

GMO detection carried out in the European Member States



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Joint Research Centre

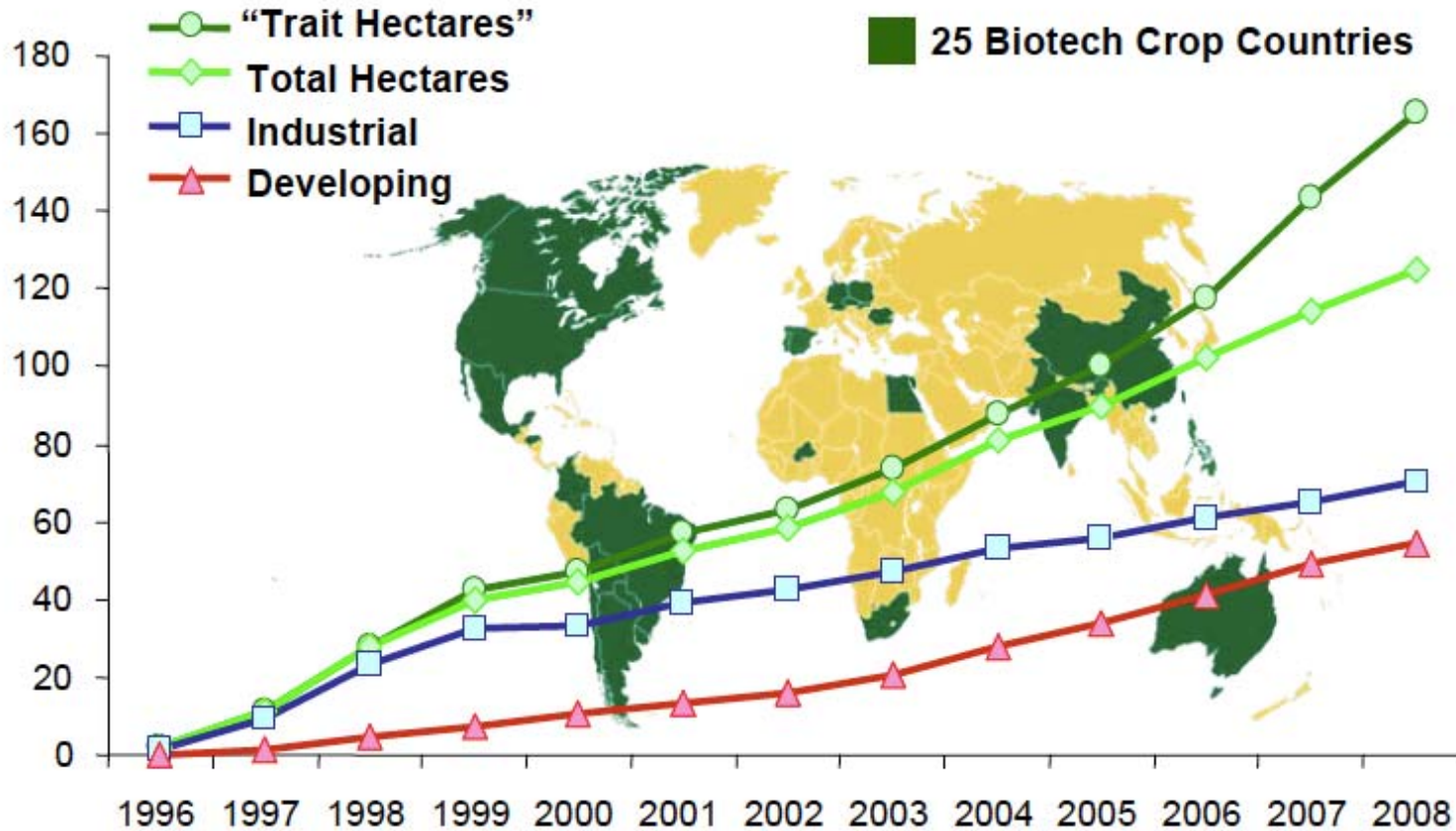
Institute for Health and Consumer Protection (IHCP)

Biotechnology and GMOs Unit







- **Global cultivation**
- **Detection strategies**
- **Protein and PCR based methods**
- **International harmonisation**

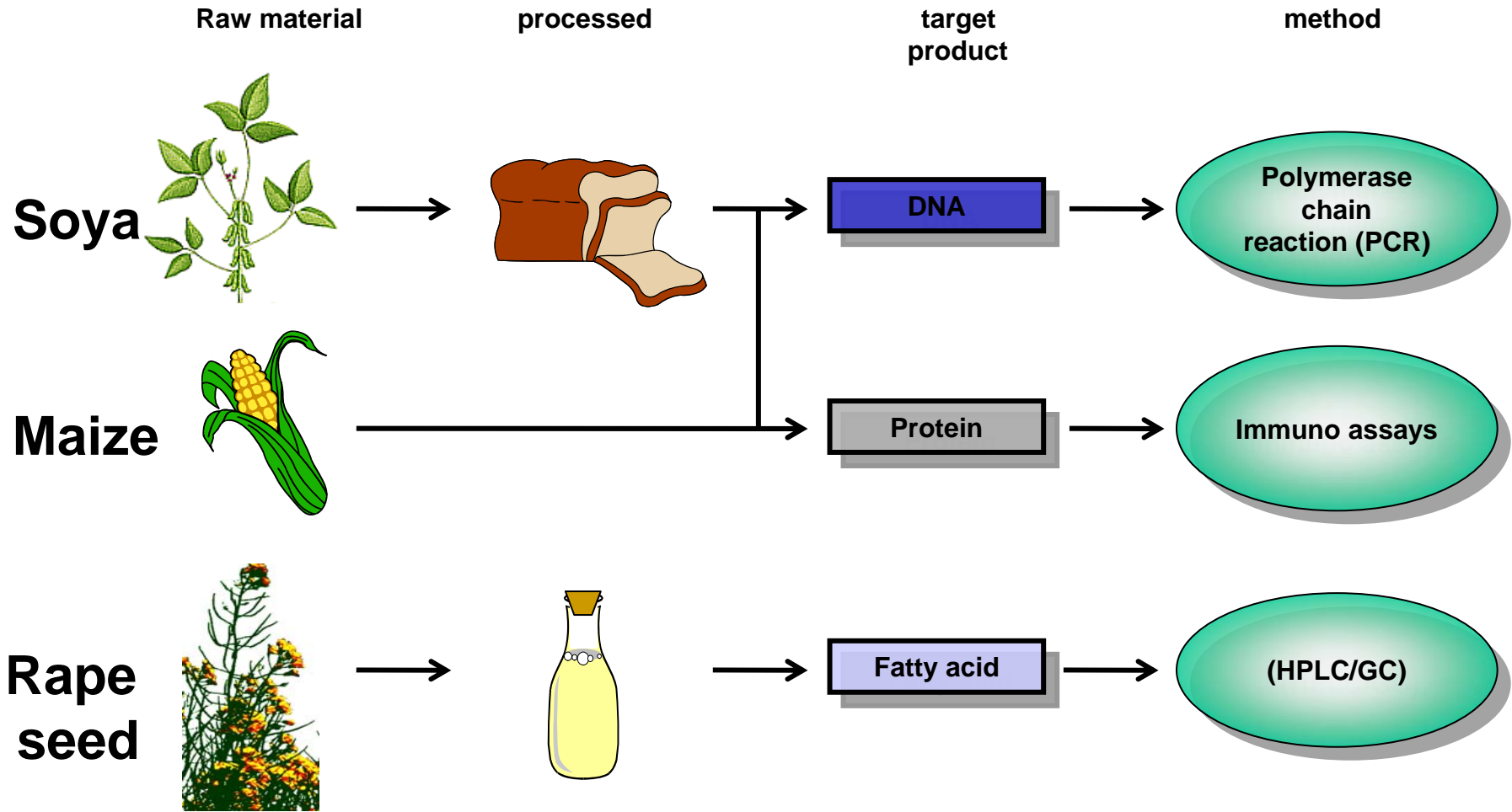
GLOBAL AREA OF BIOTECH CROPS Million Hectares (1996 to 2008)



An “apparent” increase of 9.4% or 10.7 million hectares between 2007 and 2008, equivalent to a “real” increase of 15% or 22 million “trait hectares”

Detection strategies

-  **Member States are responsible for enforcement and control**
-  **All MS have designated Competent Authorities and facilities for the control of GMOs and GM-products**
-  **Commission has responsibility for ensuring the proper functioning and development of the single European market; CRLs coordinate and manage the network of NRLs**
-  **The aim is to ensure that EC food law is enforced with equal rigor in all Member States.**





