

# Overview of Methods for the Detection and Quantification of Living Modified Organisms

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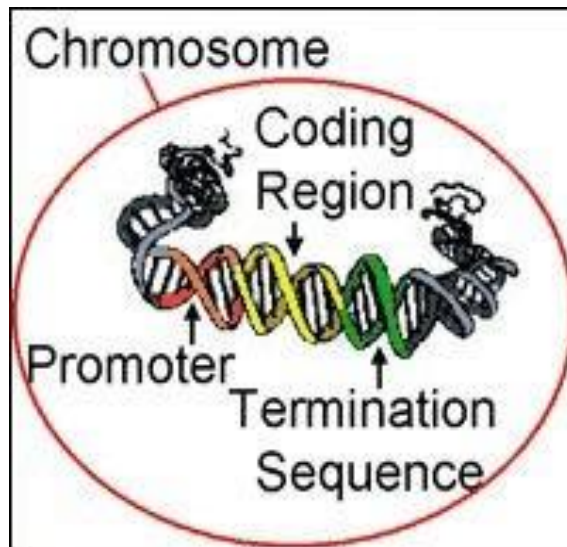
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**GM: Genetic Modification**

**GMO: Genetically Modified Organism**

**GE: Genetic Engineering**

**LMO: Living Modified Organism**



# **Introduction to GM Crop Production**

# Global Area of Biotech Crops, 1996 to 2012: Industrial and Developing Countries (M Has, M Acres)



M Acres

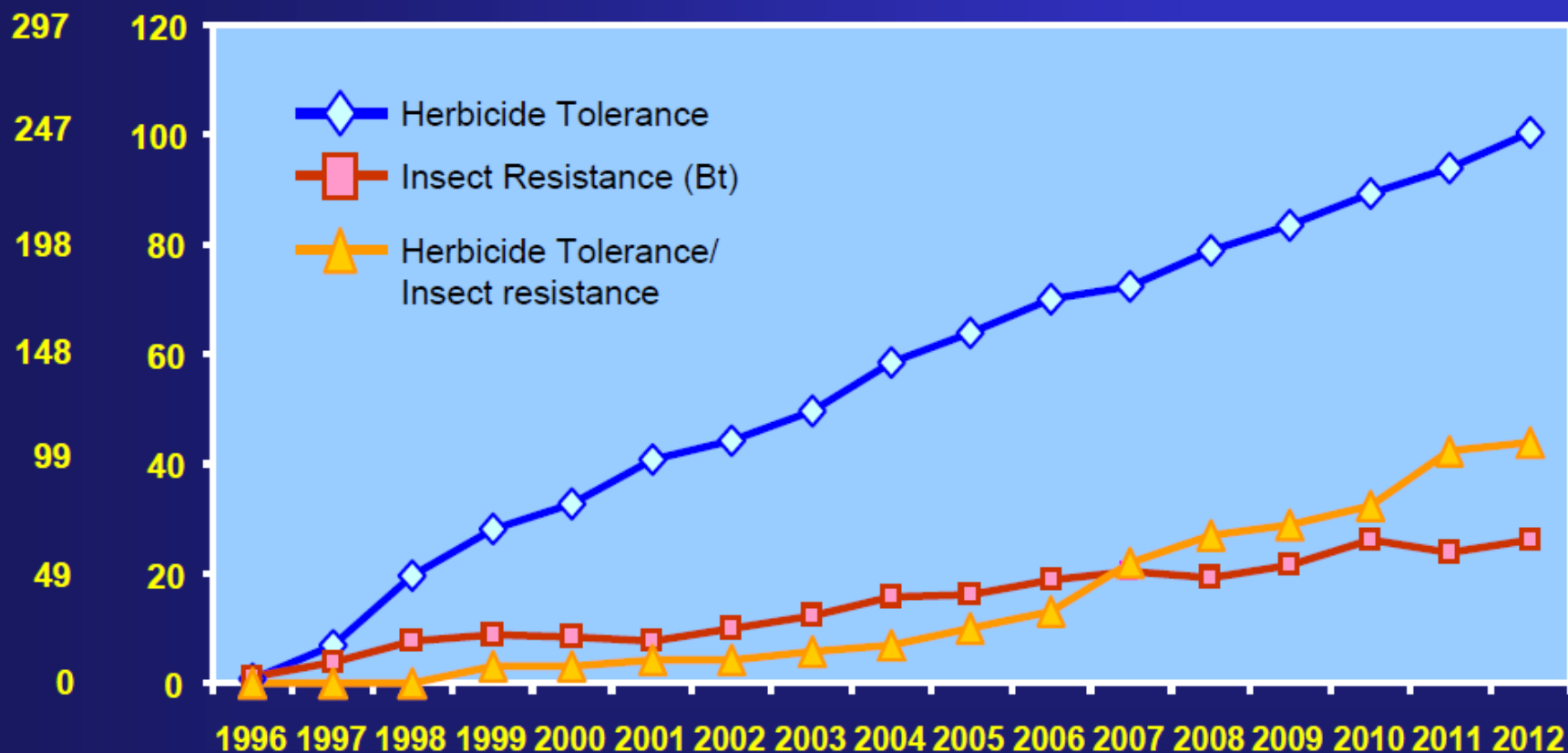


