Filter by scientific name (taxonomic status)
Recipient organism (scientific name)	<All>
Donor organism (scientific name)	<All>
Date of record	<All>
Keyword search	Enter keywords. Separate words with AND or OR.

Browse all records » Search »

Rice – *Oryza sativa*

14858	**ACS-OSØØ1-4** Liberty Link™ rice	LLRICE06
14859	**ACS-OSØØ2-5** Liberty Link™ rice	LLRICE62
46122	**Rice containing cedar pollen peptide**	
47517	**BCS-OSØØ3-7** Liberty Link™ rice	LLRICE601
101096	**Rice resistant to insects**	
101097	**Rice resistant to fungi**	
102156	**Rice vaccine against cedar pollen allergy**	
103735	**Rice Modified for Resistance to Multiple Diseases**	
104771	**Rice modified for Ultraviolet- B radiation resistance**	
104772	**Rice modified for Ultraviolet- B radiation sensitivity**	



Missing many rice events, including:

- Golden rice events
- Various Chinese events, e.g. Bt63

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What can we do to achieve a Globally harmonized detection strategy?

- Transparency and sharing of information on
  - What is being developed, field trials, etc.
  - Sequences or at least detailed methods
  - Reference materials (seeds, DNA, sequence info.)
- Centralized and stable depository
  - Sequence database, high quality control
  - Reference materials, capacity to provide RMs
  - Methods database, coupling sequence and RM
- … and finally …

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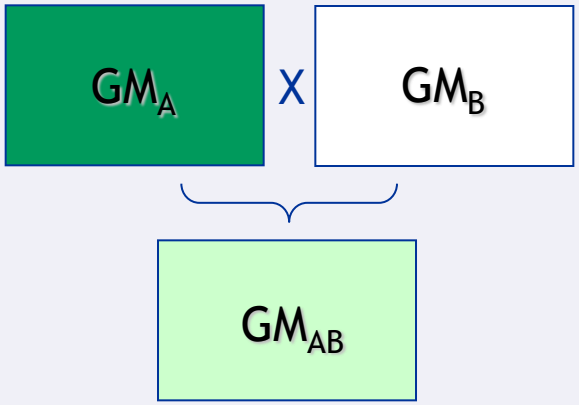
Four generations of GMOs

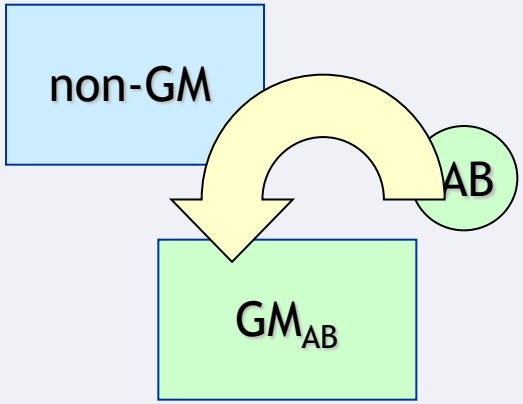
- First generation:
  - Transgene GMOs; one novel trait
- Second generation:
  - Combined transgenes (stacking)
- Third generation:
  - Near intragenic GMOs
- Fourth generation:
  - True intragenes og cisgenes

Stacking (combined GMOs)

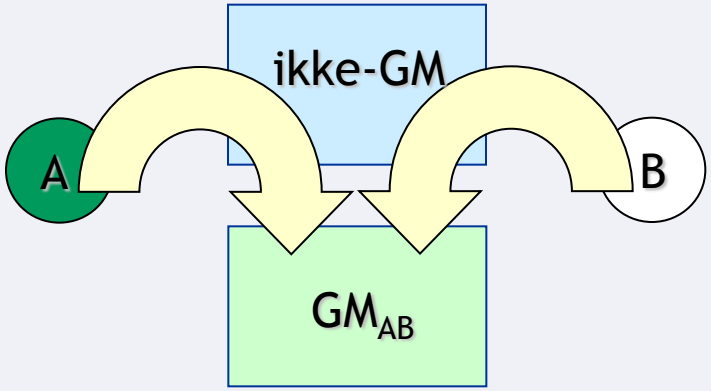
Crossing of 2 GMOs:



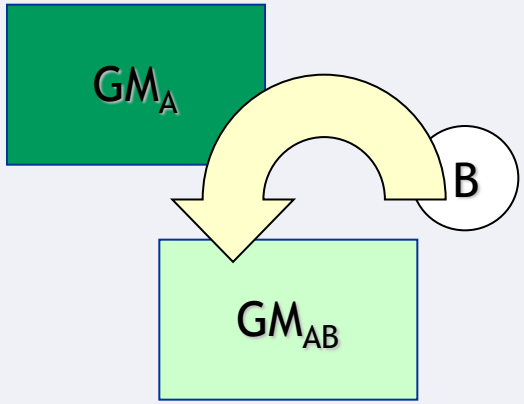
Transformation with multi gene construct:



Co-transformation with two gene constructs:



Re-transformation of a GMO:



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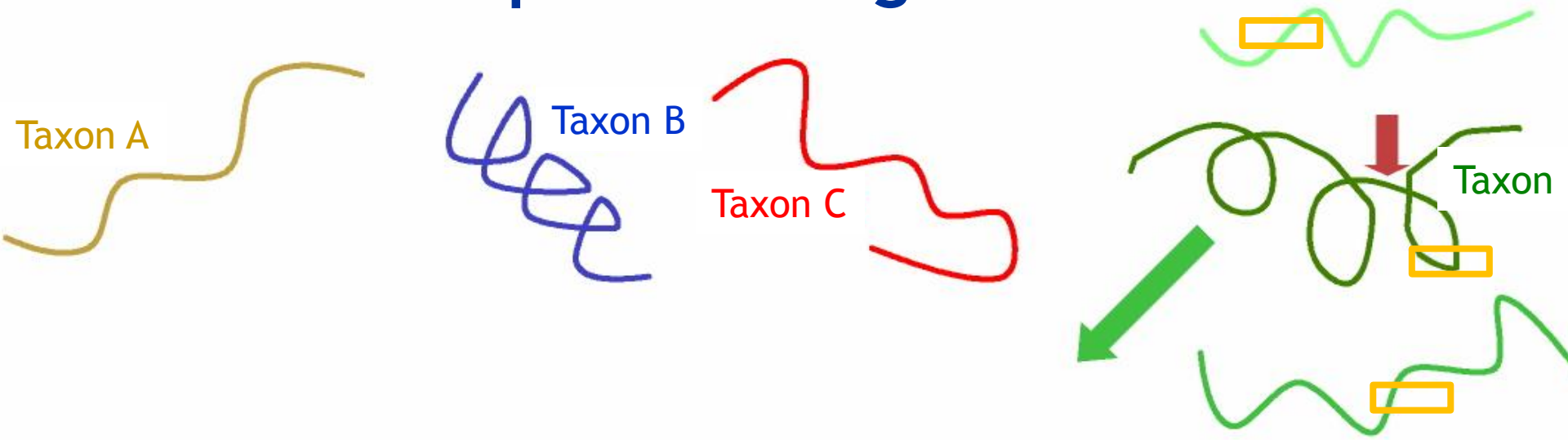