GMO detection

GMO ?
Yes/No



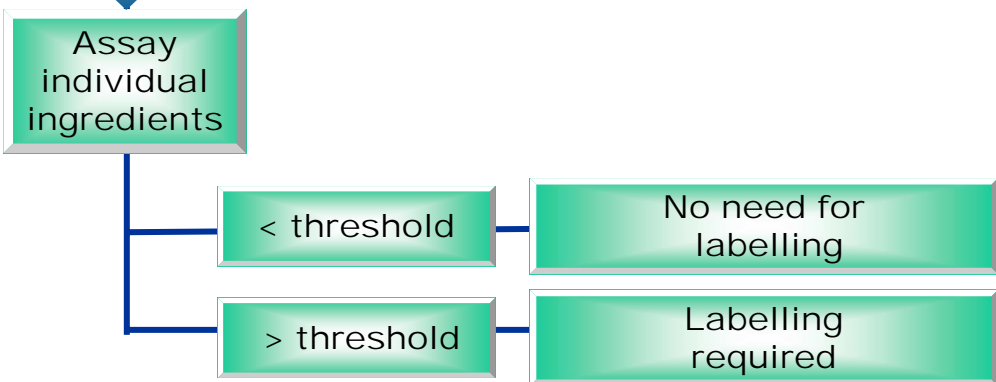
GMO identification

Are they
Authorised ?
Yes/No



GMO quantification

Need for
labelling ?
Yes/No

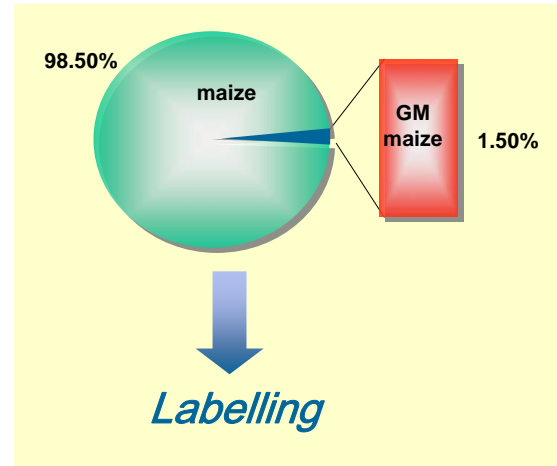
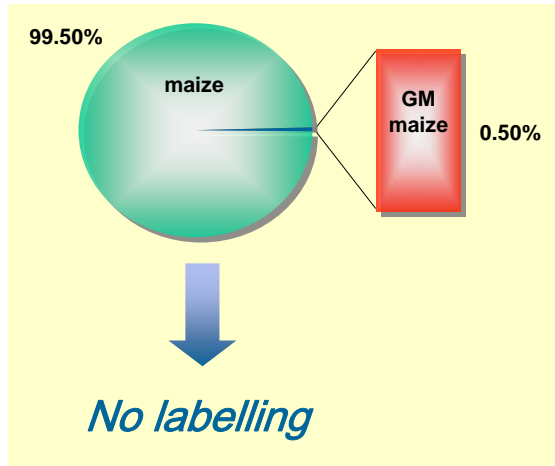




How much "maize" is present?

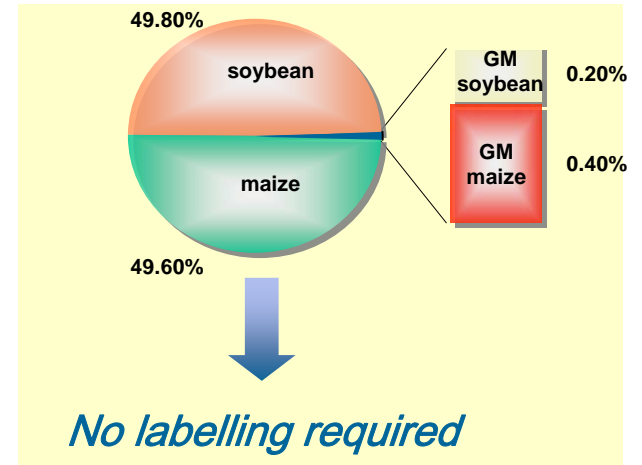
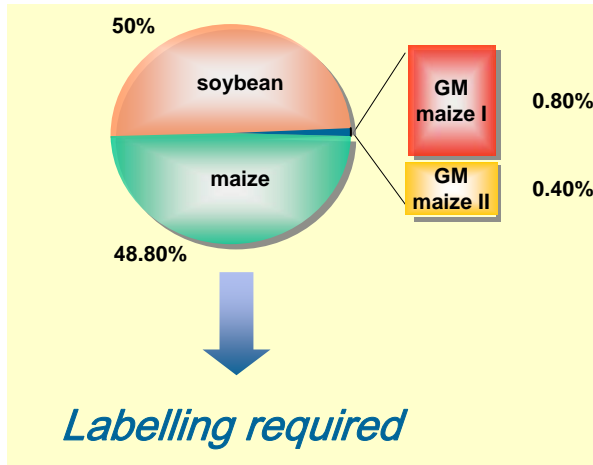
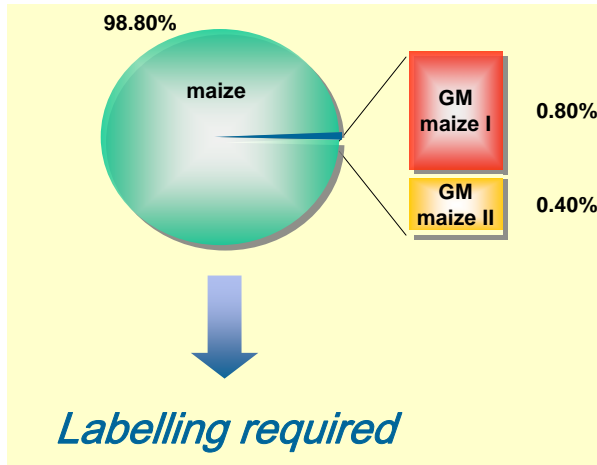
How much "GM-maize 1" is present?

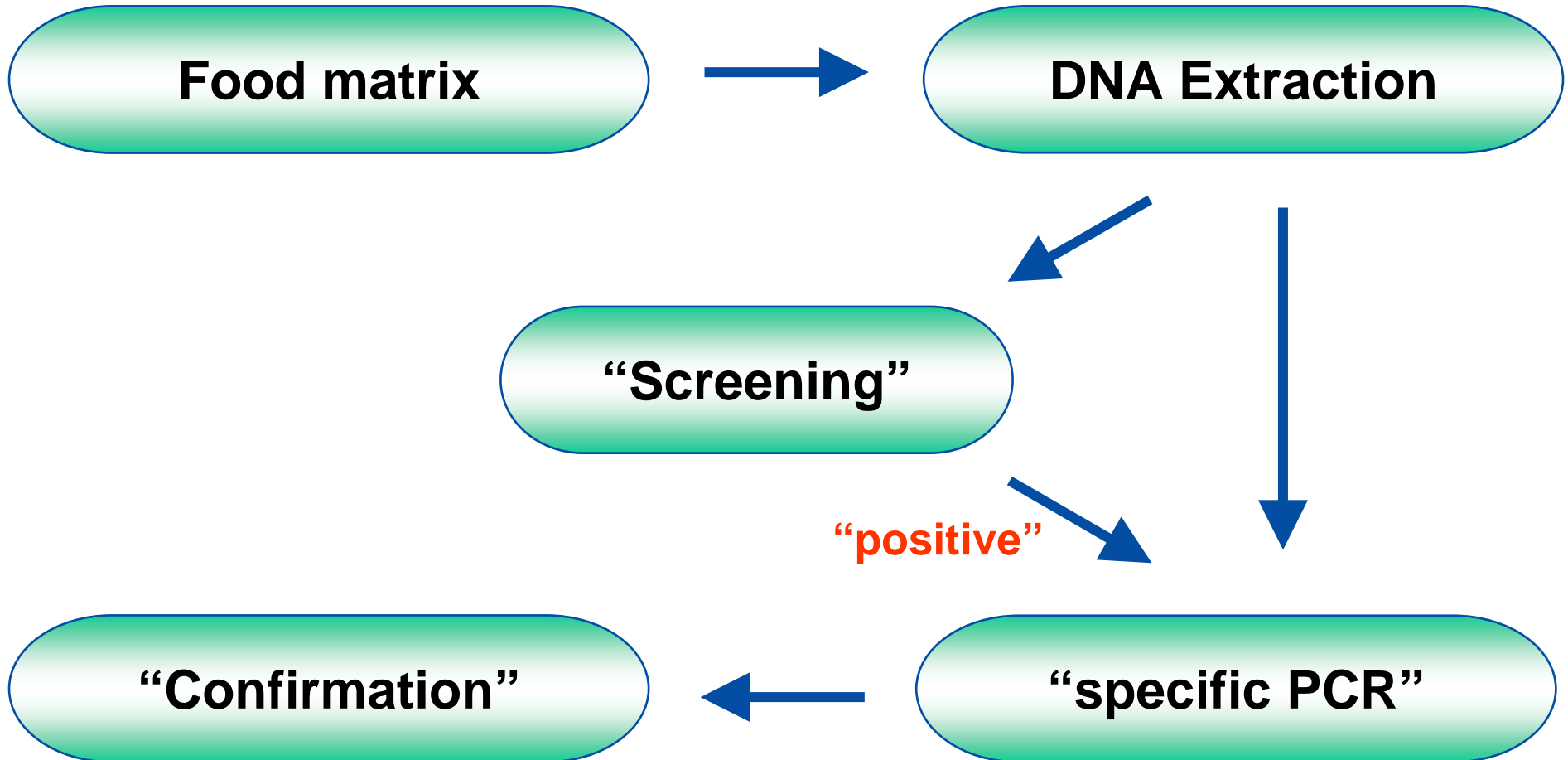
How much "GM-maize 2" is present?

