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Agricultural and environmental risk assessment

Hans-Jörg Buhk

„Food, sustainability and plant science

A global challenge“

Heidelberg

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Historical review, genetic engineering regulation

1973 First experiment with *in vitro* recombined DNA

1974 Call for a moratorium until possible risks have been discussed

1975 Asilomar-Conference: Self-restriction of genetic engineering by scientists

1976 - NIH established Recombinant DNA Advisory Committee (NIHRAC)
- guidelines define safety measures (physical and biological measures)
- development of further biological safety measures

1978 Germany established guidance similar to the NIH-Guidelines

1990 EU-Directive on the contained use of genetically modified micro-organisms (Dir. 90/219/EEC, now Dir. 2009/41/EC) and
EU-Directive on the deliberate release of genetically modified organisms (Dir. 90/220/EEC, now Dir. 2001/18/EC)

Germany: Genetic Engineering Act

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Historical review, genetic engineering regulation

1990:

- Two thousand German scientist appealed to the government to establish balanced legislation on genetic engineering.
- H. Bujard, E.L. Winnacker, and P. Starlinger presented the appeal to the government, asking for differentiated safety concept, evaluating genetically engineered organisms not solely on the grounds of being genetically modified, but on the basis oft their actual properties and risk assessment.
- The appeal included that the scientists will undertake to keep the public informed about new developments.

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Legal aspects

- EU legal framework
  - Dir. 2001/18/EC → deliberate release into the environment
  - Reg. (EC) 1829/2003 → marketing of GM food and feed
- An authorisation is required prior to market entry
  - Protection
    - Human and animal health
    - Environment
  - Risk assessment (RA)
    - Characterisation of potential adverse effects associated with the use of GM plants
      - Direct; indirect; immediate; delayed; cumulative effects
- European Food Safety Authority (EFSA)

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GM plant market authorisation applications: cultivation

- Submitted under Reg. (EC) 1829/2003 (or Dir. 2001/18/EC)
- More details → Register of Questions on EFSA website

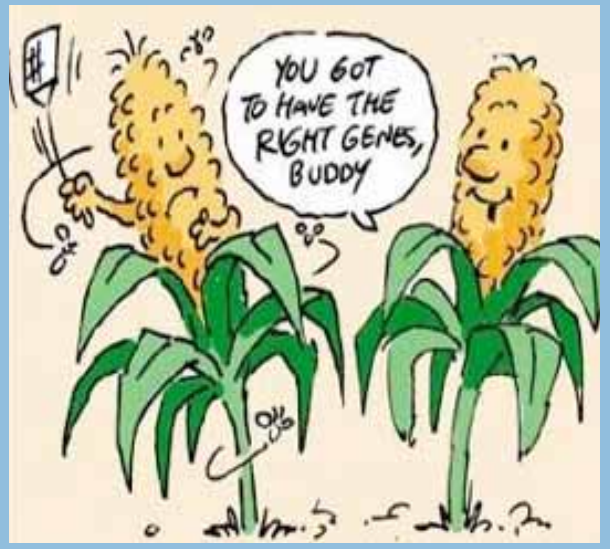
Plants:

- maize
- cotton
- soybean
- oilseed rape
- rice
- sugar beet
- potato

…

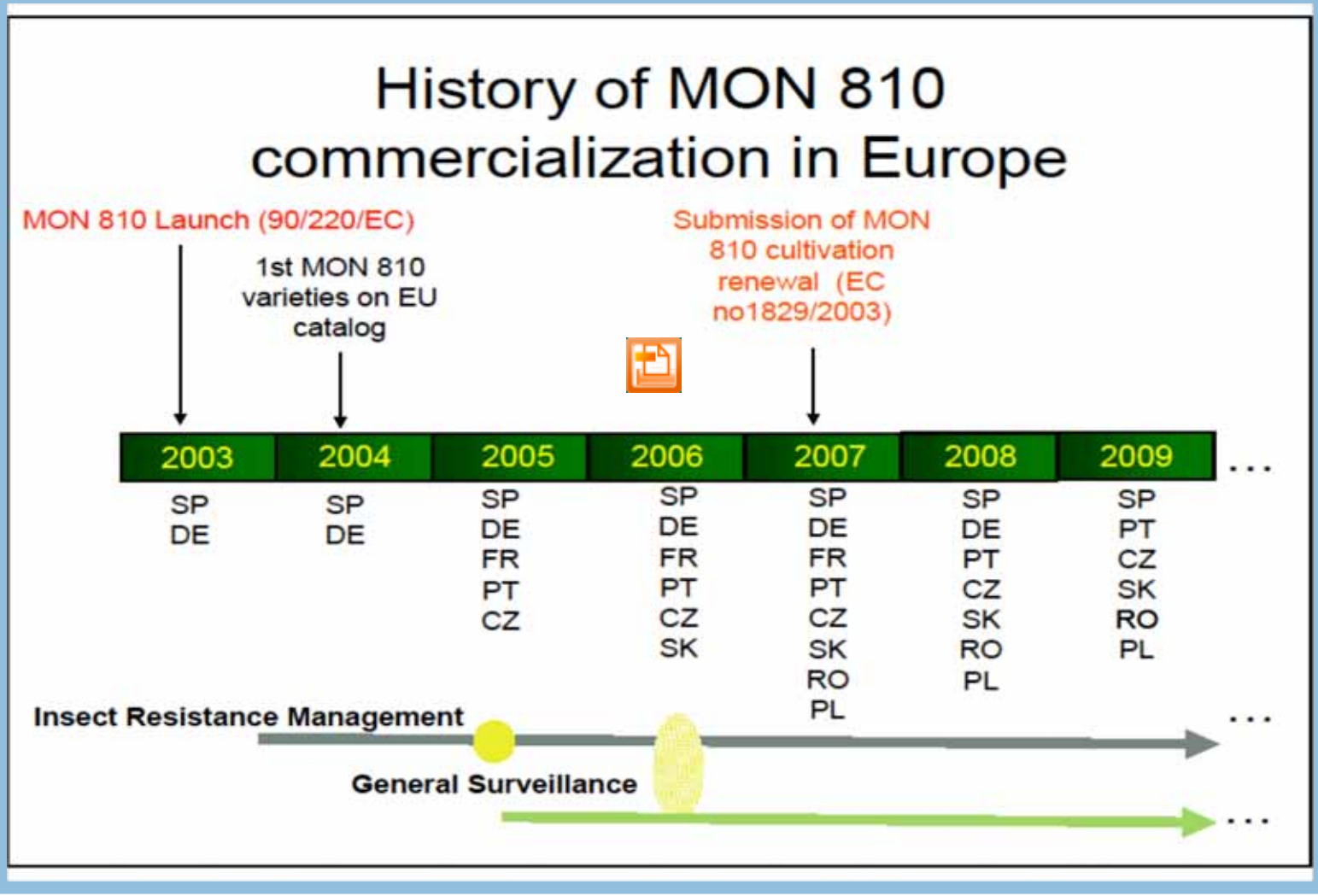
Traits:

- insect resistance
- herbicide tolerance
- other:
  - [oleic acid]
  - drought tolerance



Uses:

- food
- feed
- import
- processing
- **cultivation**


History of MON 810
commercialization in Europe
MON 810 Launch (90/220/EC)
1st MON 810
varieties on EU
catalog
Submission of MON
810 cultivation
renewal (EC
no1829/2003)
2003
2004
2005
2006
2007
2008
2009
SP
DE
SP
DE
SP
DE
FR
PT
CZ
SP
DE
FR
PT
CZ
SK
SP
DE
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Insect Resistance Management
General Surveillance

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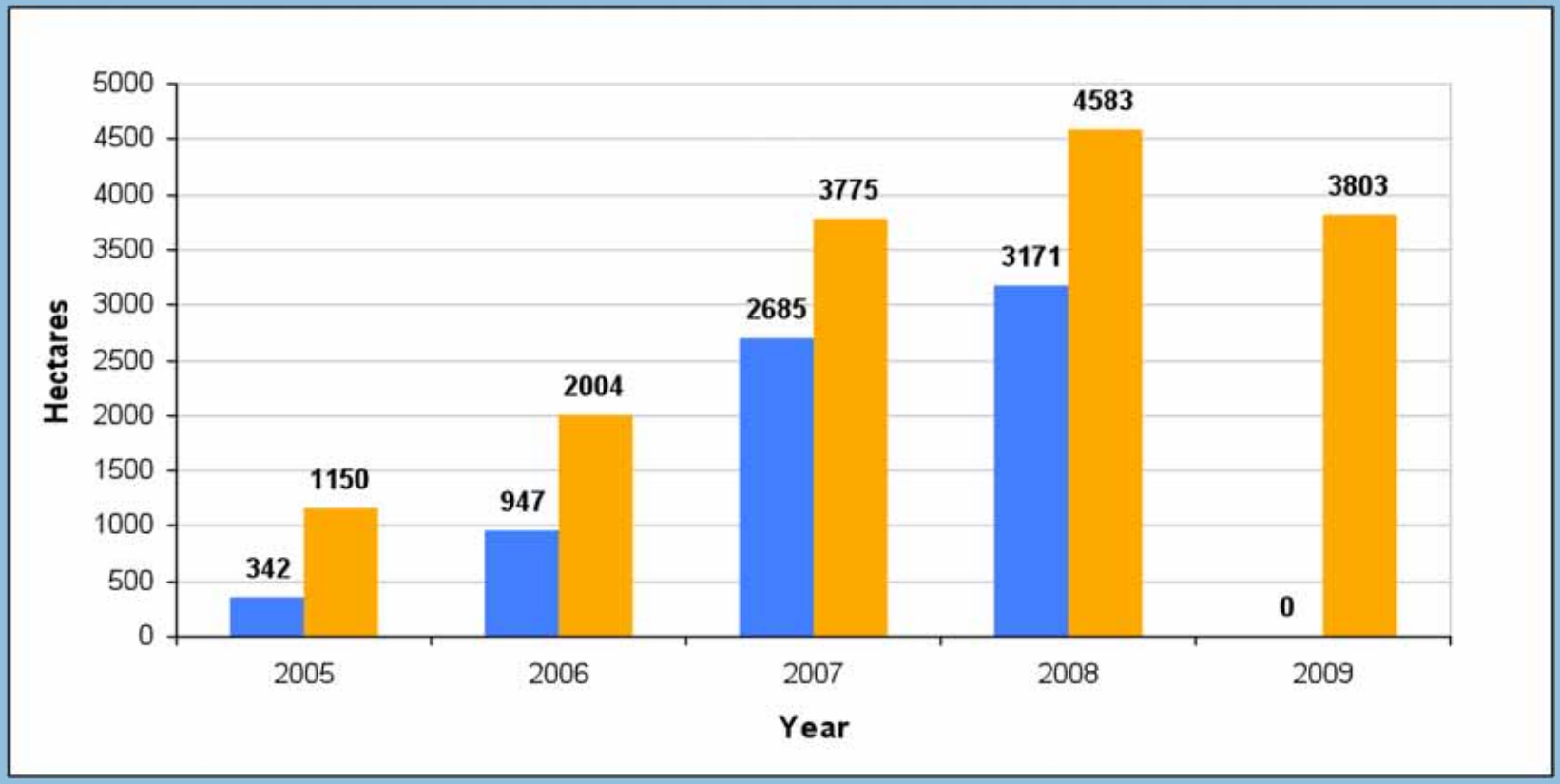
Germany: MON810 cultivation statistics



	2005	2006	2007	2008	2009
Effective Bt Cultivation	342 ha	947 ha	2685 ha	3171 ha	0 ha
Planned Cultivation	1150 ha	2004 ha	3778 ha	4583 ha	3803 ha

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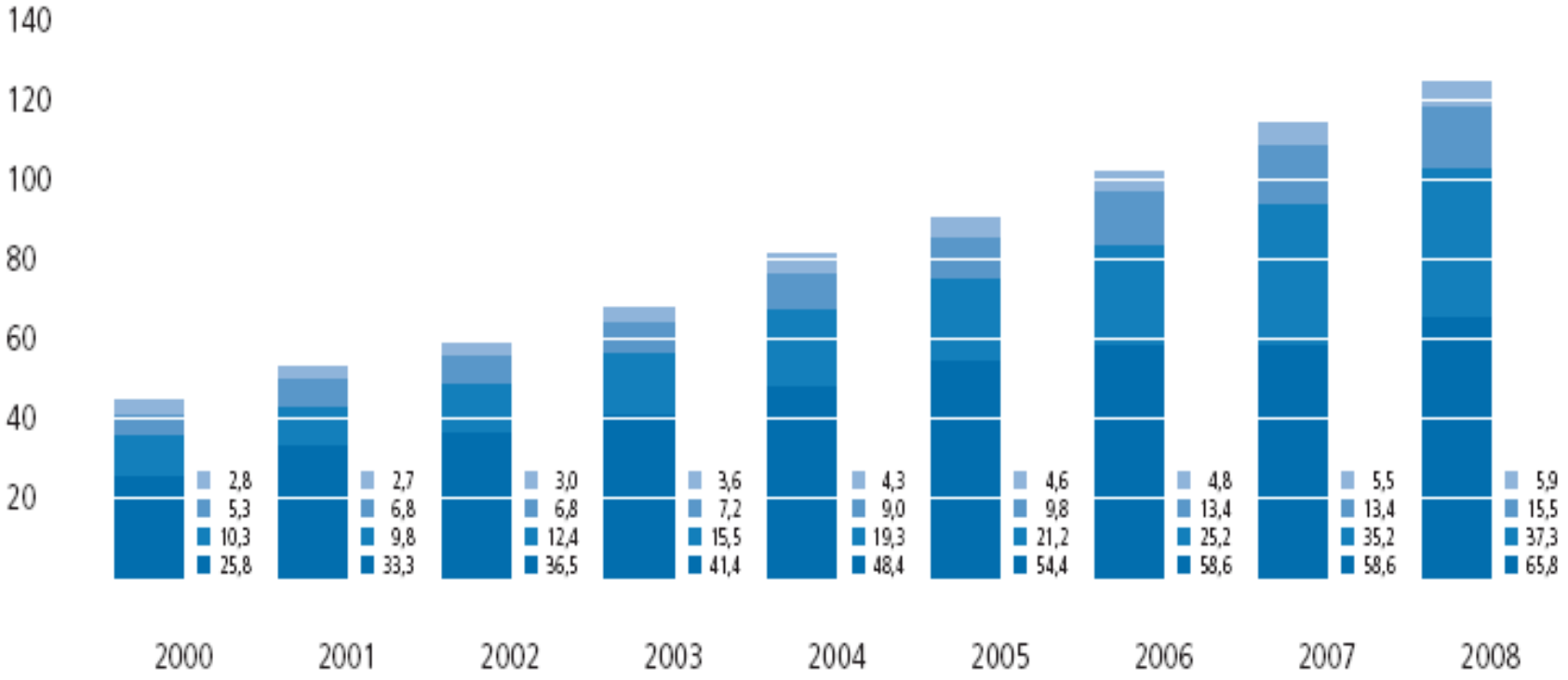
Figure 3: Hectarage of GM crops (worldwide)



in mio ha. top - down: canola cotton corn soya.

