



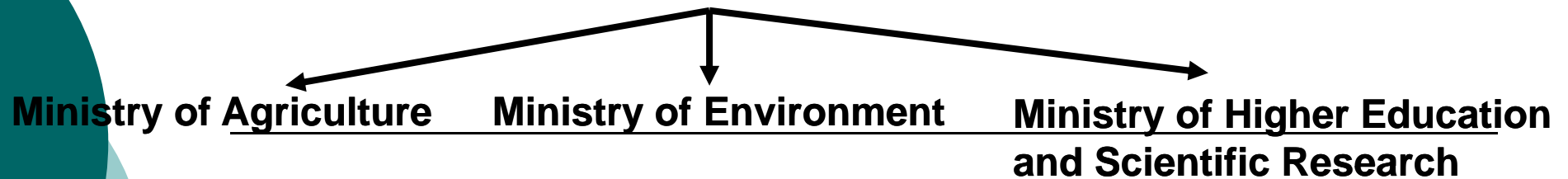
## **1st International Workshop on Harmonization of GMO Detection and Analysis in the Middle East and North Africa (MENA) Region**

**Dead Sea, Jordan: 4 - 5 June 2012**

**Biotechnology and GM crops development in Tunisia:  
Status and experience related to the implementation  
of GMO and legislation**

**Pr. Khaled Masmoudi & Dr. Faïçal Brini**  
**Centre of Biotechnology of Sfax (CBS), Tunisia**

# Germplasm improvement programs in Tunisia: the players



## ➤ Key Biotech institutions: CBS, CBBC, INAT, INRAT, FST,..

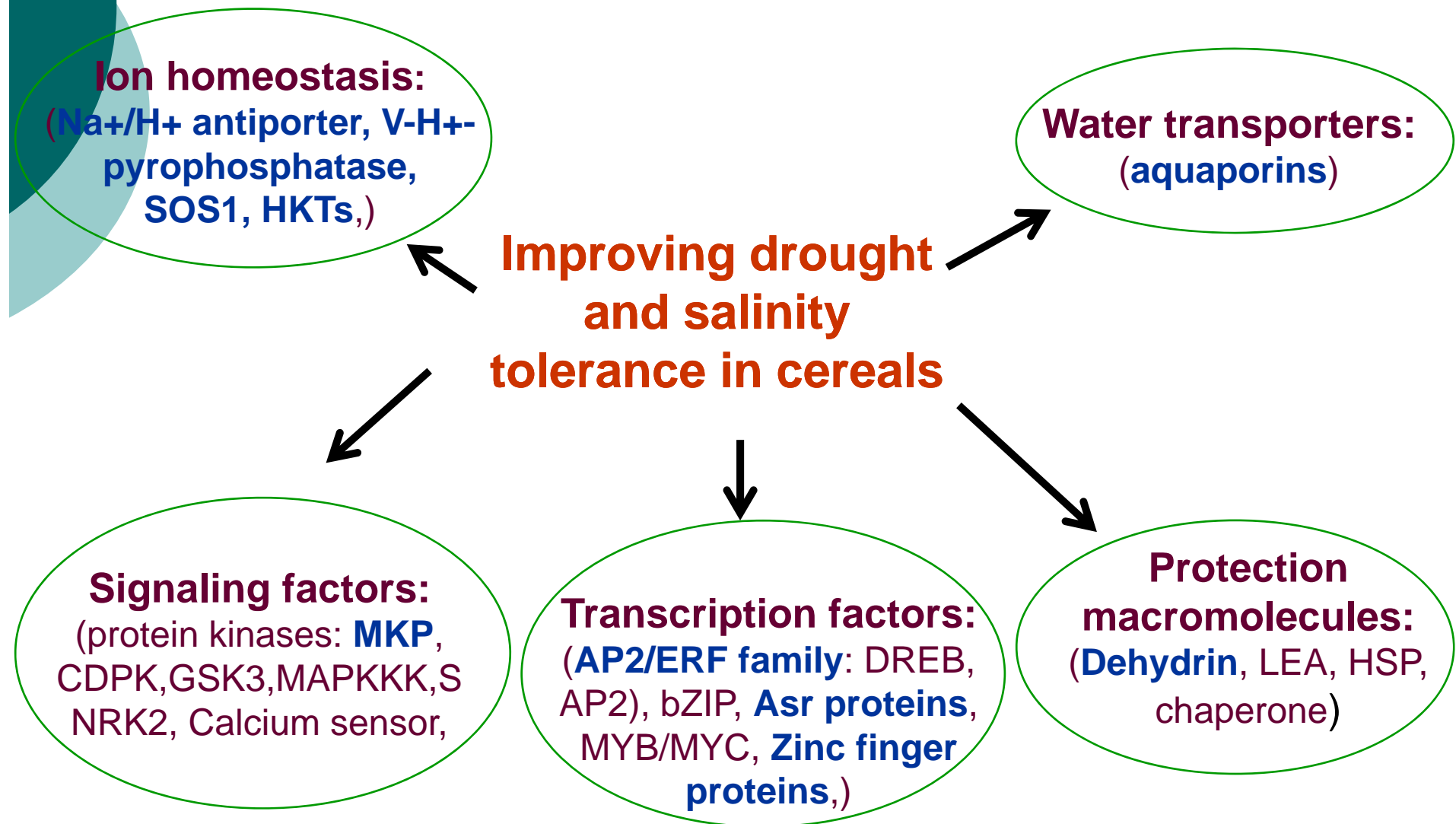
✓ Modern biotechnology tools: tissue culture techniques, DNA molecular markers, molecular genetics and genetic engineering technology, ..