Source: Clive James, 2012

# Global Area of Biotech Crops, 1996 to 2012: By Trait (Million Hectares, Million Acres)



M Acres

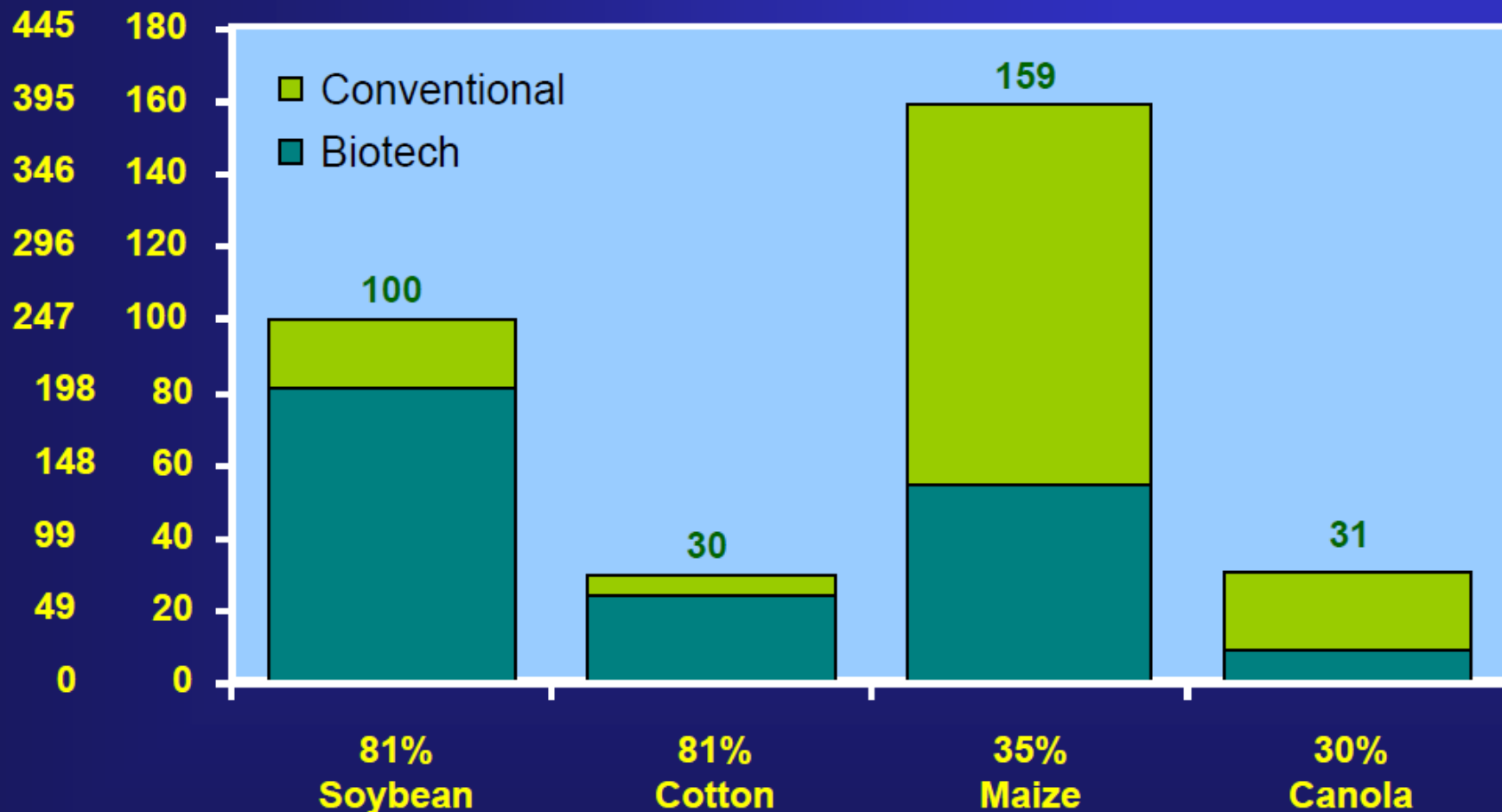


Source: Clive James, 2012

# Global Adoption Rates (%) for Principal Biotech Crops (Million Hectares, Million Acres), 2012



M Acres



Source: Clive James, 2012

# Events for Major GM Crops

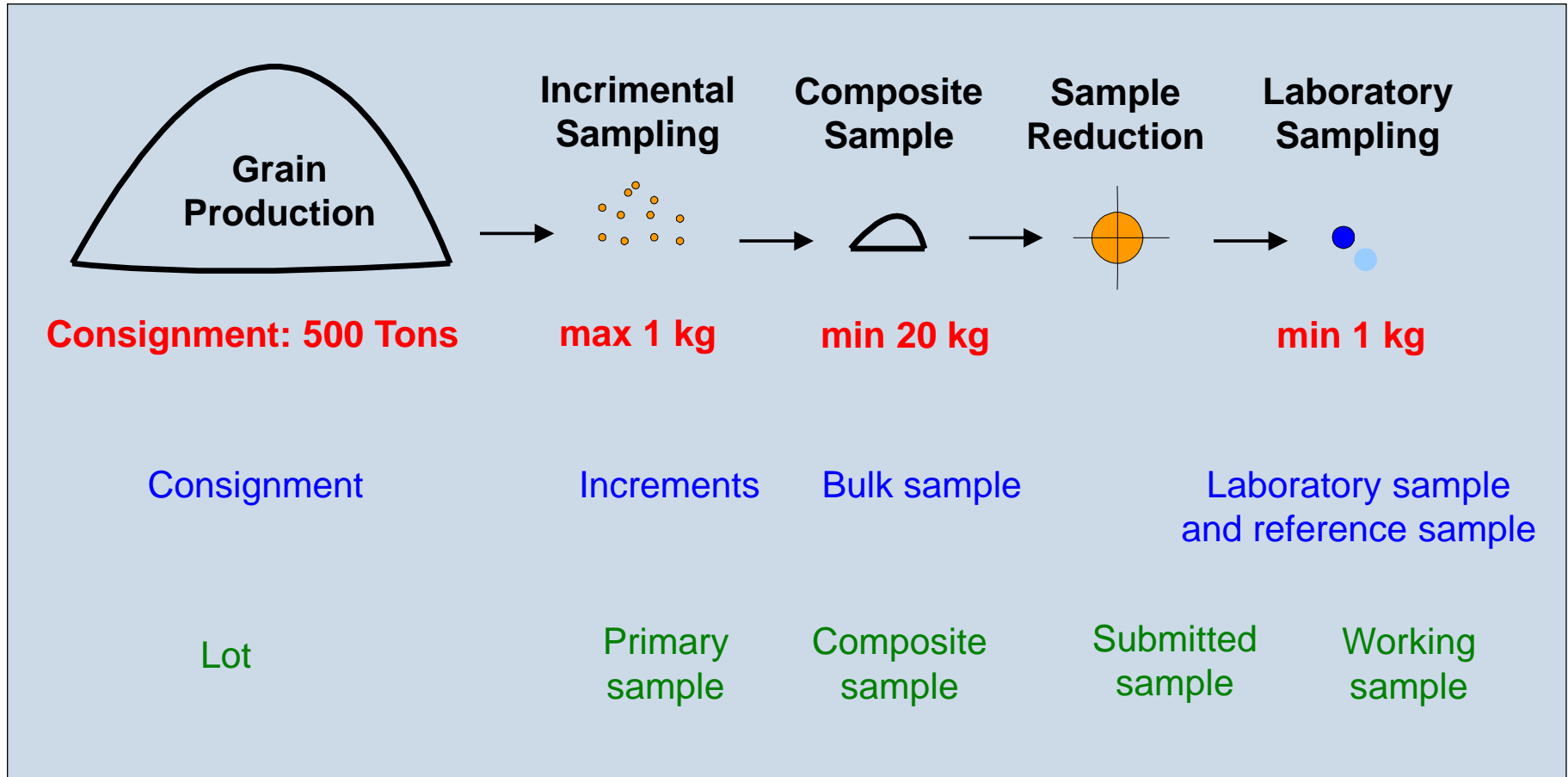
Crop	Trait	Genes	Commercial events	Countries with environmental release
Canola	FA and HT	als, bar, bxn, bay, epsps, pat	15	Australia, Canada, Japan, USA
Cotton	HT and IR	als, bar, bxn, epsps, cry1Ab, cry1Ac, cry2Ab, cry2AB2, cry1F, pat, vip3A	21	Argentina, Australia, Brazil, Burkino Faso, Colombia, India, Japan, Mexico, South Africa, USA
Maize	AA, IR and HT	als, amy797E, bar, cry1Ab, cry1A.105, cry2Ab, cry1Fa2, mcry3A, cry3Bb1, cry9C, cry34Ab1, cry35Ab1, epsps, pat	58	Argentina, Australia, Brazil, Canada, Colombia, EU, Japan, Korea, Philippines, South Africa, USA, Uruguay
Soybean	FA, HT and IR	bar, csr1-2, cry1Ac, epsps, fad2-1, gat4601, pat	13	Argentina, Brazil, Canada, Japan, Mexico, Paraguay, South Africa, Uruguay, USA

(<http://cera-gmc.org>)