Source: www.isaaa.org; Zweiter Gentechnologiebericht, 2009:262.

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From science to regulation…

What
does a regulator
need to know
for decision making
?

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EFSA Checklist ERA

Sections in the EFSA Guidance Document

Potential changes in the interaction of the GM plants with the <u>biotic environment</u>

- persistence, invasiveness,
- selective advantage or disadvantage
- gene transfer
- interaction plant-TO
- interaction plant – NTO
- effects on human health
- effects on animal health
- effects on biogeochemical process
- impact specific cultivation, management and harvesting techniques (including GM HT crop guidance)

Potential interaction with the <u>abiotic environment</u>

Long-term effects

briefing GM crops

Long-term effect of GM crops serves up food for thought

Declan Butler and Tony Reichhardt

NATURE | VOL 398 | 22 APRIL 1999 | www.nature.com

The media has inflamed public fears about the risks of genetically modified crops for human health and biodiversity. But many responsible scientists agree on the need for more research to identify potential long-term problems.

[…]

Some researchers have proposed specific monitoring strategies. Hans-Jörg Buhk, director of the Robert Koch Institute in Berlin, has called for the creation of a 'gene register' to track which genes and constructs have been introduced into the crop gene pool. This precautionary measure, he argues, would improve the ability of researchers to predict interactions between genetic modifications.

[…] He points to a tomato developed by the British company Zeneca that was designed to have an increased shelf life, using antisense technology directed against the polygalacturonase gene that causes ripening. According to Buhk, the best performing line was in fact caused by an unpredicted 'sense' event (gene activation). "This was a rare event, either a contamination or a chance turnaround [in the genome]," he says.

[…] "This is gene silencing," says Buhk. "There is interaction going on at the RNA level that we do not understand."

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Biological and
Ecological
Evaluation
Towards
Long-term
Effects

Report

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Objectives of the BEETLE project

- GM crops were assessed with respect to potential long-term (10-20 years) adverse effects on environment and health

- **Main focus**
  - **Maize** insect resistance (IR)
  - **Oilseed rape** herbicide tolerance (HT)
  - **Sugar beet** herbicide tolerance (HT)
  - **Potato** starch modification (SM)

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BEETLE general conclusions (1)

- > 20 years of experimental field releases
- > 10 years of commercial cultivation
- Adverse long-term effects reported in the scientific literature concern
  - (i) the development of resistance in Bt crop target organisms and
  - (ii) tolerance in weeds to complementary herbicides used in HT crops.

  } already anticipated from ERA
- No other adverse long-term effects have yet been established.
- However, other potential long-term effects are discussed in the relevant scientific literature and in scientific fora in general.

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BEETLE general conclusions (2)

- Due to the nature of *potential* long-term effects, it is not yet possible to *quantify* the long-term risks associated with GM crops.
- However, the BEETLE study has identified a *qualitative* **priorization concerning the processes linked to GM plants** that could have long-term effects on the environment (including biodiversity) and health.

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BEETLE general conclusions (3)

- Long-term effects on **animal or human health** linked to GM crops **have not yet been identified**.
- However, forthcoming generations of GM crops will include more **complex genetic modifications, e.g. more stacked events** (several GM traits in the same crop variety) …

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BEETLE general conclusions (4)

- A tool for providing pre-market information on GMO characteristics is a database including novel bioinformatic applications guiding assessment of **potential interaction between different genetic modifications, e.g. synergistic effects of stacked events (intended or unintended).**
- **Possible synergistic effects of proteins from intended and unintended combination of different GMOs** should be considered during the ERA to improve the prognostic power of the long-term effect assessment.

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BEETLE general conclusions (2)

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- However, the BEETLE study has identified a *qualitative* **priorization concerning the processes linked to GM plants that could have long-term effects on the environment** (including biodiversity) and health.

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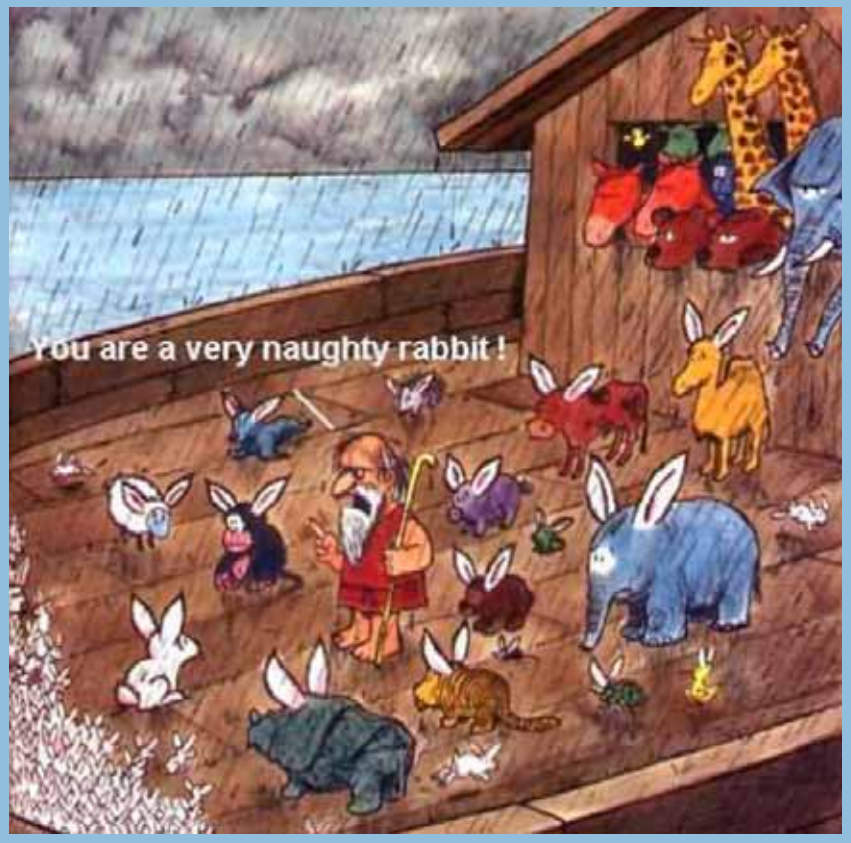
BEETLE: Highest priority

Potential adverse effects due to 'Cultivation and Management' issues:

- likely to be caused indirectly through changes in cultivation and agricultural management of HT crops and consequently affecting wider biodiversity.
- **The use of complementary herbicides can potentially change the management practice.**
- Effects will depend on crop/trait combinations cultivated and possibly regional aspects.