✓ Genetic engineering is applied for research only and this is due to lack of human capacity, financial support and biosafety framework.

✓ In Tunisia, there are no GMOs grown in open field at commercial level

✓ Tunisia is party to the Convention on Biological Diversity and the Cartagena Protocol on Biosafety

# Current state of art in our laboratory at CBS





# **Status of GMO crops developed in Tunisia for research purpose**

---

## **Institutions:**

- ✓ **CBS**
- ✓ **CBBC**
- ✓ **FST**

- ✓ **Potato: PVY- CP, scFv**
- ✓ **Tomato: TYLCV, NHX-PPase,**
- ✓ **Durum wheat: NHX-PPase, DHN5, AISAP,..**
- ✓ **Grapevine: GFLV, Rd22,**

# Project of law regulating GMOs

---



**Confined  
utilisation**

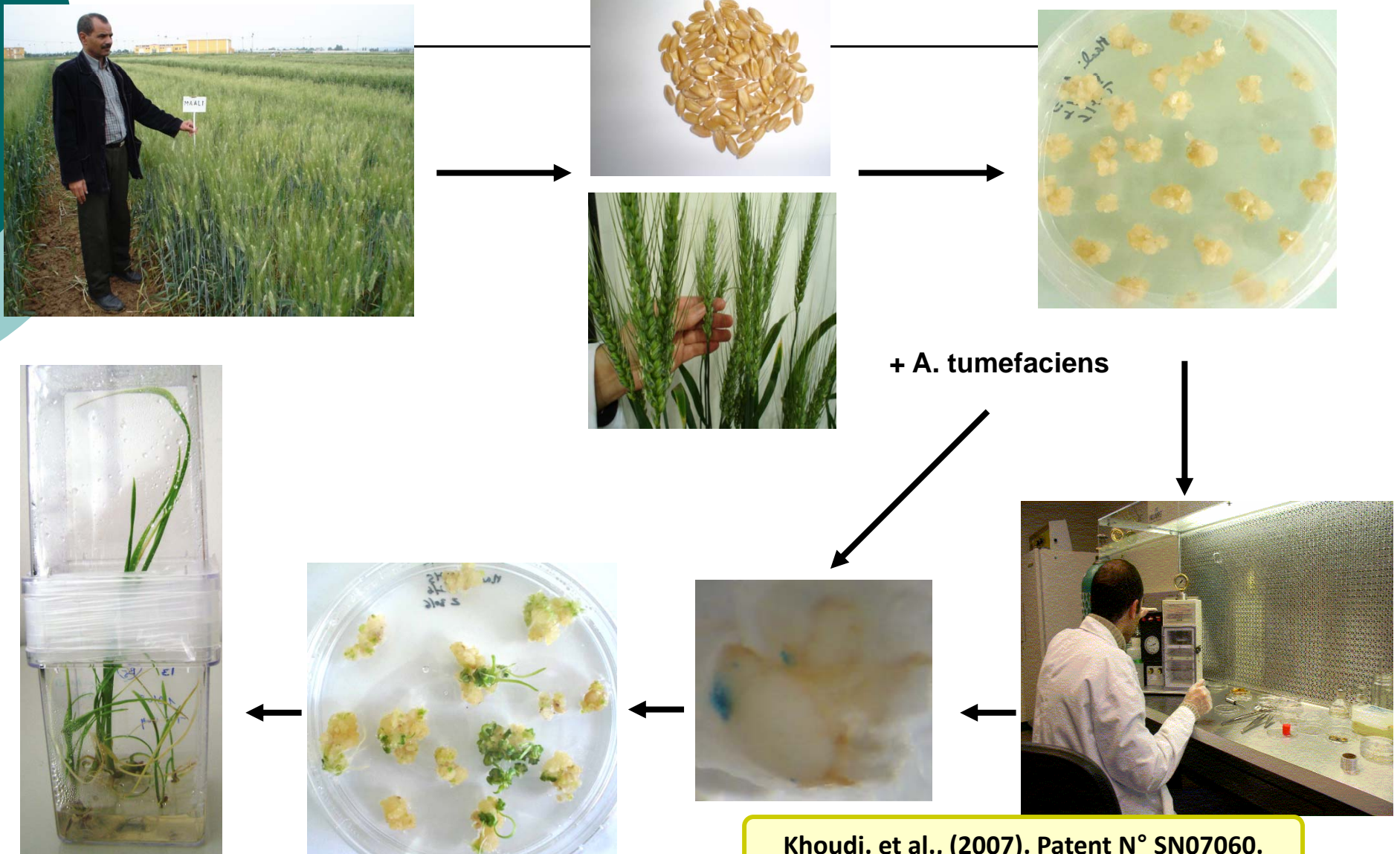
**Volunteer  
dissemination**

**Commercialisation**

**Importation  
& Transit**

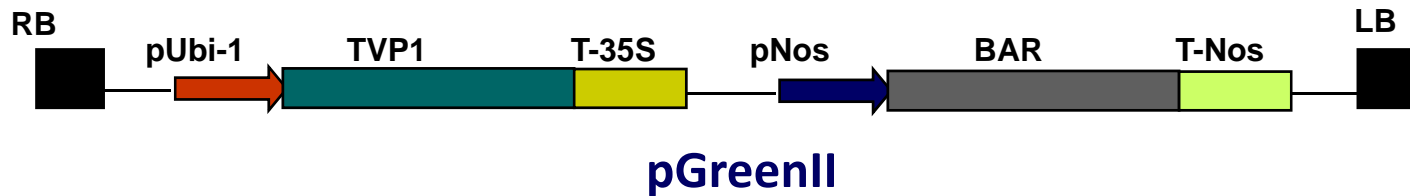


# Durum wheat and barley transformation procedure using the Bio-Rad PDS-He1000 or *Agrobacterium tumefaciens*

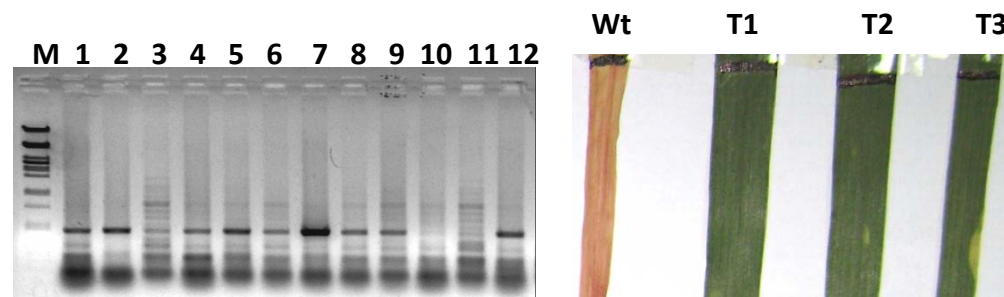




# Molecular and physiological analysis of transgenic durum wheat plants



Transgenic wheat plants



PCR screening

Leaf painting with Basta