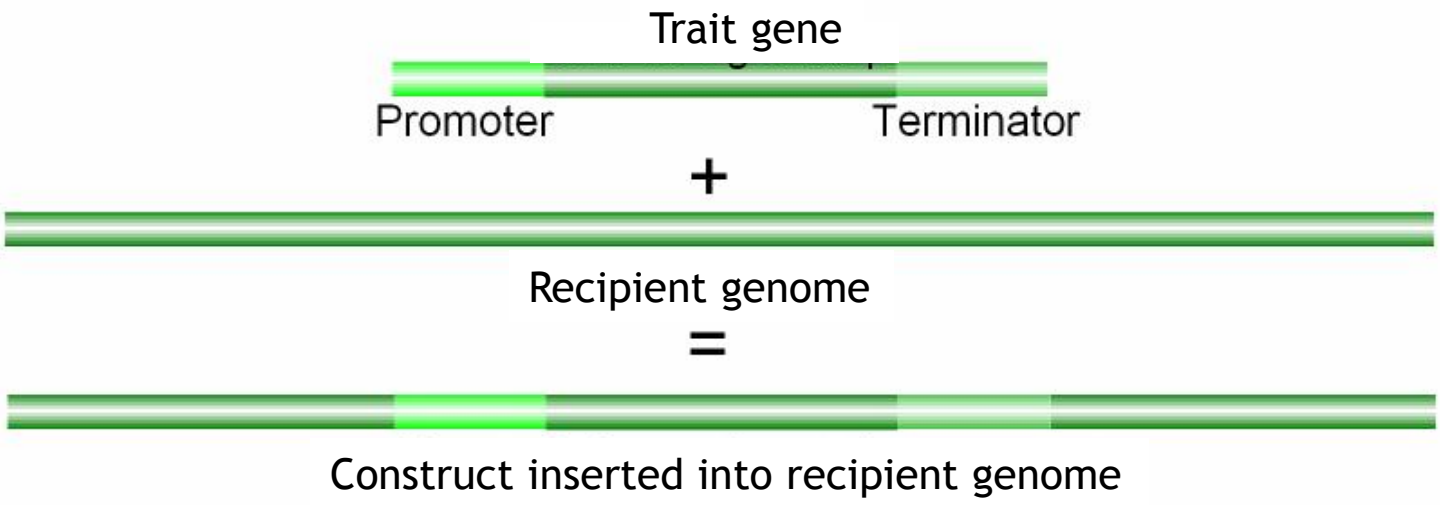
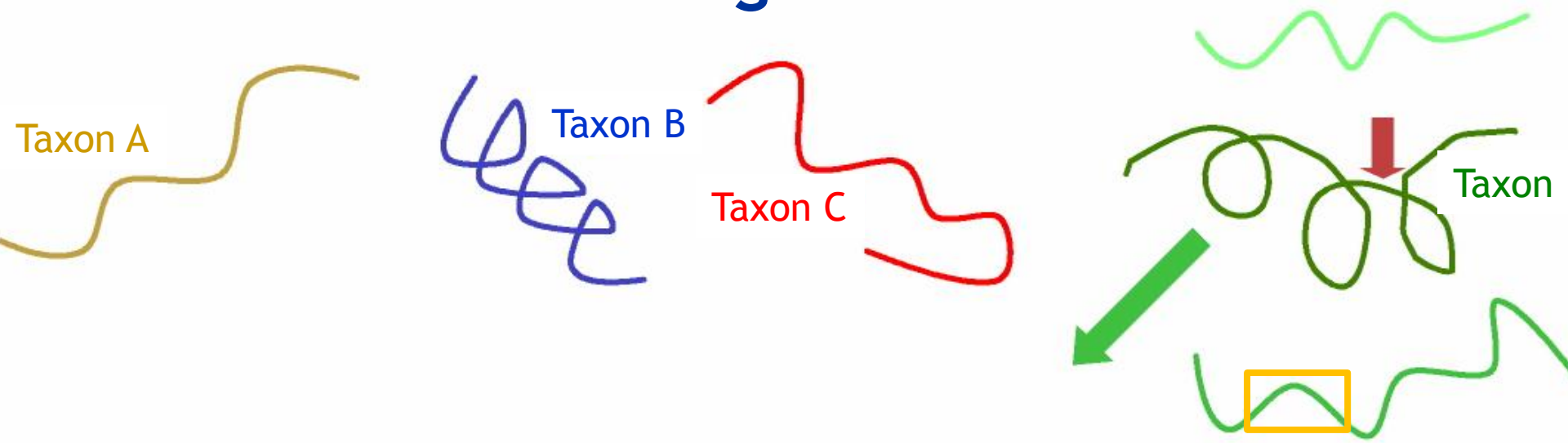
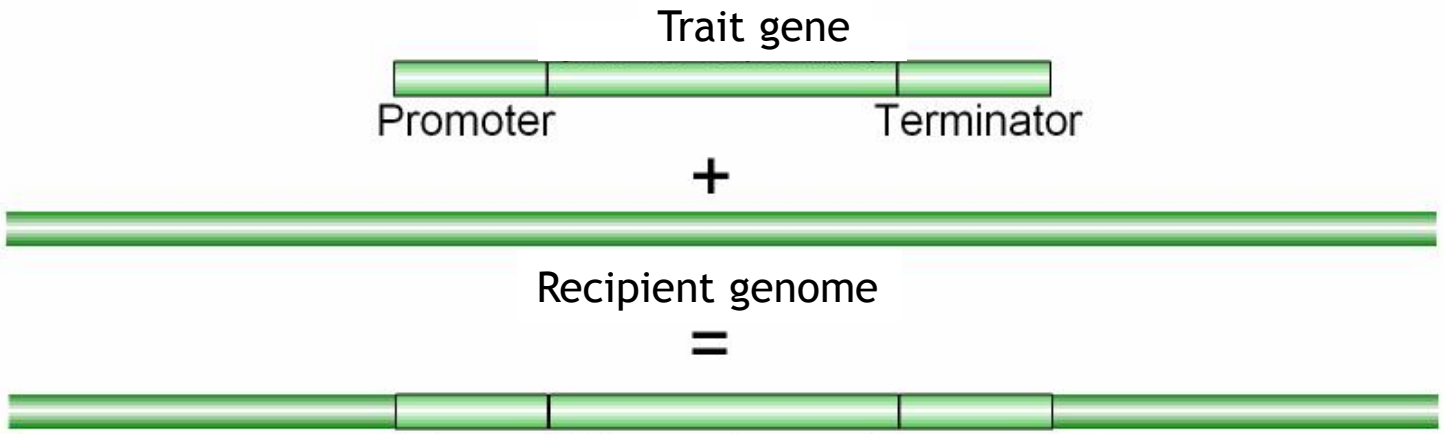
Example of intragenic scenario