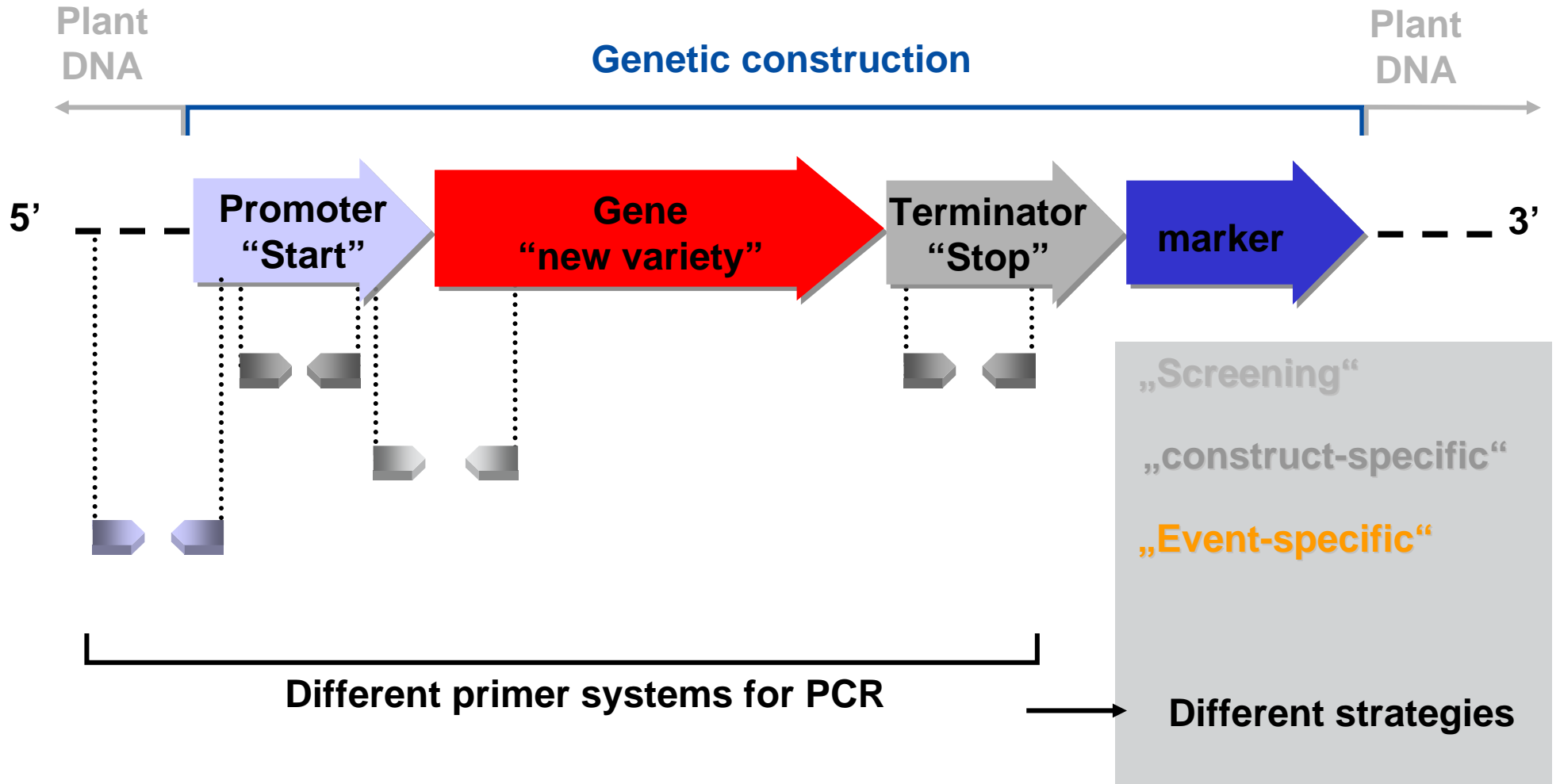


Make calculations

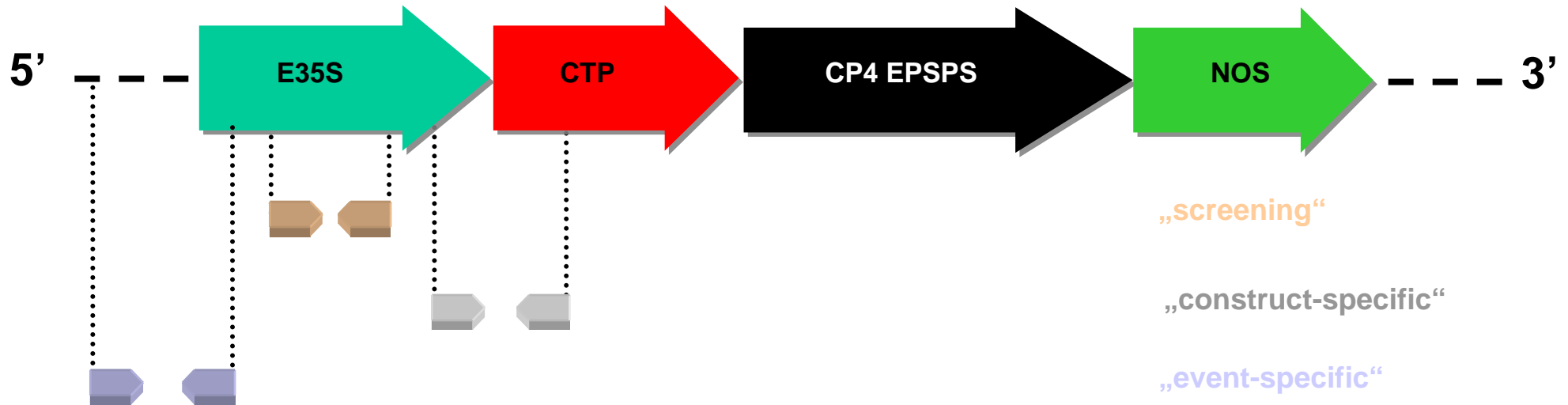
- maize
- soybean
- GM maize I
- GM maize II
- GM soybean

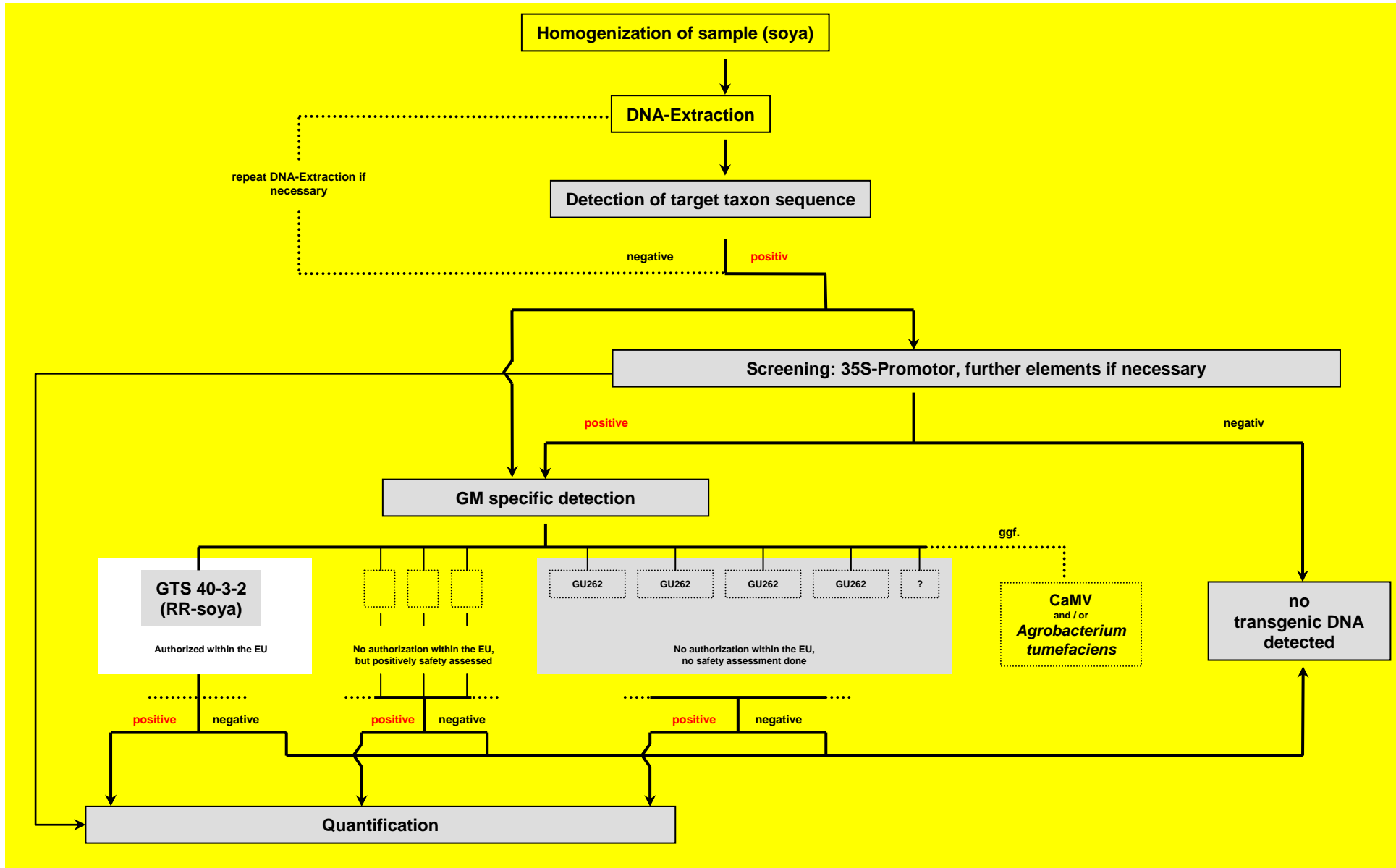






Roundup Ready[®] Construct





Protein based approach

Monoclonal

- ✓ Lot-to-lot consistency
- ✓ Indefinite supply
- ✓ Highly specific
- ✓ Longer lead time
- ✓ Higher initial costs

Polyclonal

- ✓ Lot-to-lot variability
- ✓ More broadly reactive
- ✓ Often more sensitive
- ✓ Shorter lead times
- ✓ Lower initial costs