## Salinity tolerance phenotype of different transgenic durum wheat lines compared to sensitive non transformed plants



WT

TVP1.1



WT

TVP1.1

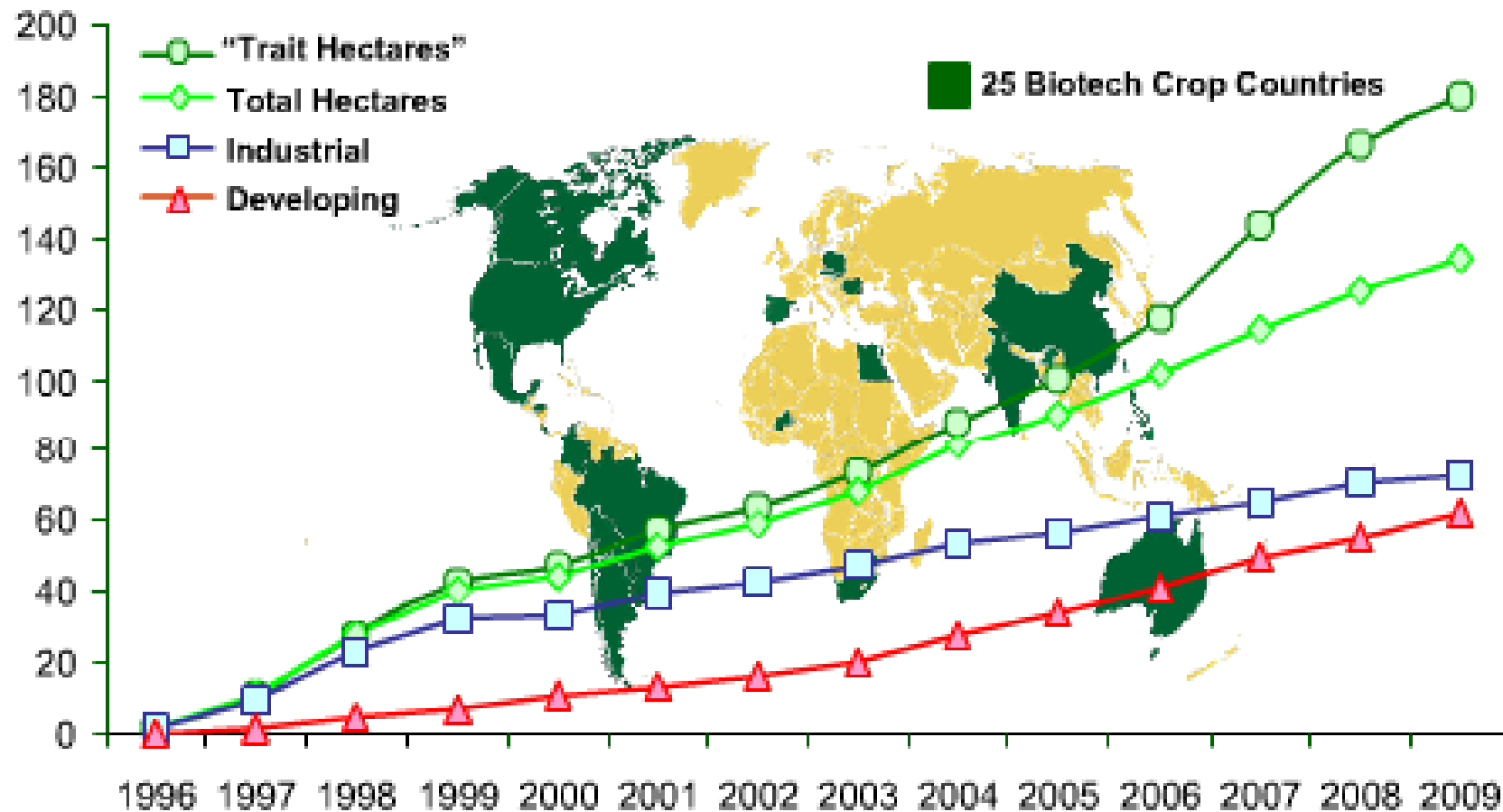
TVP1.2

**200 mM NaCl**

**Gouiaa et al. in preparation**



### GLOBAL AREA OF BIOTECH CROPS Million Hectares (1996 to 2009)



*A record 14 million farmers, in 25 countries, planted 134 million hectares (330 million acres) in 2009, a sustained increase of 7% or 9 million hectares (22 million acres) over 2008.*

Source: Clive James, 2009.

# Risk assessment of GM plants

## 1. Contained use: laboratory, greenhouse, closed facilities

It covers:

- ✓ General information on the project
- ✓ Risk assessment for environmental protection
- ✓ Risk assessment for human health and safety

## 2. Deliberate environmental release

Application with the following information's:

- The GMO (e.g. recipient organism, details of modification and novel trait)
- Proposed release and the receiving environment
- Interactions between GMO and the environment
- Monitoring regime and procedures for controlling the release
- A statement evaluating the impacts and risks posed by the GMO to human health and the environment from the uses envisaged

# GMO or not GMO?

... Towards harmonization on:



- **Sampling**

- **Sample preparation**

- **Extraction methods**

- Matrix
    - Processing
    - Protocols

- **Analytical choice**

- **DNA-based methods**

- **Protein-based methods**

- Protocols
      - Specificity
      - Sensitivity

- **Interpretation and reporting**

- **Laboratory facilities**

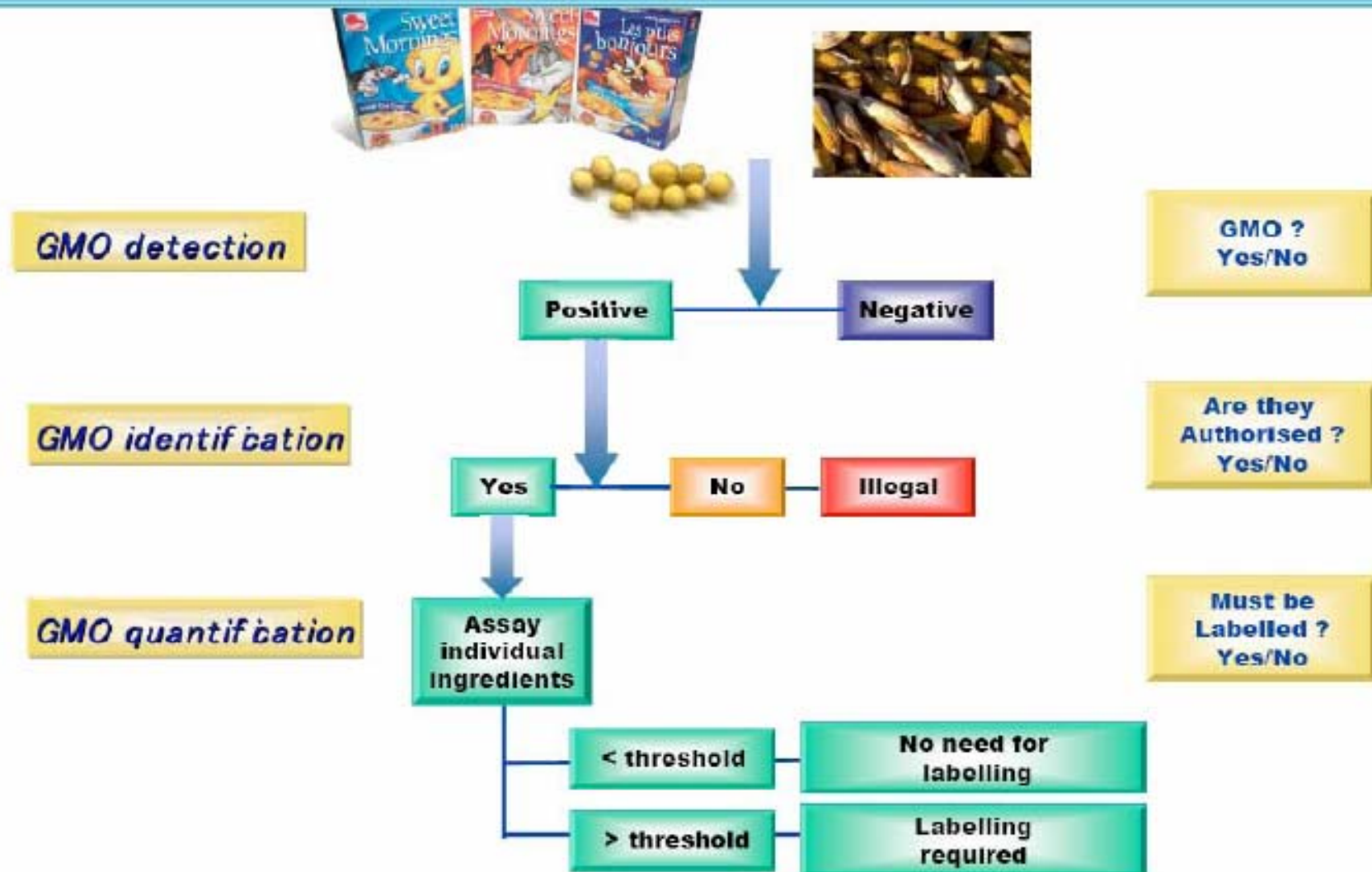