Potential for gene transfer

Vertical gene flow: plant to plant; plant to wild/weedy relatives



- Routes of exposure
  - Pollen flow
  - Seed dispersal
  - Introgressive hybridisation
- Impact (harm)
  - Altered fitness
    - Extinction
    - Invasiveness
    - Weed/pest control failure
  - Adverse effects on non-target organisms

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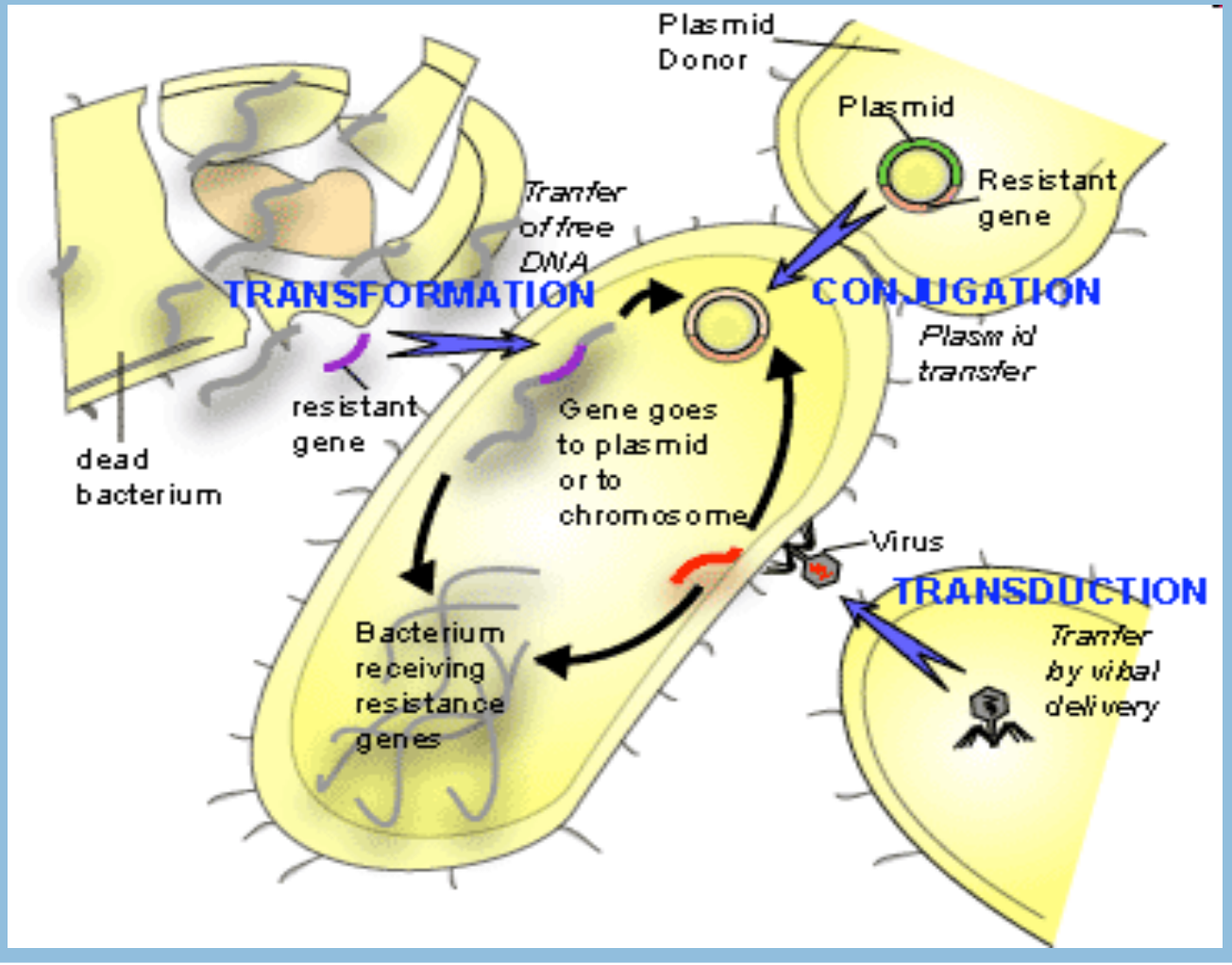
Horizontal gene transfer

Mechanisms of horizontal gene transfer (HGT) in bacteria:



Source: http://www.textbookofbacteriology.net/resantimicrobial_3.html

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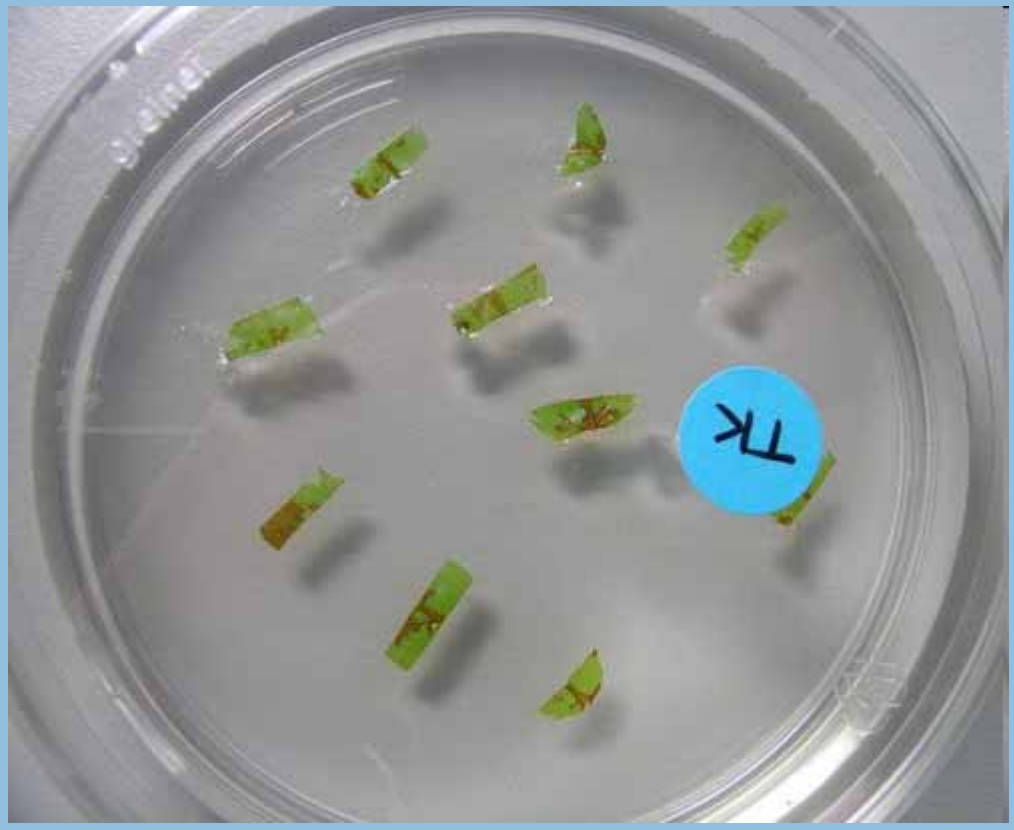
Horizontal gene transfer



Antibiotic resistance genes are besides the genes of interest introduced into plant cells. Only plant cells transformed successfully can grow on media containing the antibiotic.