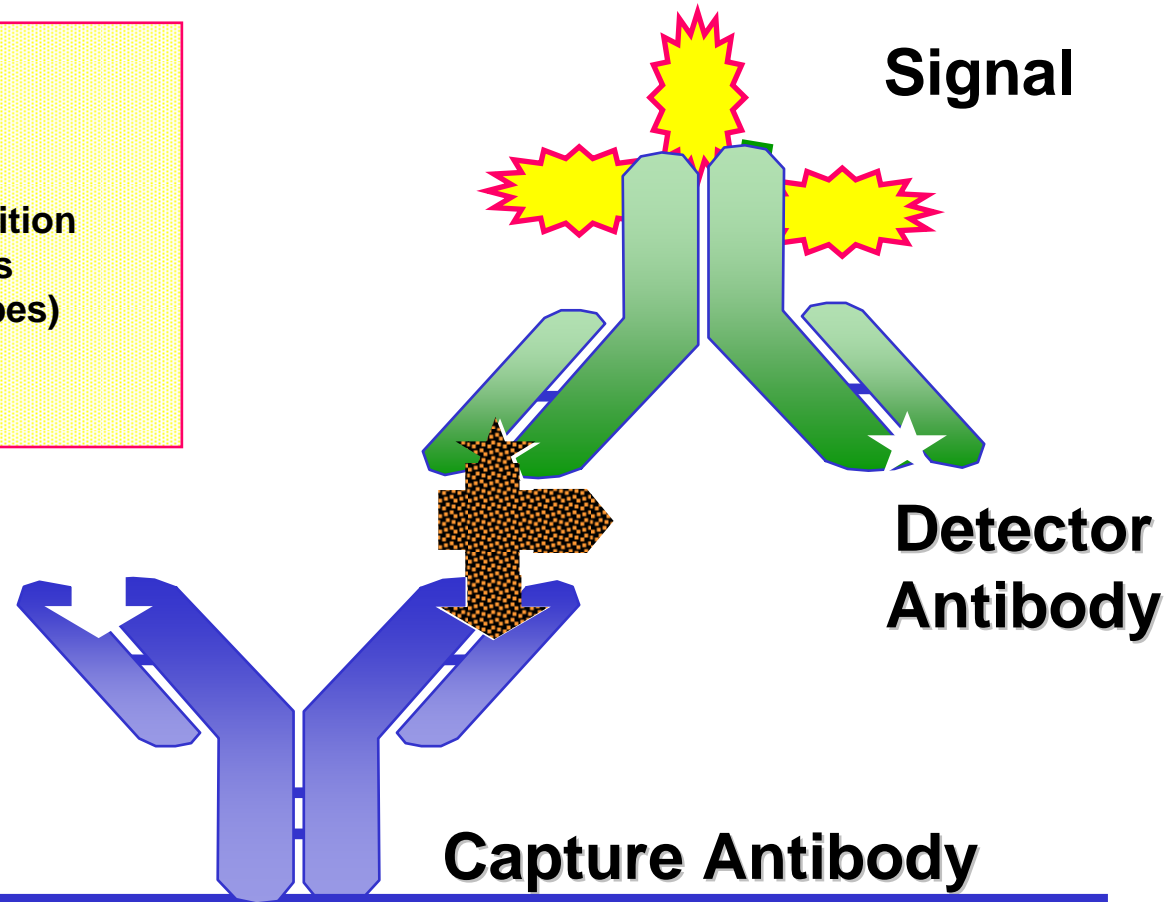
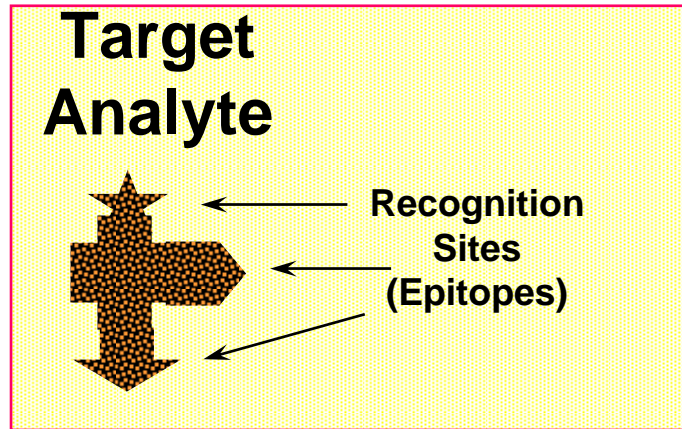
Selection is based on application, time and money

‘Solid Phase’ Antibodies – Separation and washing

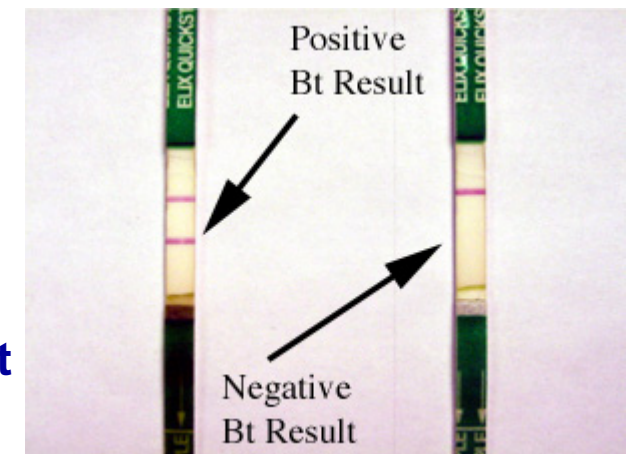
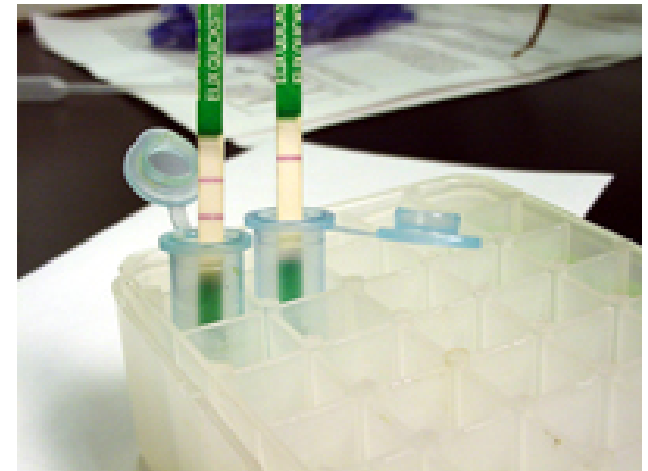
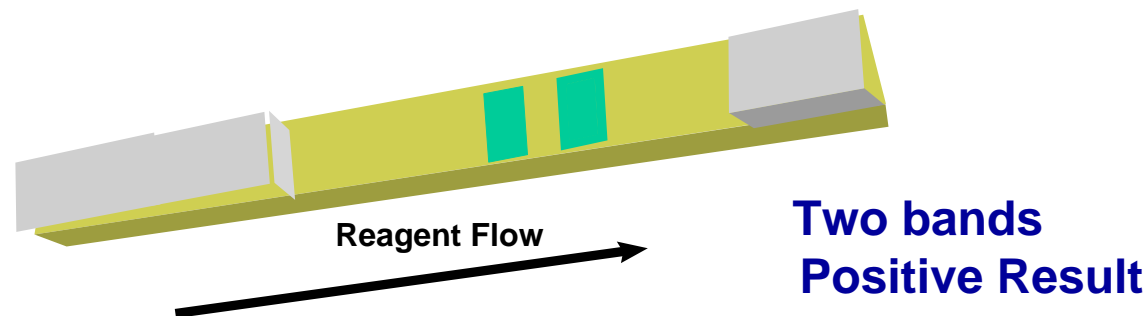
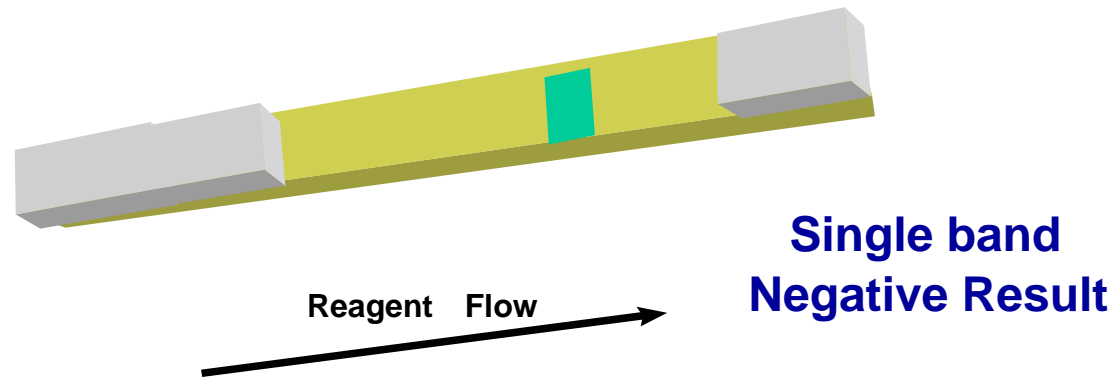
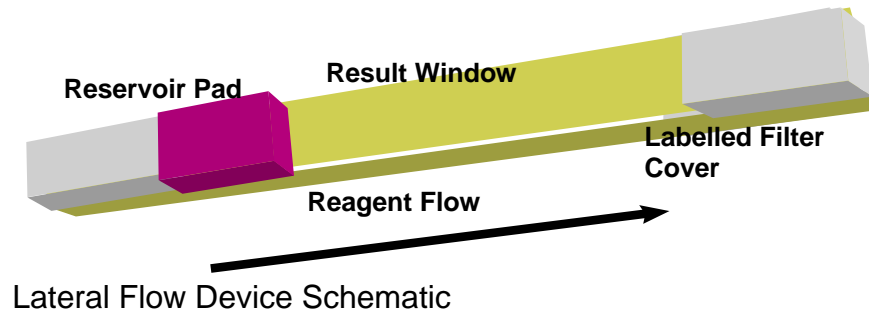
- Plastic wells, tubes, capillaries
- Membranes
- Magnetic particles