# **Sampling for GMO Detection**

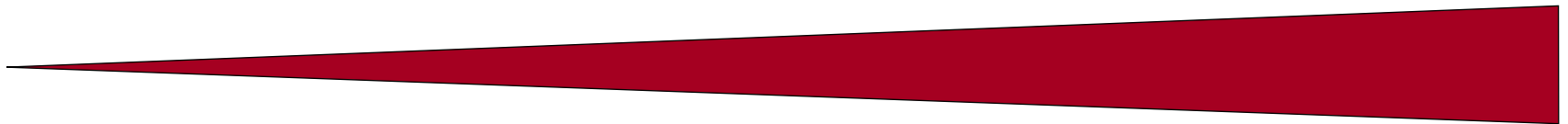


# GMO Sampling

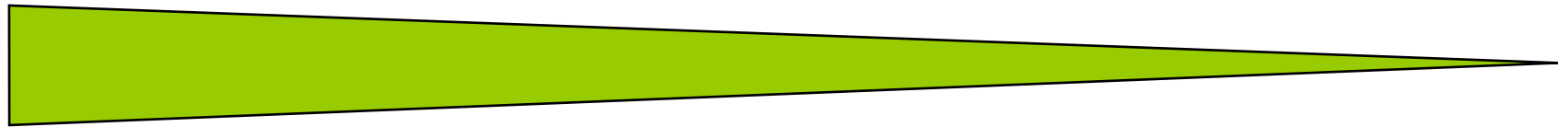


# Sample Homogeneity vs Particle Size

Increasing homogeneity / representation



Particle Size



# Probability Table for Sample Size

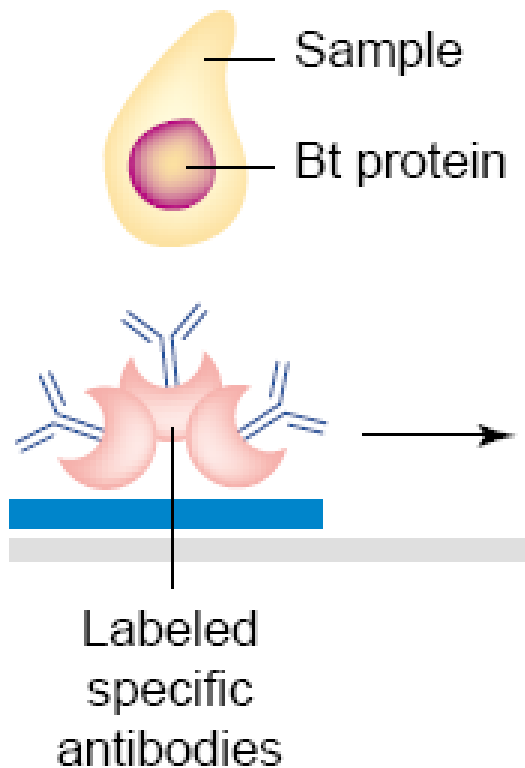
Table 1: Determining Laboratory Sample sizes using the binomial distribution

Nr of grains	Probability level		
	90%	95%	99%
100	2.28%	2.95%	4.50%
200	1.14%	1.49%	2.28%
300	0.76%	0.99%	1.52%
400	0.57%	0.75%	1.14%
800	0.29%	0.37%	0.57%
1200	0.19%	0.25%	0.38%
2000	0.12%	0.15%	0.23%
2500	0.09%	0.12%	0.18%
3000	0.08%	0.10%	0.15%
6000	0.04%	0.05%	0.08%
10000	0.02%	0.03%	0.05%

# **GMO Testing Methods**

- **Protein Detection (Antibody Recognition)**
- **DNA Detection (DNA Sequence Recognition)**

# Protein Antibody Recognition



# Protein Testing

- **Strip test**
  - Qualitative
  - Can be used at point of entry
  - LOD ( $>0.1\%$ )
- **ELISA**
  - Qualitative / Quantitative
  - Requires a plate reader
  - LOD/LOQ ( $\approx 0.1\%$  to  $0.2\%$ )



# Protein Testing

- **Advantages**

- Easy / Moderately Easy
- Reasonably Low Technology Input
- Qualitative / Quantitative