- **Quality assurance**

- **Accreditation**

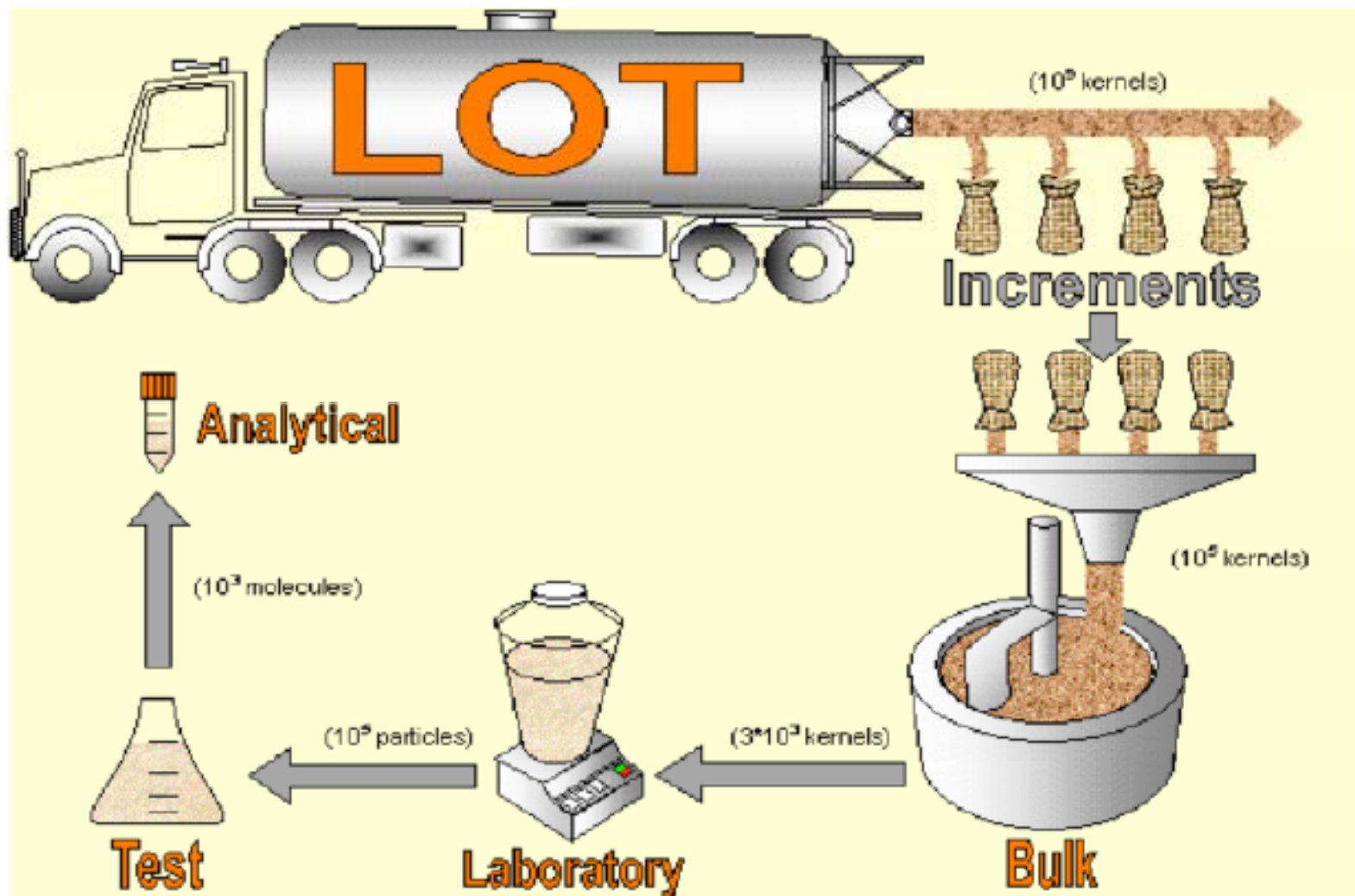
- **Validation**

# Operation of GMO detection Laboratories :

## To comply with national legislation



## Sampling steps: general scheme





# Steps in GMO analysis: sources of errors

20

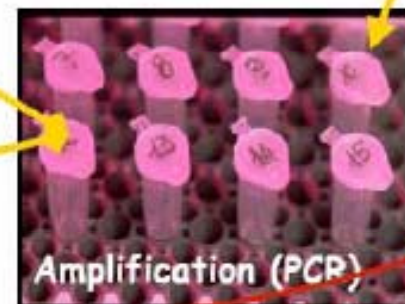
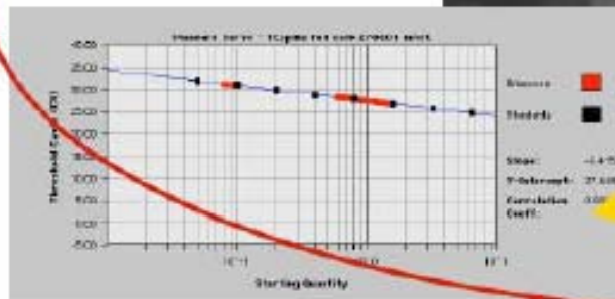