‘Labeled’ Antibodies - Detection

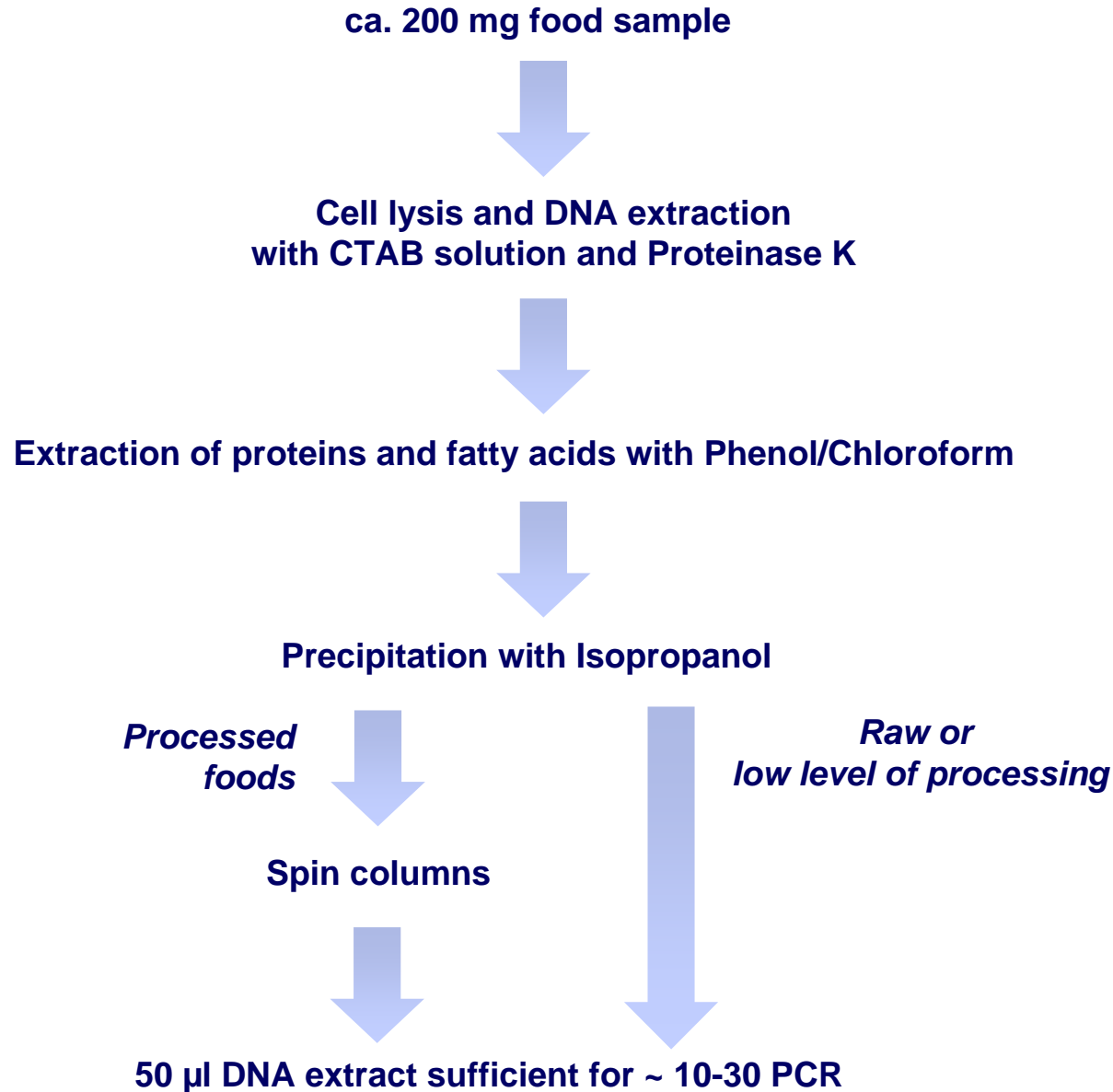
- Colored particles (e.g., colloidal gold, latex)
- Enzymes
- Fluorescent molecules
- Chemiluminescent molecules

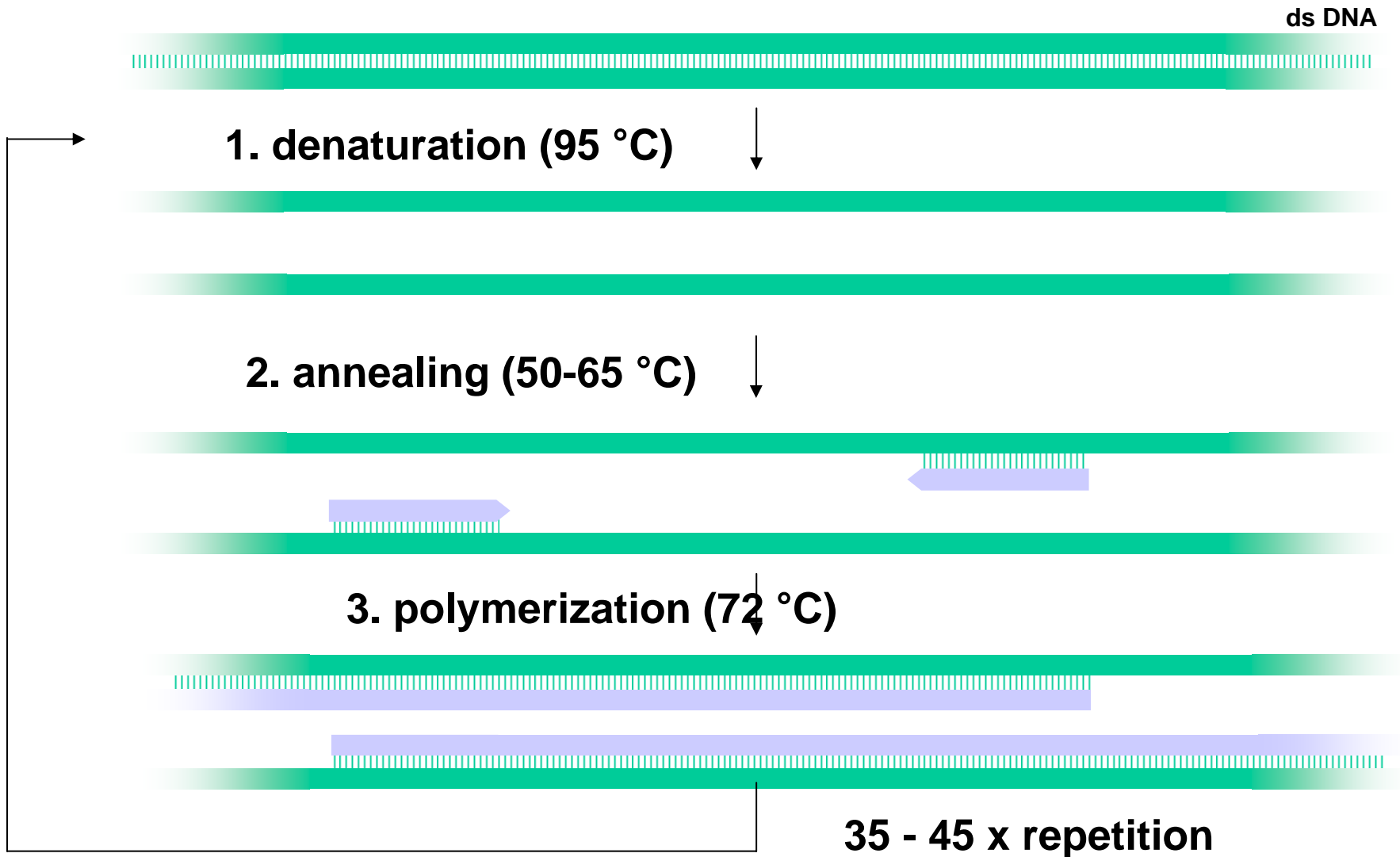


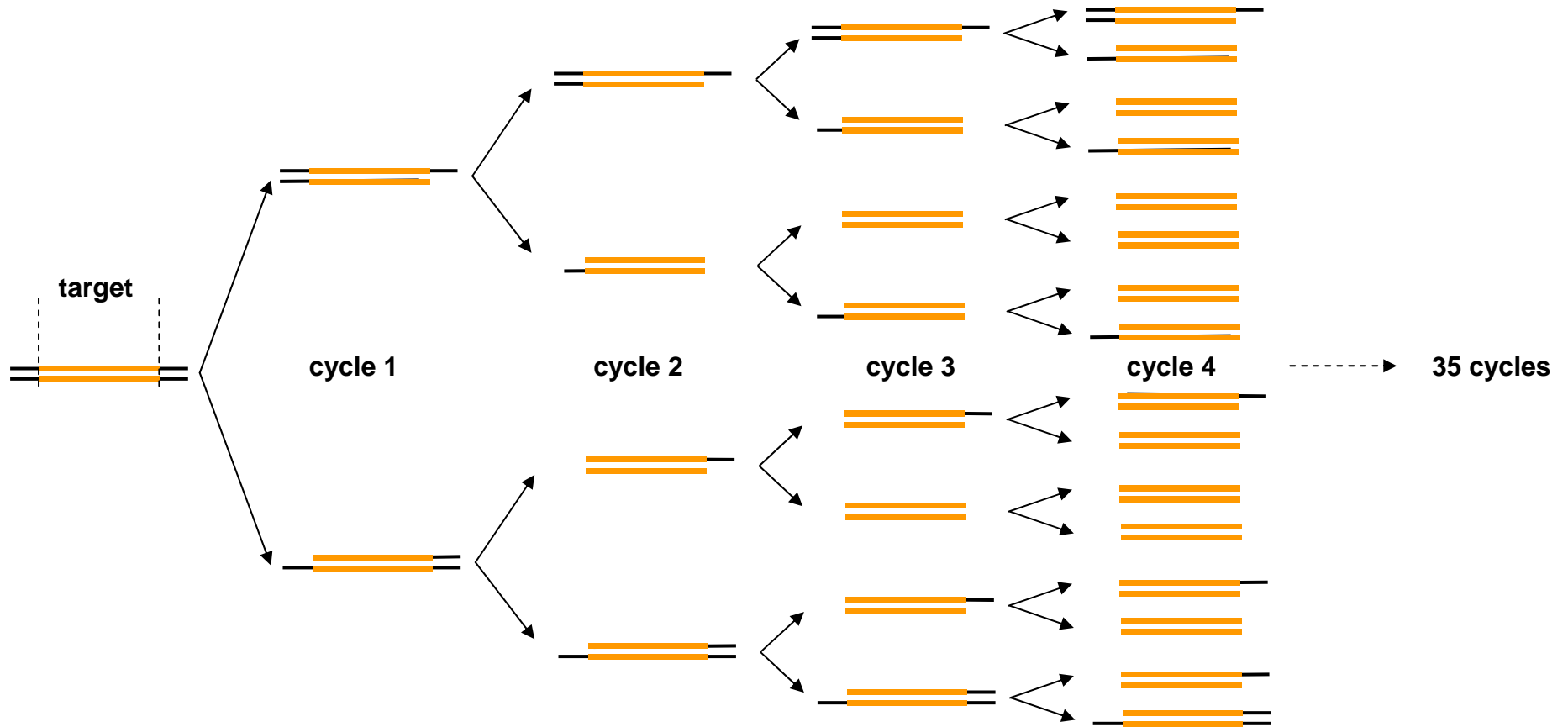
Protein-based method: Lateral-Flow Strips, 5 minutes test