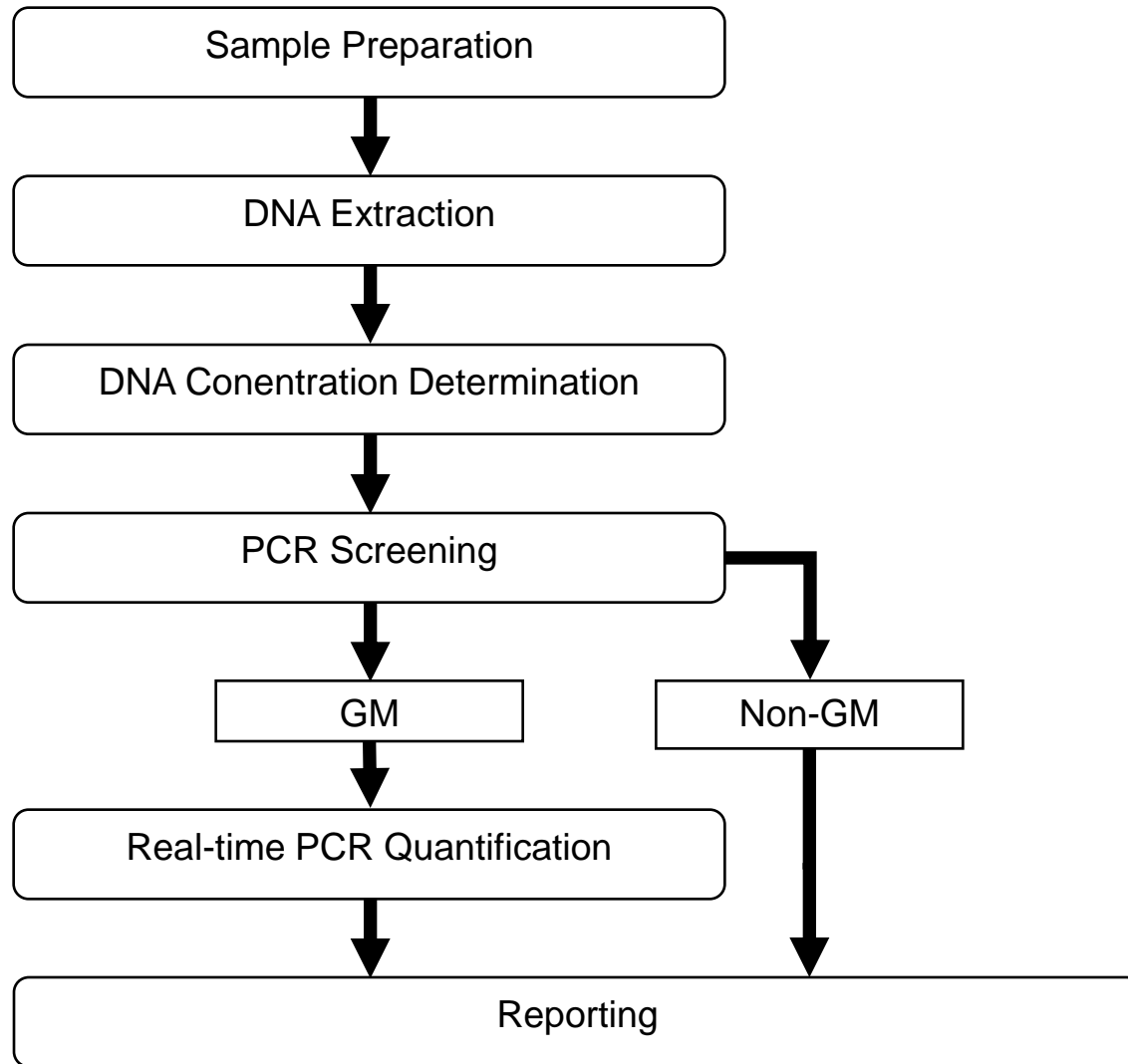
- **Disadvantages**

- Specific for a Particular Transgene Protein
- Relies on Commercial Availability of Antibodies

# **DNA Detection**



# Workflow for DNA Based Testing



# DNA Extraction

- **Matrix Specific Extraction Methods**
- **Ensure Quality and Quantity of DNA**