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Horizontal gene transfer, plant to bacteria

- **The conditions for a horizontal gene transfer must be optimized artificially in experiments:**
- release of the antibiotic resistance gene in an intact form from the plant cells,
- competent bacteria must take up the DNA
- the transferred DNA fragment must become established in the bacteria cell
  - by integration into the genome (homologous recombination or homology-mediates illegitimate recombination)
  - in rare cases by circularization into a plasmid if the transforming DNA fragment carries plasmid replication functions;
- successful expression of the transferred antibiotic resistance gene.

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Horizontal gene transfer

According to calculations by Schlüter and Potrykus (1996) the **probability of transforming soil bacteria with the *npt*II** gene from harvest remains of genetically modified plants into **bacterial recipients possessing no homology** to the taken up DNA is between **2×10^{-11} and 2.7×10^{-17}**.

No such transfer was observed in analyses of antibiotic resistance gene transfer with e.g. *npt*II or *bla* from plants to bacteria **under natural conditions** (Badosa et al., 2004; Gebhard and Smalia, 1999), **or** where the **corresponding homology does not exist** in the bacteria (de Vries et al., 2001; de Vries et al., 2003; de Vries et al., 2004; Nielsen et al., 1998).

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ZKBS opinion on horizontal gene transfer

ZKBS, 2008:

Potential adverse effects from a possible gene transfer are **not expected** because the genes transferred to a bacterium are **returning to their existing natural gene pool.**

Genetic modifications from bacteria introduced into crop plants, e.g.

- ***cry* gene** (Bacillus thuringensis, Bt) → self-protection against european corn borer
- **herbizide tolerance** (soil mico-organisms) → increased tolerance to **glyphosate** or **glufosinate**
- ***npt*II** gene (micro-organisms) → antibotic resistance marker gene

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GM plant market authorisation applications, cultivation and cross-polination

Applications	Crops	Events	MS(*); status	EFSA status
NL-2005-24	soybean	**40-3-2**	DE; ✓	ongoing
DE-2008-63	sugarbeet	**H7-1**	DE; ongoing	ongoing
NL-2009-69	potato	**AV43-6-G7**	SW; -	CC(**)

(*) One Member State (MS) performs the initial environmental risk assessment evaluation

(**) Completeness check by EFSA GMO Unit

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GM plant market authorisation applications, cultivation and cross-polination

Applications	Maize transformation events	MS(*); status	EFSA status
UK-2005-17	1507 x NK603	ES; ✓	ongoing
NL-2005-22 & RX-NK603	NK603	ES; ✓	✓
NL-2005-23	59122	NL; ✓	ongoing
NL-2005-26	MON810 x NK603	ES; ongoing	ongoing
NL-2005-28	1507 x 59122	NL; ✓	ongoing
UK-2006-30	59122 x 1507 x NK603	BE; ongoing	ongoing
NL-2007-46 & RX-T25	T25	UK; ongoing	ongoing
CZ-2008-54	MON88017	BE; ongoing	ongoing
UK-2008-60	GA21	CZ; ongoing	ongoing
BE-2009-71	MON89034 x MON88017	BE; -	CC(**)
NL-2009-72	MON89034 x NK603	NL; -	CC(**)
RX-MON810	MON810	ES; ✓	✓

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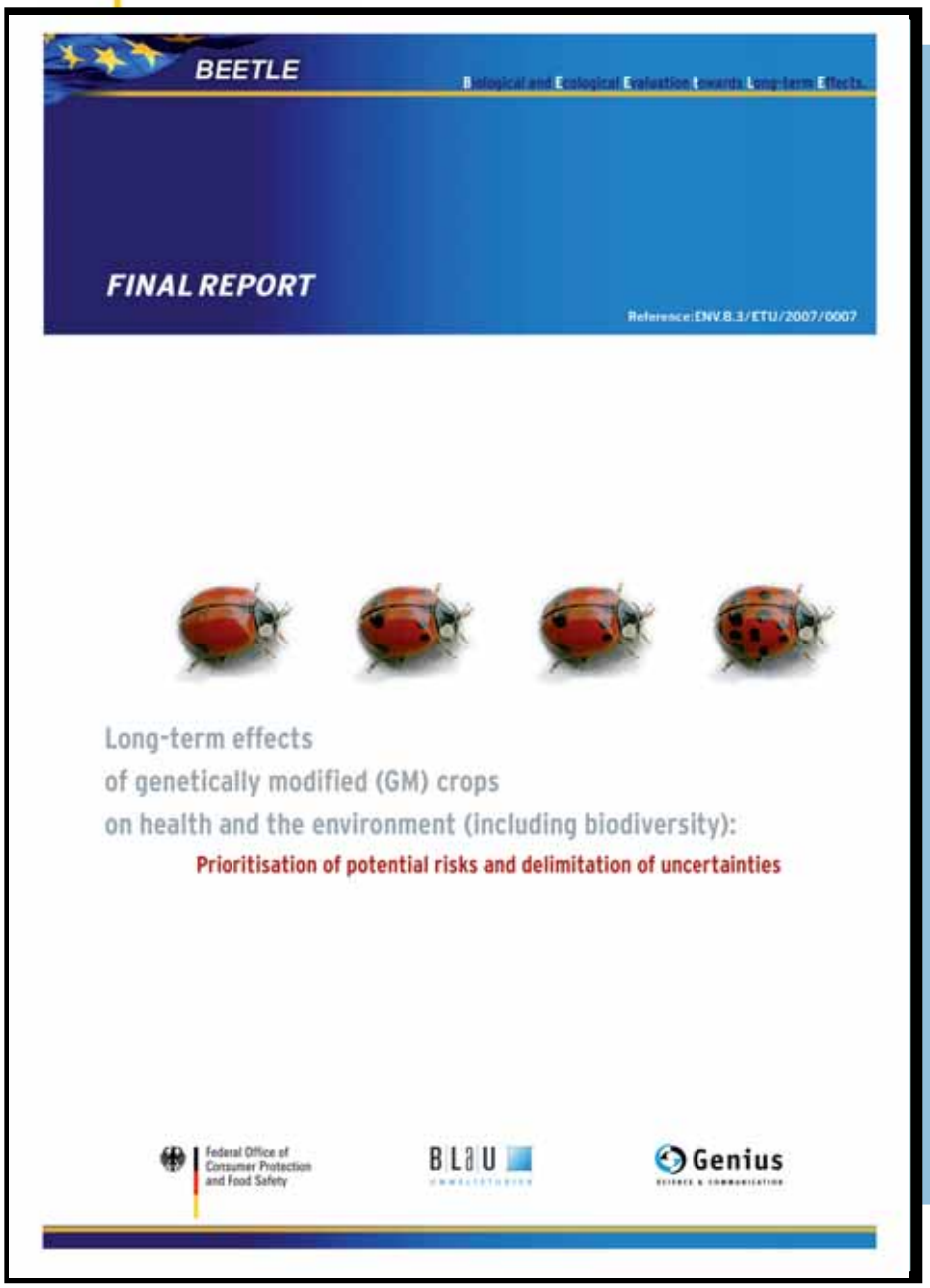
Cross-pollination