**Sampling error**



**DNA extraction**

**Analytical error**

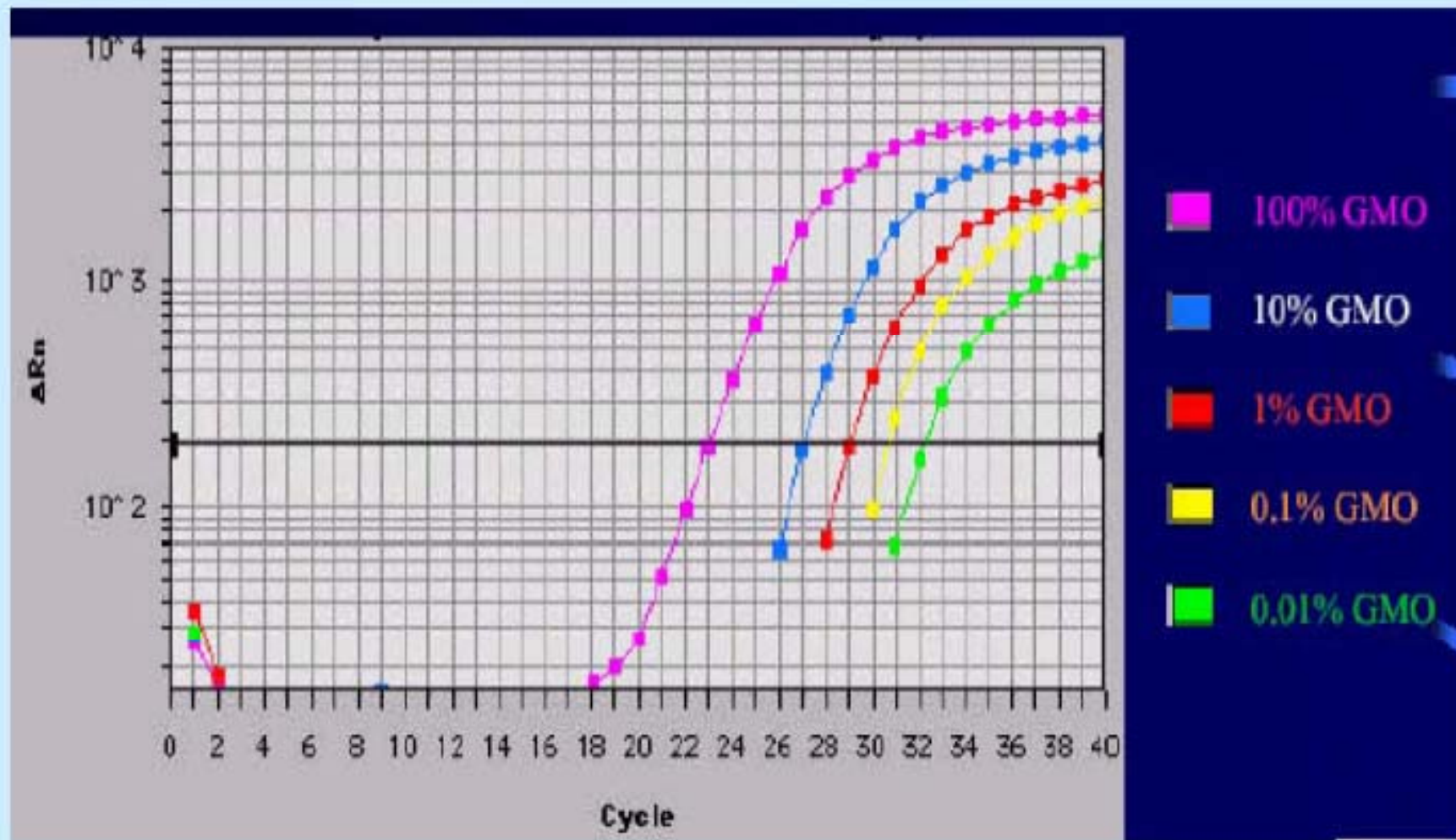


**Amplification (PCR)**

# Requirements for GM-detection

- **Target molecules must be present:**
  - DNA (template) or protein
- **Capture molecules must be developed:**
  - For DNA: primers and probes
  - For proteins: antibodies
- **Reference materials**
  - Positive and negative controls
  - Calibrants for quantitation

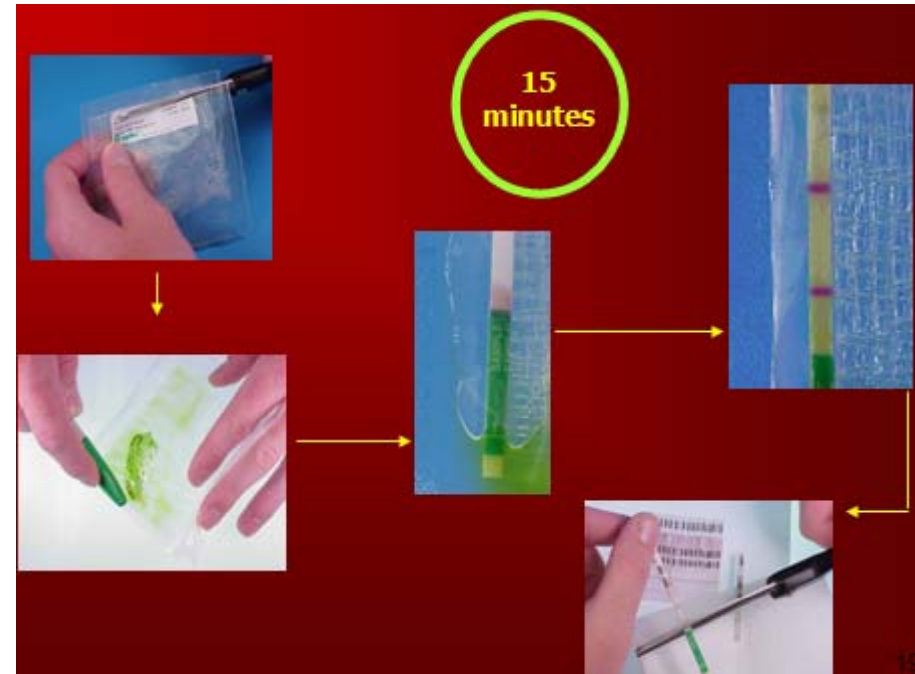