# DNA Based Testing

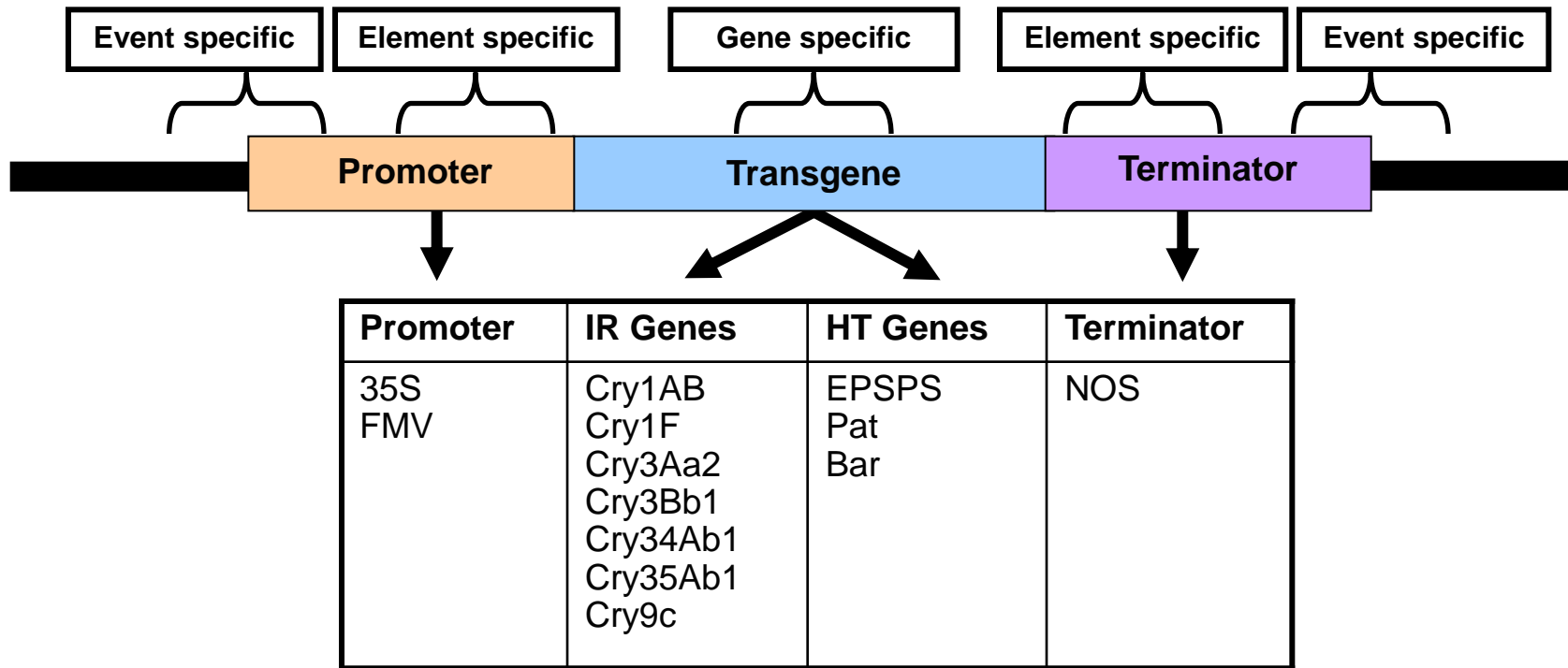
- **Qualitative PCR Screening**

- Element
- Gene
- Construct
- Event

- **Quantitative Real-time PCR**

- Element Quantification (Limited Application)
- Event Quantification

# The Transgene Construct



# Detection Scheme

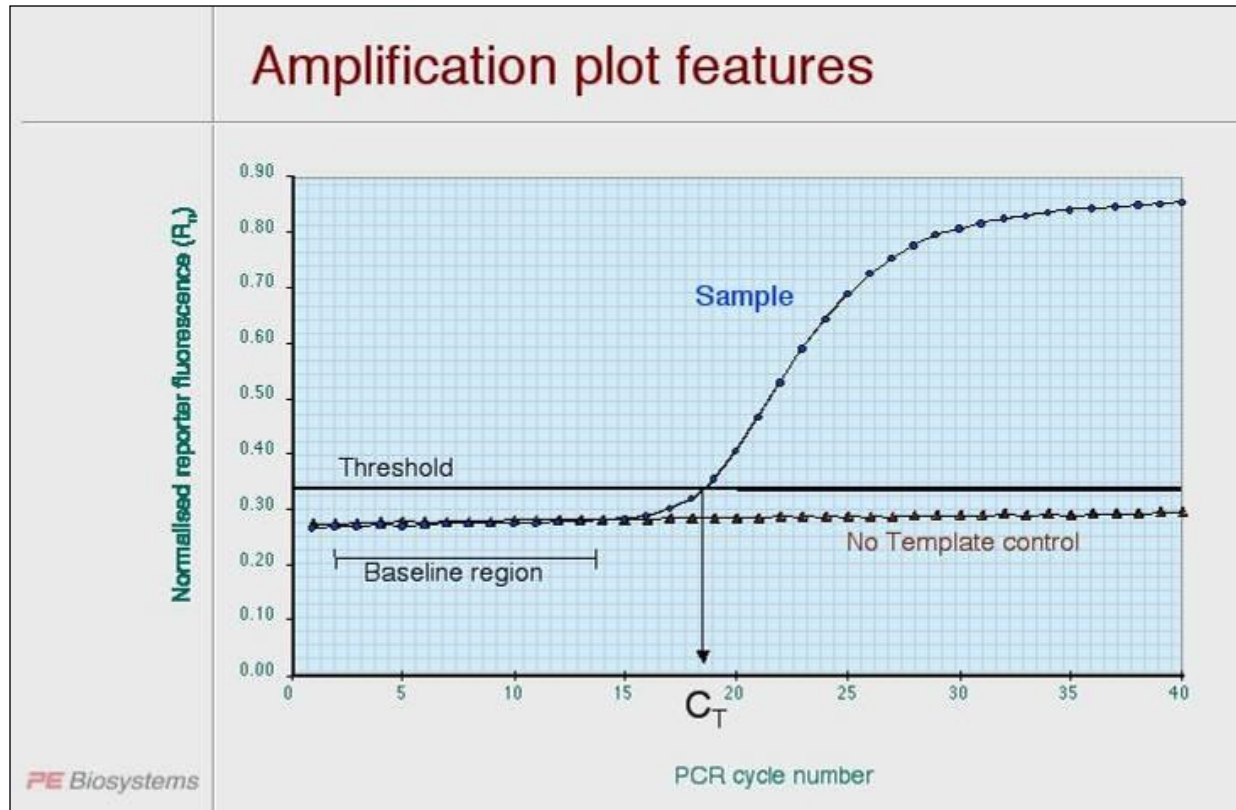
Event	Unique Identifier	Element		Gene		Event
		35S	NOS	NPTII	D-SYN	
GA21	MON-00021-9		+			+
MON810	MON-00810-6	+				+
NK603	MON-00603-6	+	+			+
MON863	MON-00863-5	+	+	+		+
LY038	REN-00038-3				+	+
MIR604	SYN-IR604-5		+			+