Conclusion:

- A tool for providing pre-market **information on GMO** characteristics is a **database** including **novel bioinformatic** applications guiding assessment of **potential interaction between different genetic modifications**, e.g. synergistic effects of **stacked events (intended or unintended).**

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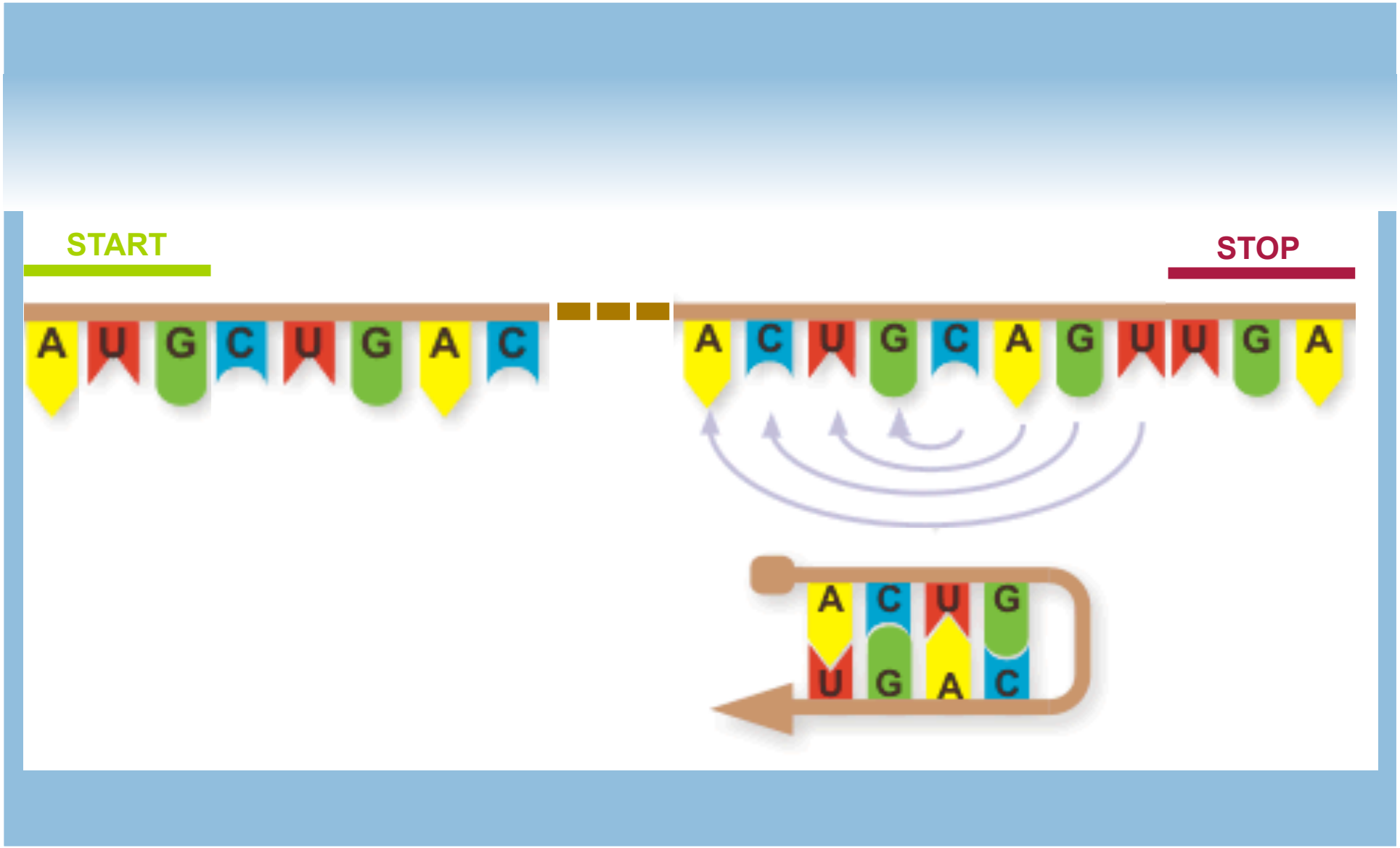
Interference by microRNA



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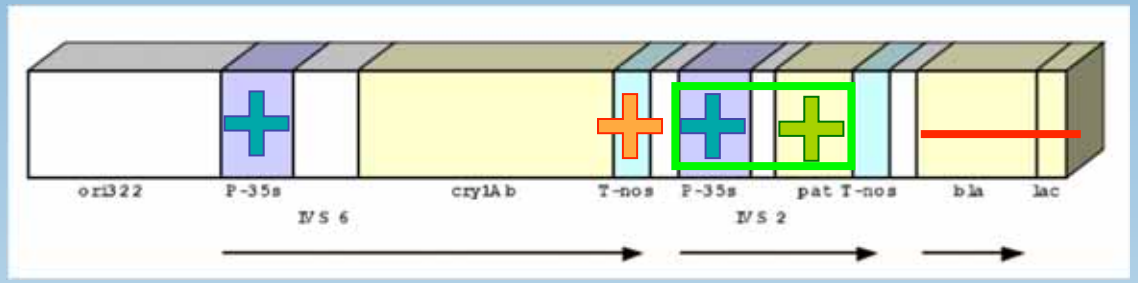
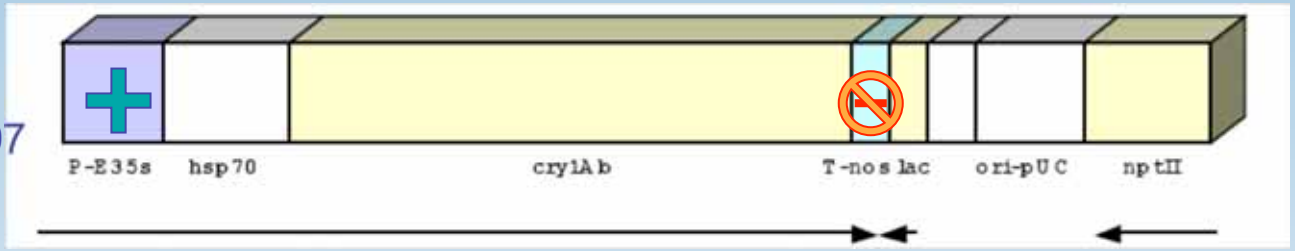
Molecular characterisation