# Real Time PCR - GMOs Detection Thresholds



# Flashkits® OGM



- Easy to use
- Fast- results in few minutes
- Easy to interpret
- Cheap, economic
- Accreditation by GIPSA-USDA





## Example of detection threshold

---

Variété testée	Protéine détectée	Limite de détection
Résistance à l'herbicide RoundUp Ready	CP4 EPSPS	1 grain CP4 EPSPS dans 1000 grains (0.1%)
Résistance à la pyrale	Bt-Cry1Ab1Ac	1 grain Bt- Cry1Ab/1Ac dans 100 grains (1%)



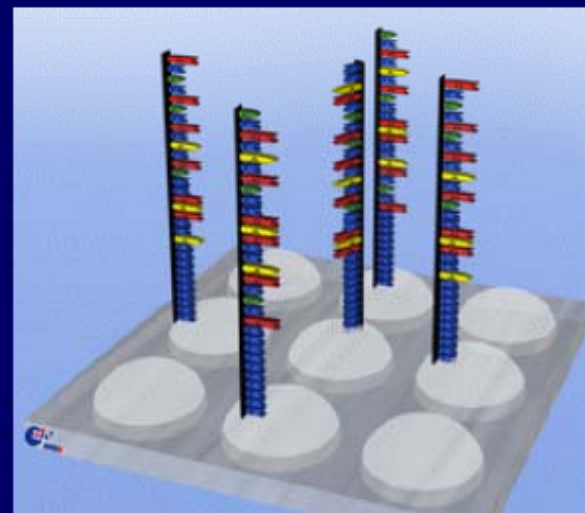
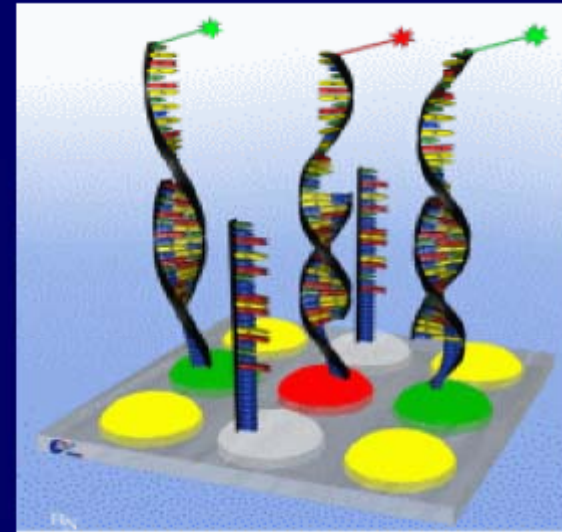
# GMO CHIPS