PCR based detection







Exponential amplification:

$$2^1 = 2 \text{ copies}$$

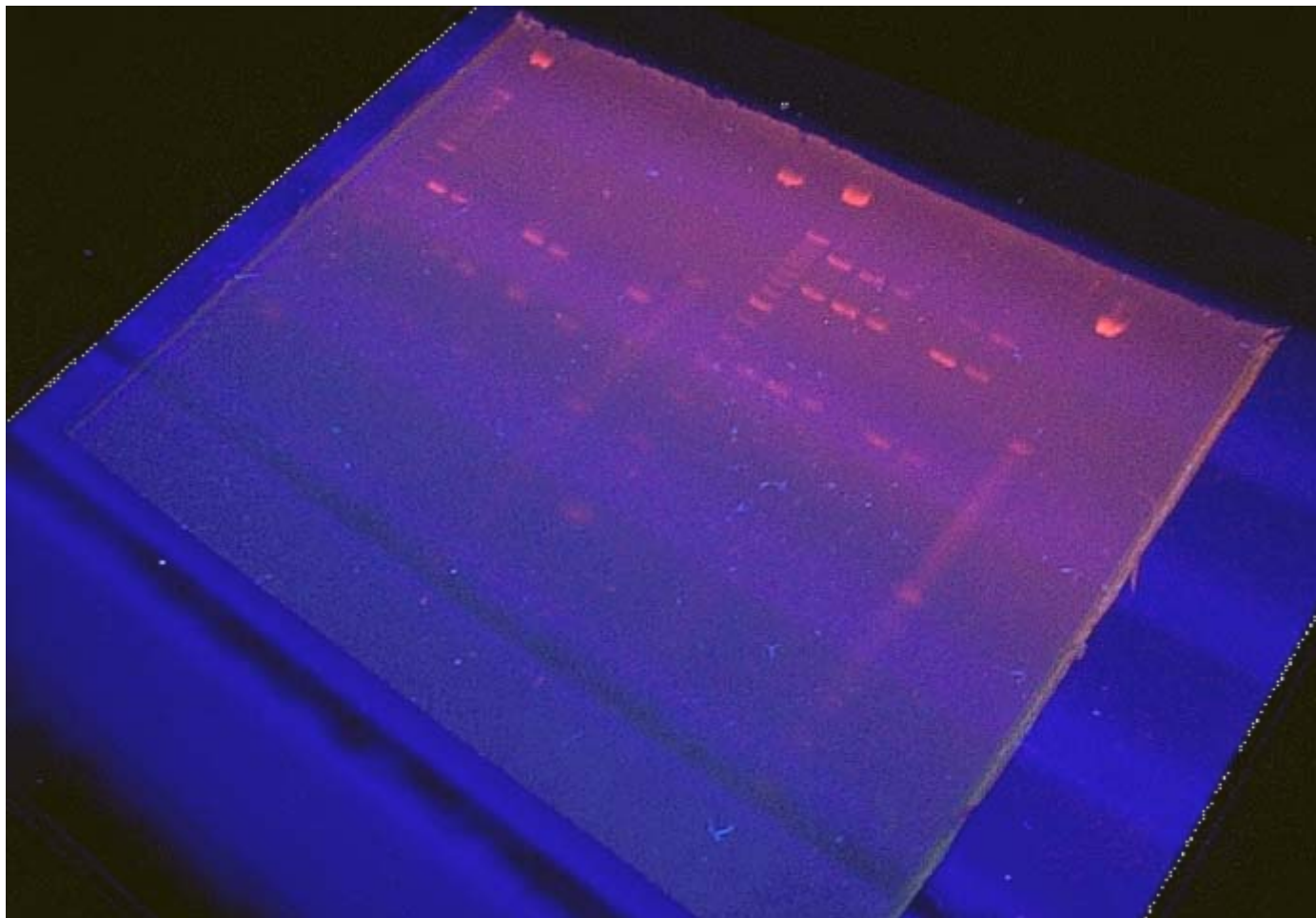
$$2^2 = 4 \text{ copies}$$

$$2^3 = 8 \text{ copies}$$

$$2^4 = 16 \text{ copies}$$

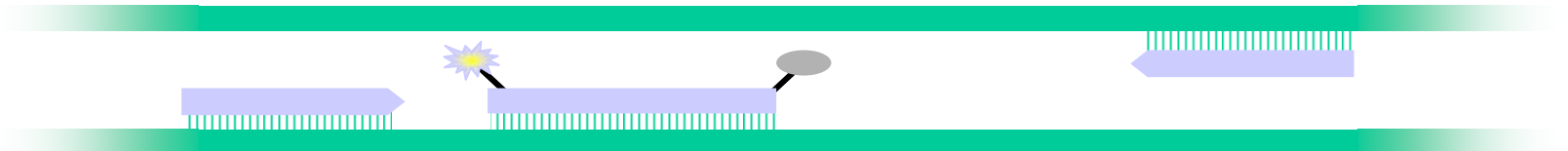
$$2^{35} = 34 \text{ billion copies}$$

n = number of cycles

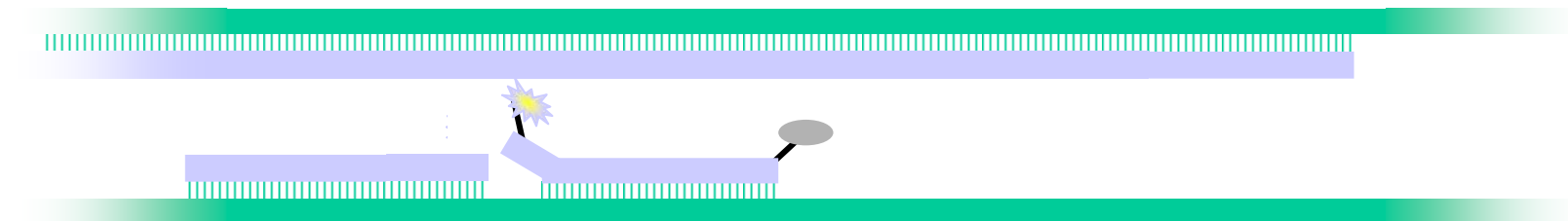


1. denaturation (95 °C)

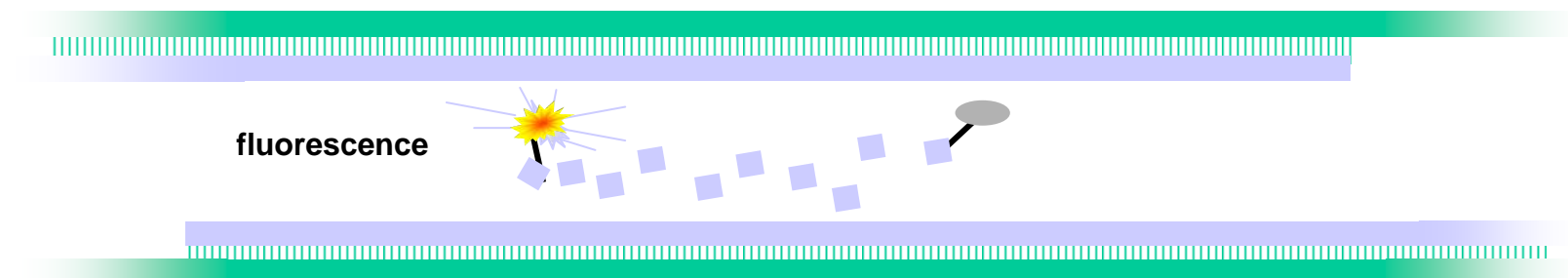
2. Primer- and probe (60 °C)