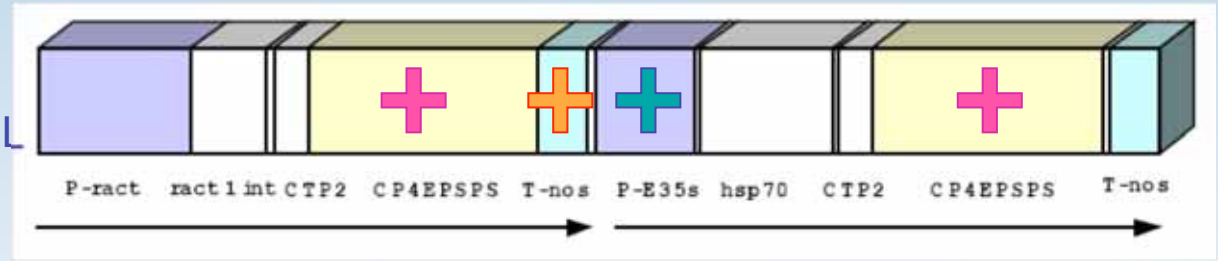
Bt11
pZO1502



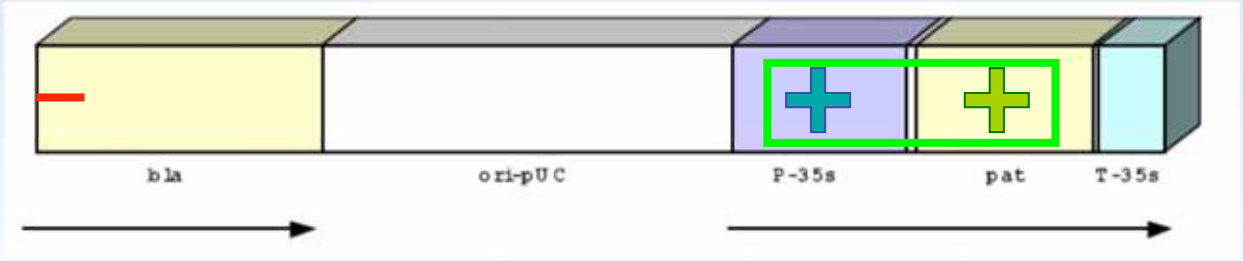
Mon810
PV-ZMBK07



NK603
PV-ZMGT32L



T25
p35S/Ac



P35S | pat | 35S-pat | T-nos | EPSPS

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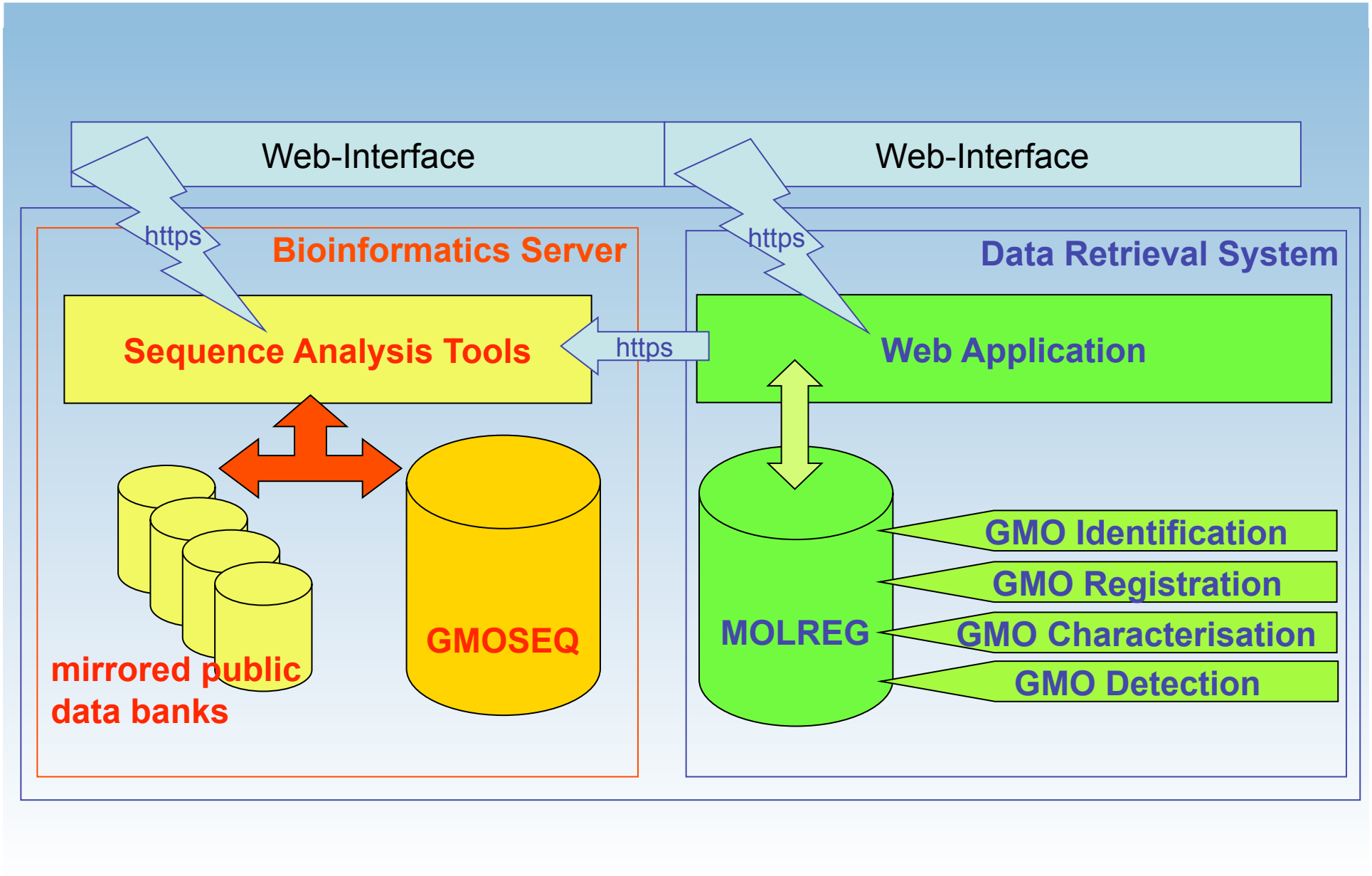
GMO data bank and applications