Based on classical DNA hybridization principle

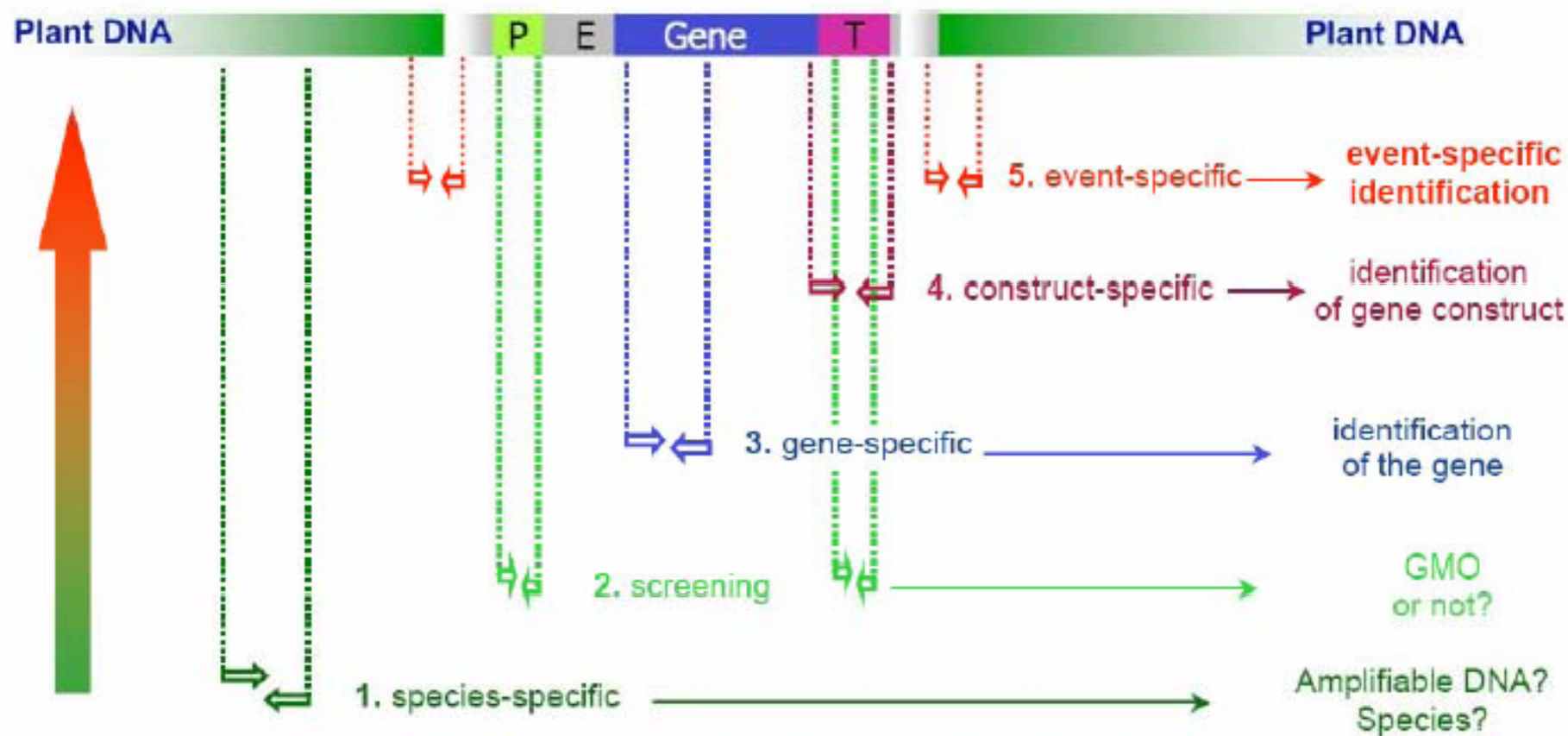
Many specific probes are attached to a solid surface

Screen & identifies GMOs in raw material and processed food, currently available on soybean, maize, oilseed, rape, rice, CaMV, RR-soybean, *Bt* etc.,

Allows screening for all GMOs with the CaMV 35S promoter, Nos-terminator, *bar*-gene and *pat*-gene



## Levels of specificity – GMO target sequences





**Thank you for your attention!**