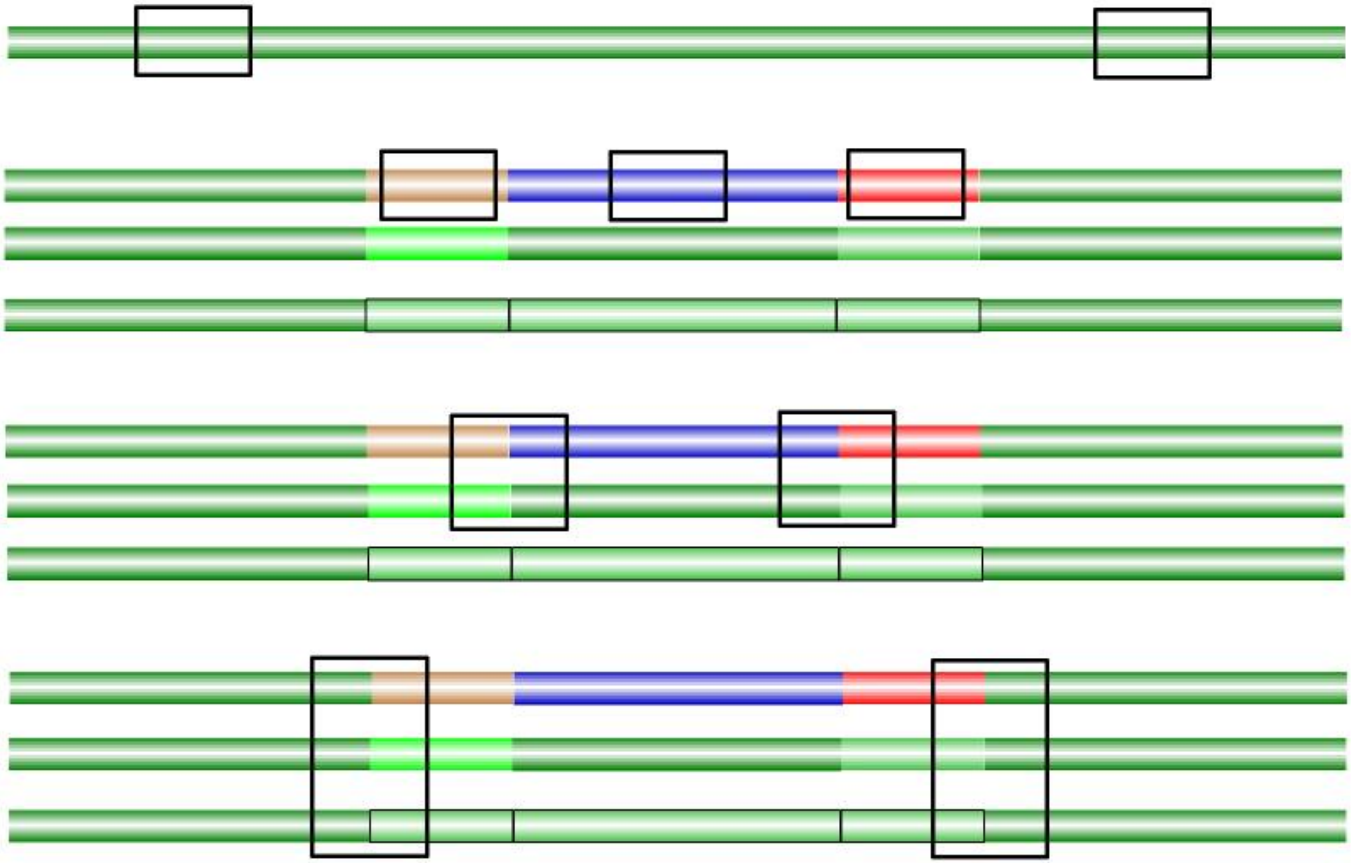




True cisgenic scenario







Taxon specific motif
(Any chromosome)

Element specific motif
(Only for screening)

Construct specific motif
(= GMO but not for ID)

Event specific motif
(Certain ID of GMO)

- ISK-clas detection:
  - ISK-1: All motifs
  - ISK-2: Not “event”
  - ISK-3: Only element
  - ISK-4: Unknown target!
- Source based detection:
  - Transgene: All motifs
  - Intragene: Not element
  - Cisgene: Only “event”

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What can we do to achieve a Globally harmonized detection strategy?

- Transparency and sharing of information on
  - What is being developed, field trials, etc.
  - Sequences or at least detailed methods
  - Reference materials (seeds, DNA, sequence info.)
- Centralized and stable depository
  - Sequence database, high quality control
  - Reference materials, capacity to provide RMs
  - Methods database, coupling sequence and RM
- Harmonized methodology possible?
  - Does it exist or do we need to develop it?

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