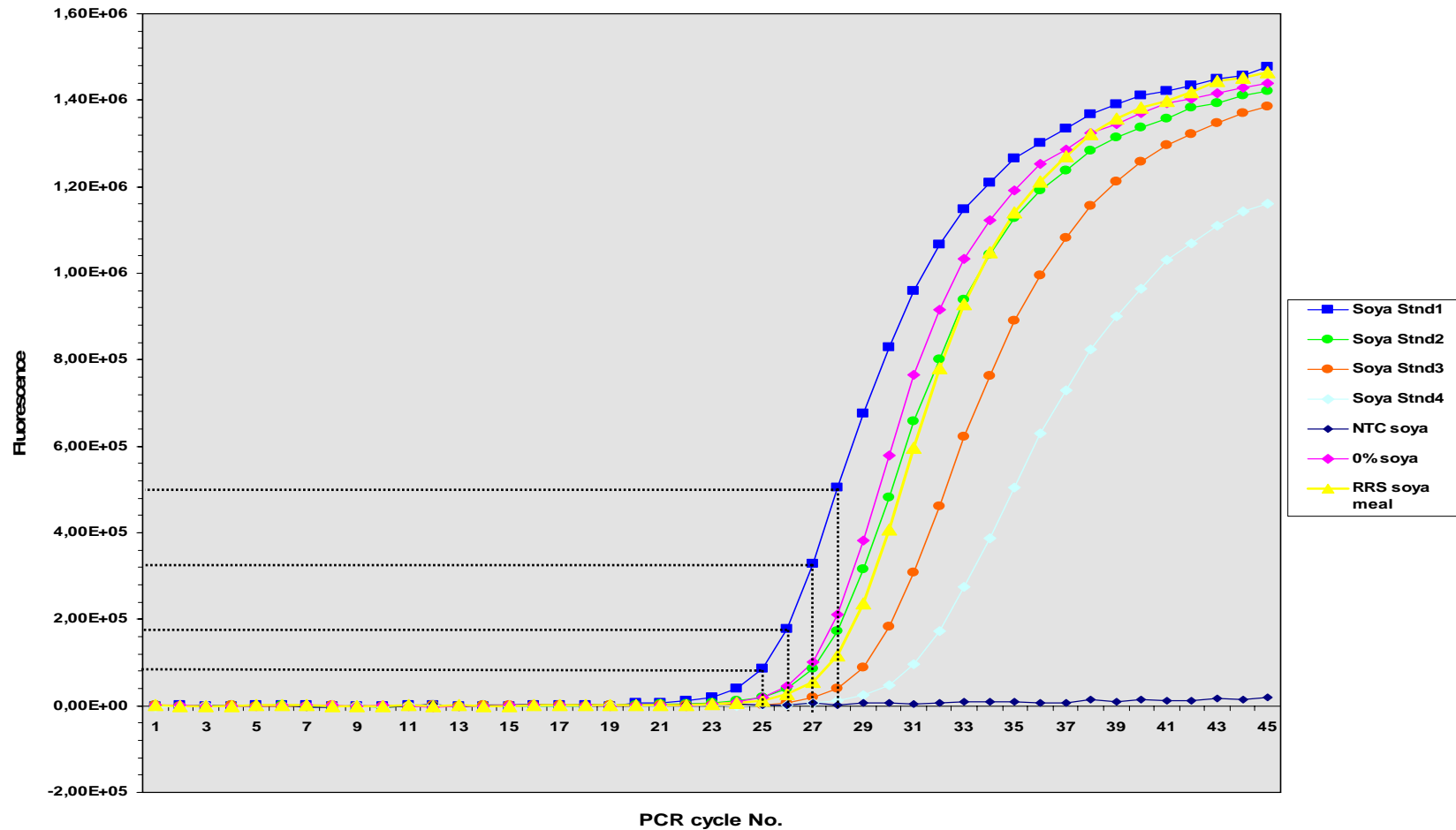


3. Polymerization and replacing the probe (60 °C)

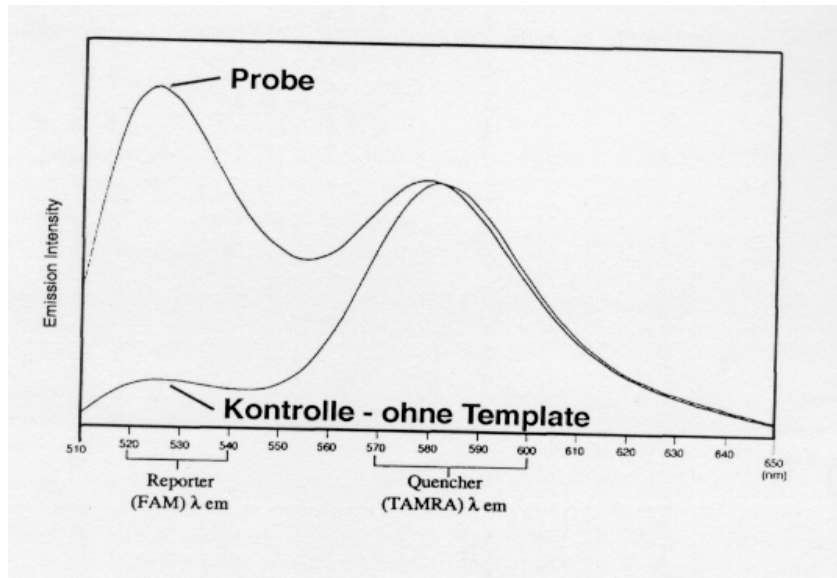


4. Exonuclease activity (60 °C)

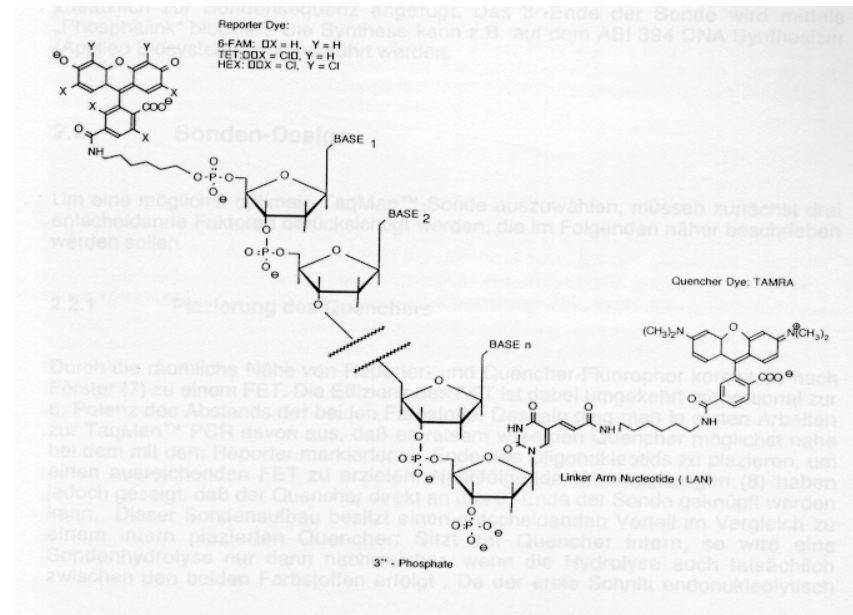




Emission-Scan of samples with or without target, post-PCR



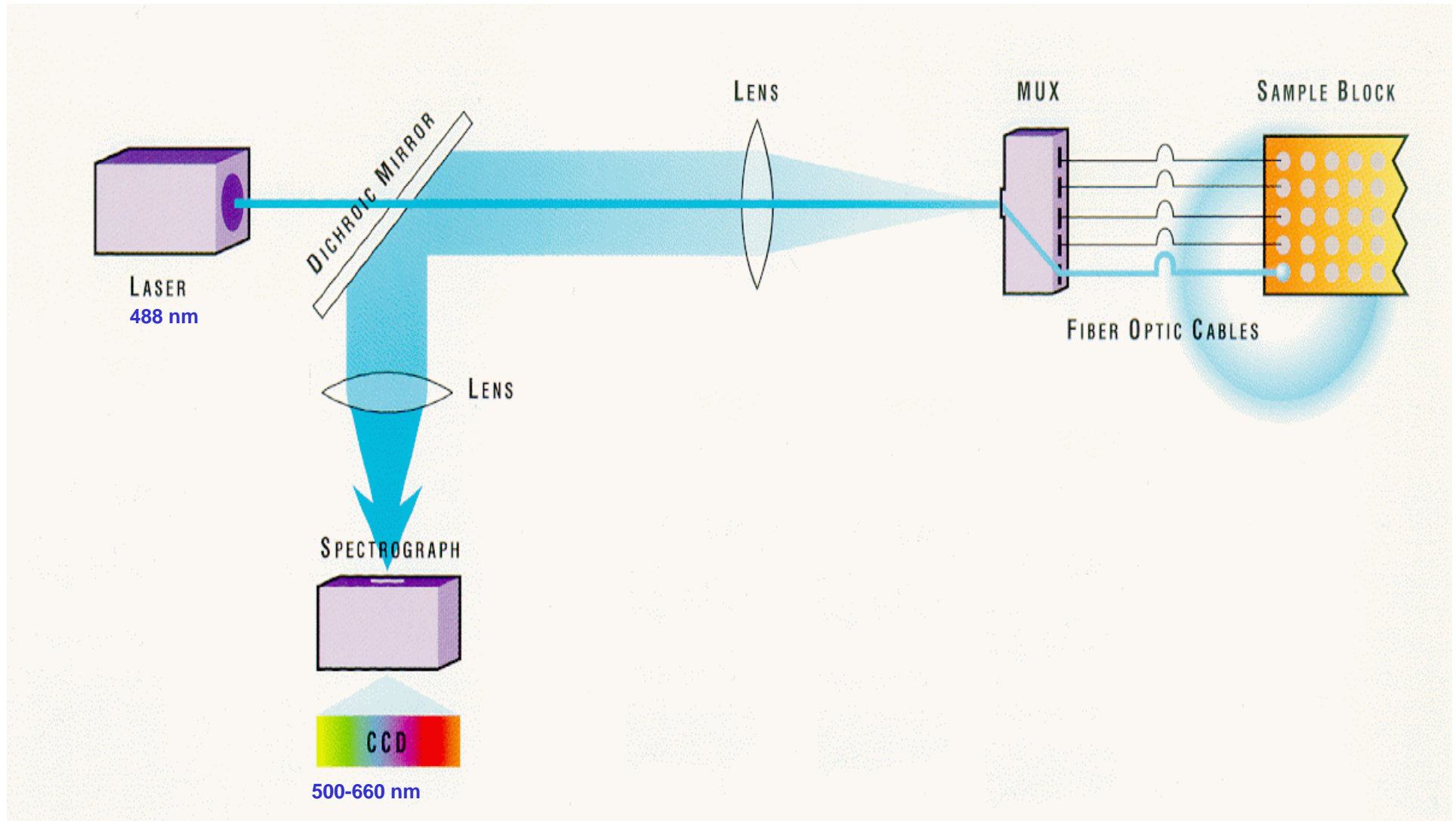
Chemical structure of TaqMan probe

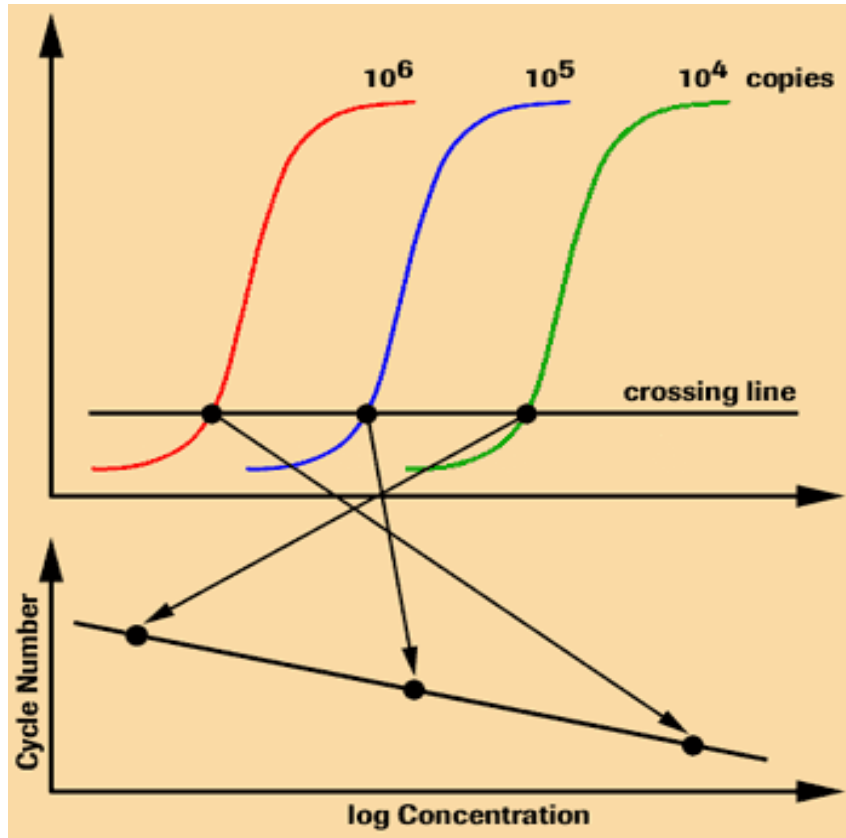


- **Applied Biosystems: ABI Sequence Detection Systems**
 - ABI PRISM 7000, 7700, 7900
 - ABI GeneAmp 5700
- **Roche: Light Cycler**
- **MJ Research: DNA Engine Opticon**
- **Bio-Rad: iCycler**



⇒ **different detection procedures**

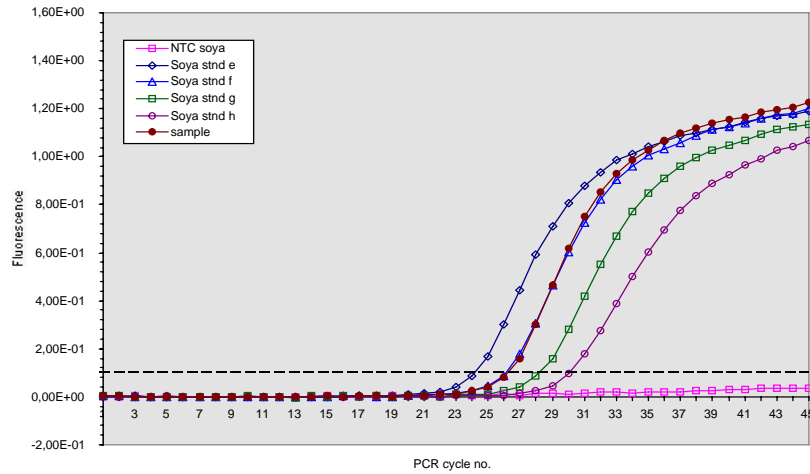




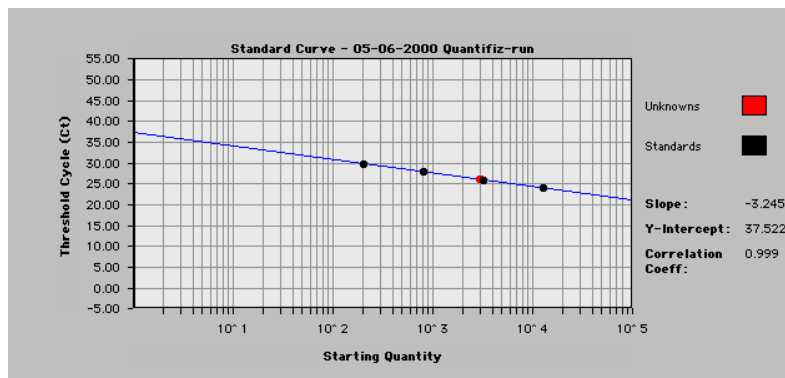
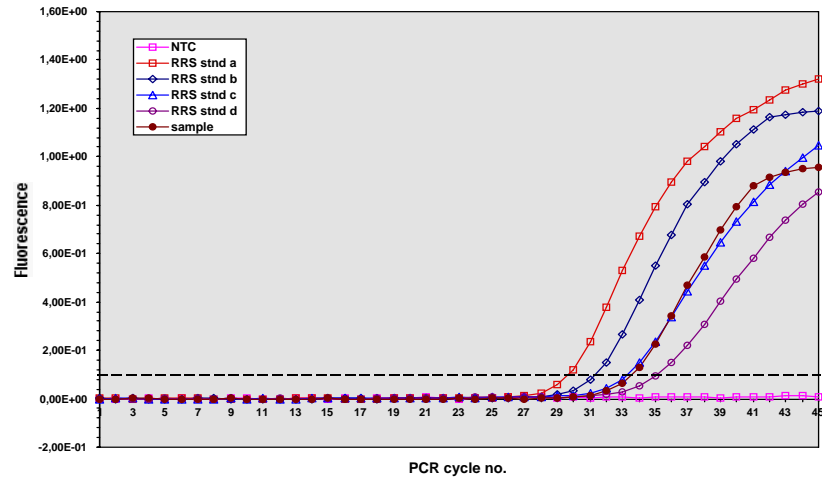
Exponential amplification = $10^{(-1/\text{slope})}$

Efficiency = $[10^{(-1/\text{slope})}] - 1$

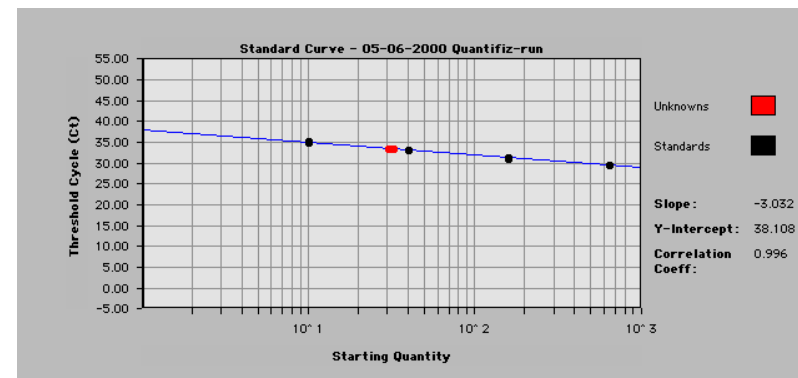
Target taxon specific



RRS specific

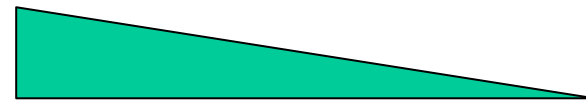


App. 5×10^3 copies

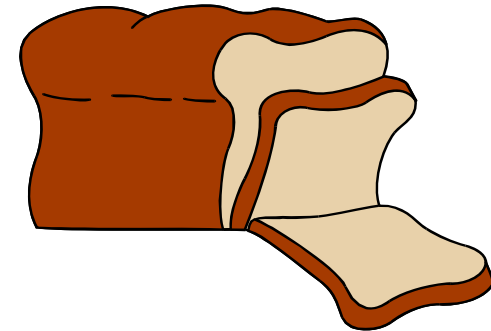


App. 50 copies

- ✓ **PCR: app. 10 genome copies**
- ✓ **correspond to 0.05% GM material**
- ✓ **depending from processing**



Decrease of sensitivity:
– fragmentation
– elimination



- ✓ **need for calculation of the practical LOD for each sample under investigation**

Pre-spotted plates coated with primer/probes for several GM events: a JRC initiative contributing to effective high throughput detection of unauthorised GMOs

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	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	LLCotton2 5 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
E	HMG Maize Ref	SAH7 Cotton Ref	PLD Rice Ref	CruA Oilseed Ref	Lectin Soybean Ref	GS Sugarbeet Ref	UGPase Potato Ref	Bt11 Maize	NK603 Maize	GA21Maize Monsanto	MON863 Maize	1507 Maize
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G	LLCotton2 5 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
H	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

Methodological approach: real-time PCR (probe based)