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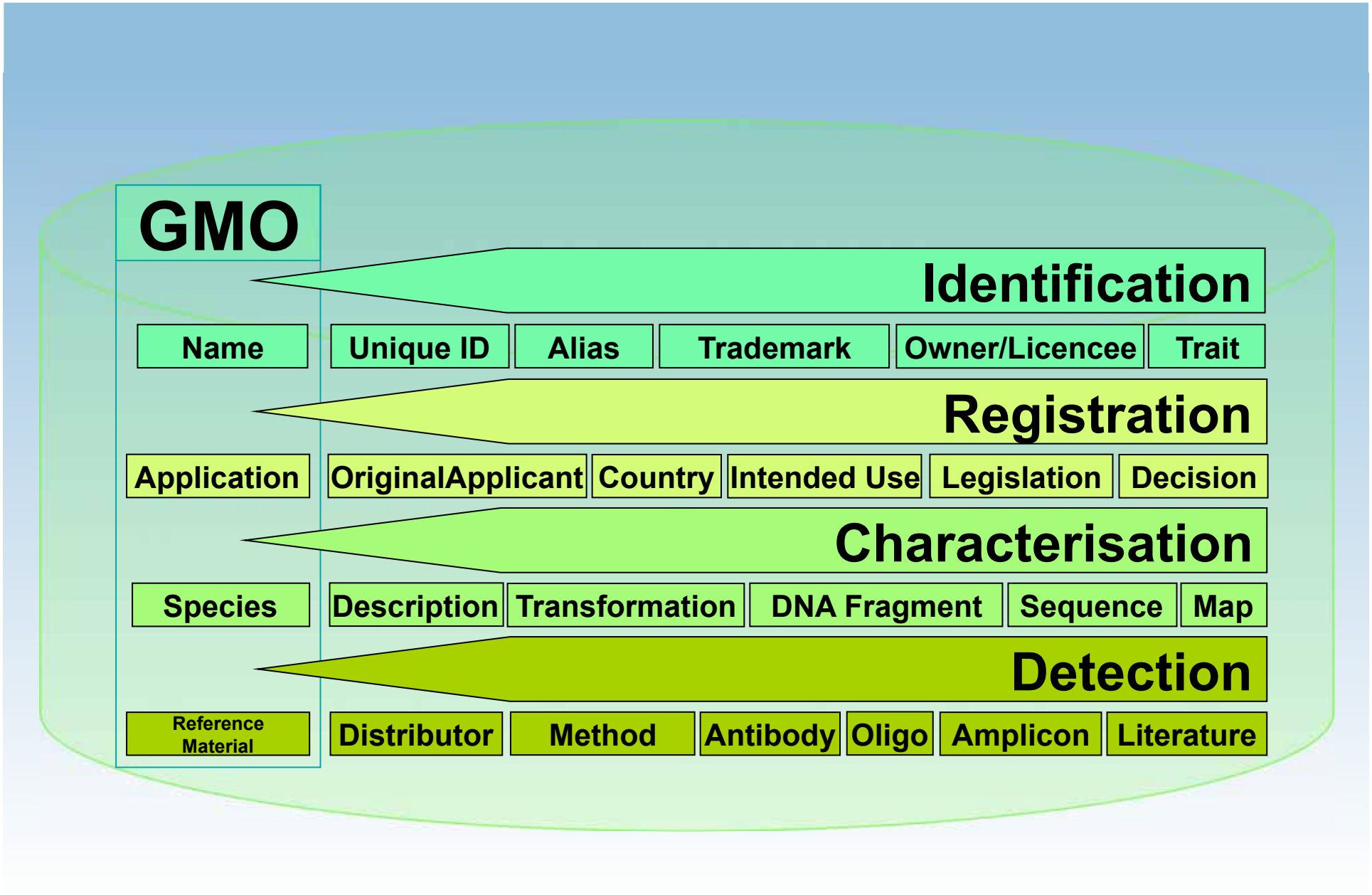
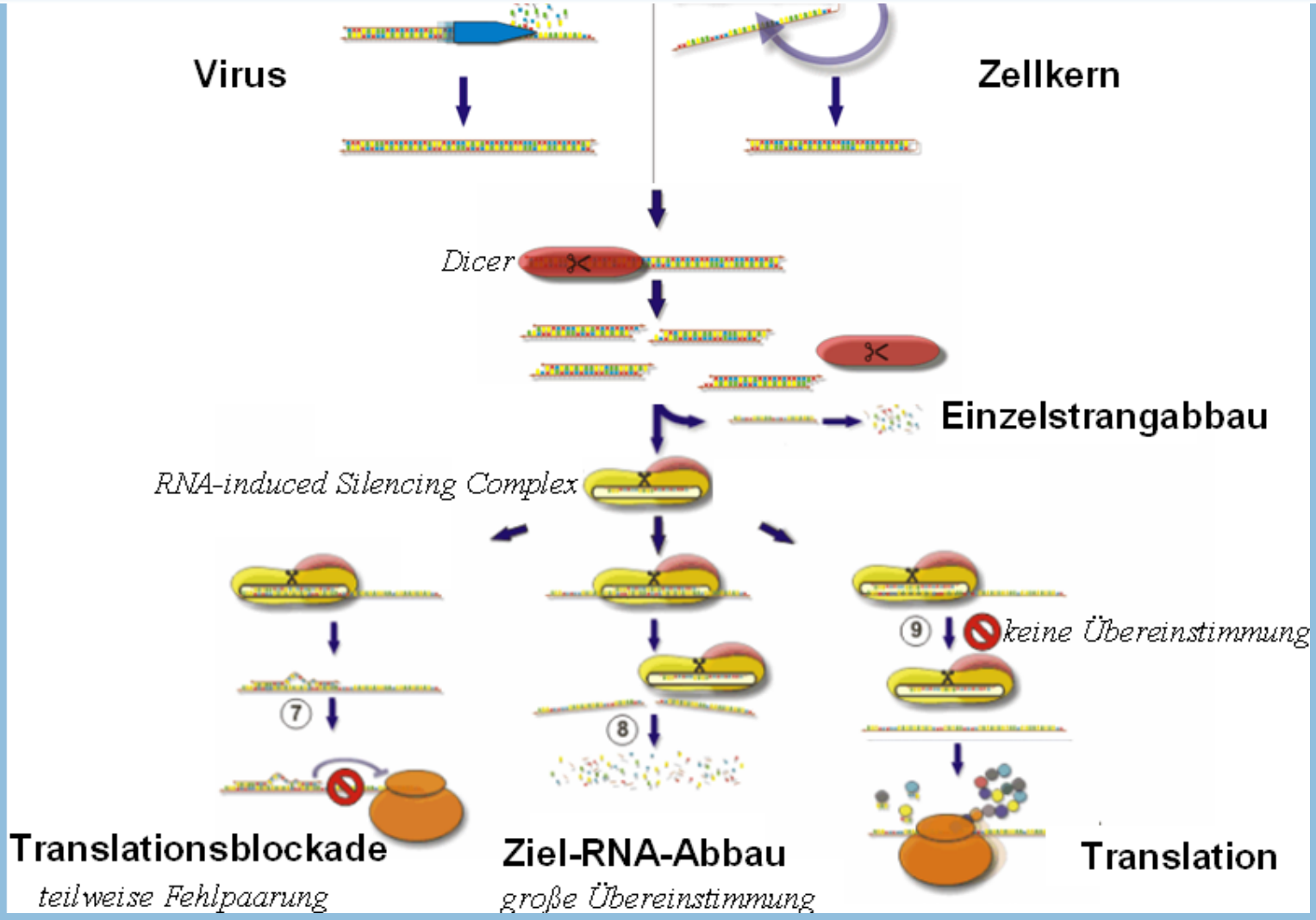
Data retrieval



Interference by small RNAs



microRNA: green giant

Abb.: *Links eine Wildtyp-Pflanze kurz vor dem Blühen; rechts eine Pflanze, die unter gleichen Bedingungen gewachsen ist. Die rechte Pflanze ist mehr als doppelt so alt, blüht noch immer nicht, hat aber das mehr als dreifache Gewicht der linken Pflanze. Diese Effekte sind auf die Überproduktion der microRNA156 zurückzuführen.*

Bild: MPI für Entwicklungsbiologie

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Thank you for your attention