Format: 96-well plate format

Analytical target(s): Event-specific targets of EU approved and unapproved GM events

Product format: Ready-to-use pre-spotted plates containing, in lyophilized format, all primers and probes for the individual detection of 39 GM events and of the corresponding 7 plants species (maize, cotton, rice, oilseed rape, soybean, sugar beet, and potato).

Just add DNA and cycle !!

We need to get information about a food/feed item by submitting the sample to analysis, applying a specific method;

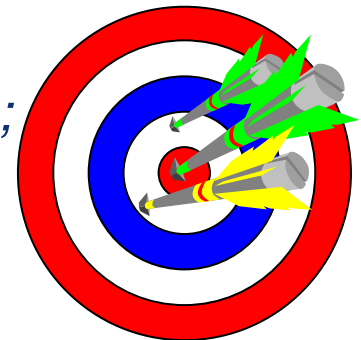
The analytical problem defines the purpose of the method;

Conducting a validation study is a tool to check whether the method is fit for the purpose;

The validation study delivers *performance characteristics*;

How to validate the analytical method?

- **By performing an in-house validation**
- **By conducting a collaborative study**



Convener: Dr. Marianna Schauzu, Federal Institute for Risk Assessment, Berlin
Secretary: Carola Seiler, DIN, Germany

EN ISO	Topic	Stage	Details
21572	Foodstuffs - Methods for the detection of genetically modified organisms and derived products - Protein based method	Standard ratified in November 2003	Corrigendum to change the status of the Annex from "normative" into "informative" has been published by ISO and is under way in CMC
21571	Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction	Standard ratified in February 2005	
21569	Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods	Standard ratified in June 2005	
24276	Foodstuffs - Nucleic acid based methods of analysis for the detection of genetically modified organisms and derived products - General requirements and definitions	Standard ratified in January 2006	
21570	Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Quantitative nucleic acid based methods	Standard ratified in October 2005	
21568	Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products – Sampling	European Technical Standard 2006	No agreement within ISO

<http://gmo-crl.jrc.ec.europa.eu/>