# **Real-time PCR**

## **Detection / Quantification**

- **PCR in “Real-time”**
- **One-step Detection and Verification**
- **Detection using Fluorescent Probes**

# PCR Amplification Curve

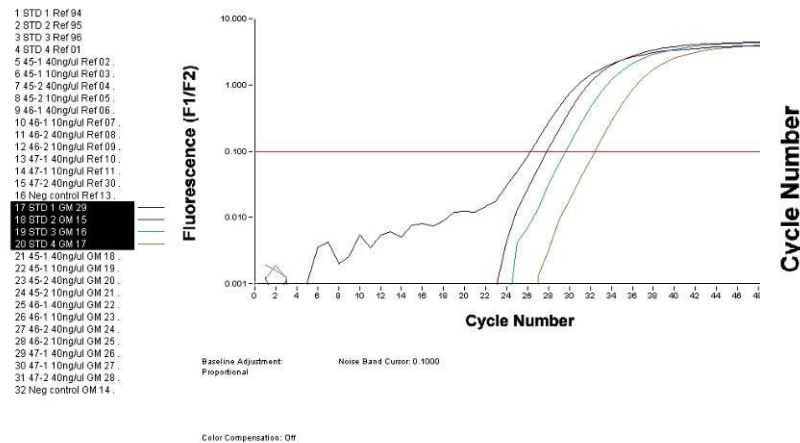


$C_t$ : Threshold Cycle

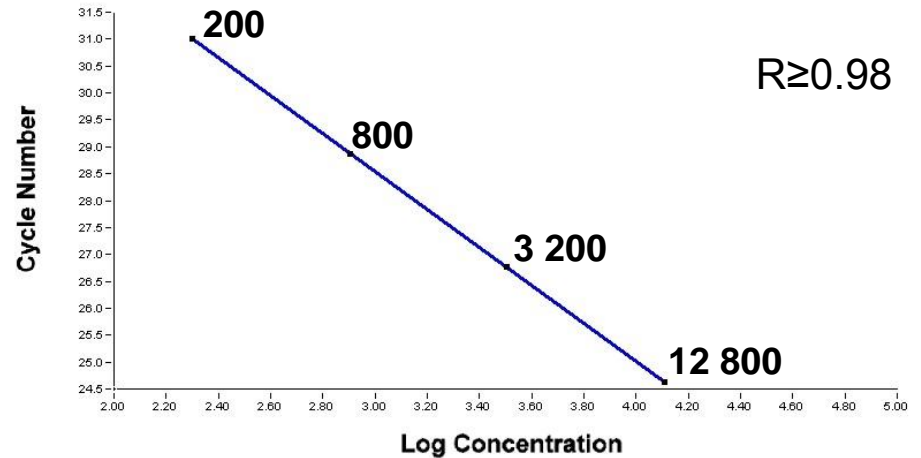
# PCR Amplification Curve

## Reference Amplification Curve

File: C:\LightCycler3\Users\Administrator\Data\QMD Testing Facility\QMD Quantification\03-04-2003\Quant1.ABT Program: Cycling Run By: Administrator  
Run Date: Apr 03, 2003 16:43 Print Date:



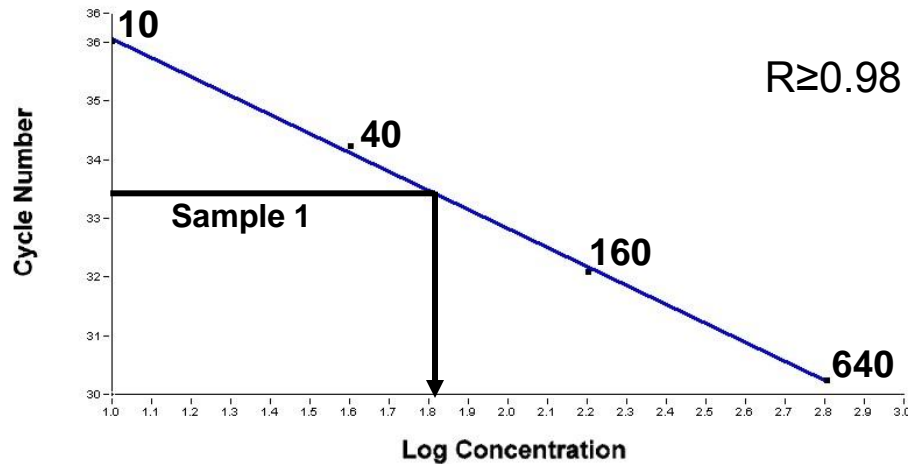
## Reference Standard Curve



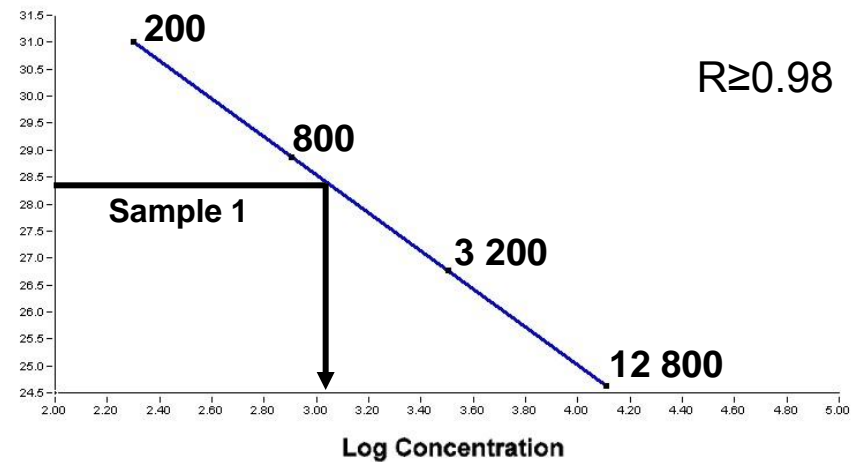


# Standard Curves

## GM Standard Curve




## Reference Standard Curve



$$\% \text{GMO} = \frac{\text{GM Copy Number}}{\text{Ref Copy Number}} \times 100$$

# **Considerations for PCR Based GMO Testing**

- **Inhibition of PCR**
  - **Prevention of Contamination**
  - **Method Validation**
- 
- 
- A solid red horizontal bar spanning the width of the slide at the bottom.

# Challenges for PCR Based GMO Testing

- Availability of Transgene Sequence
  - Availability of Reference Material
  - Difficulty in Detecting Unknown / Unexpected GMOs
-

## TRAINING COURSE ON

### THE ANALYSIS OF FOOD SAMPLES FOR THE PRESENCE OF GENETICALLY MODIFIED ORGANISMS

## USER MANUAL

Edited by Maddalena Querci, Marco Jermini and Guy Van den Eede

This publication is also available online at:  
<http://mbq.jrc.ec.europa.eu/capacitybuilding/documentation.htm>

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WORLD HEALTH ORGANIZATION  
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WELTGESUNDHEITSORGANISATION  
REGIONALBÜRO FÜR EUROPA



ORGANISATION MONDIALE DE LA SANTÉ  
BUREAU REGIONAL DE L'EUROPE

ВСЕМИРНАЯ ОРГАНИЗАЦИЯ ЗДРАВООХРАНЕНИЯ  
ЕВРОПЕЙСКОЕ РЕГИОНАЛЬНОЕ БЮРО

**EU-RL GMFF Home**

**Legal basis**

**Tasks and duties**

**Guidance documents**

**Status of dossiers**

**Methods database**

**Capacity building**

**ENGL**

**Emergencies/  
Unauthorised GMOs**

**Contacts**

## GMOMETHODS:

**EU Database of Reference Methods for GMO Analysis**

**Home**

Search  for   Select by GMO Unique Identifier:

### Quantitative GMO detection PCR methods

- GMO specific
  - Event specific
    - Maize
    - Soybean
    - Cotton
    - Oilseed rape
    - Potato
    - Rice
    - Sugar beet
  - Construct specific
  - Element specific
- Taxon specific
  - Validated independently
  - Validated in combination with other method(s)

### Qualitative GMO detection PCR methods

- GMO specific
  - Event-specific
  - Construct-specific
  - Element-specific
    - Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
    - Figwort Mosaic Virus 35S promoter (P-FMV)
    - Neomycin phosphotransferase II gene (nptII)
    - Nopaline synthase terminator (T-nos)
    - Phosphinothricin N-acetyltransferase gene (bar)
- Taxon specific
  - Validated independently
  - Validated in combination with other method(s)
  - Plant-specific

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

### Main Menu

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### GMO reference materials

According to EC regulations food/feed and food/feed ingredients containing genetically modified organisms (GMOs) authorised in Europe in concentrations above 0.9 % must be labelled appropriately. Additionally, feed may contain 0.1 % of GMOs for which an authorisation procedure is pending or the authorisation of which has expired.

The implementation of [Regulation \(EC\) No 1830/2003](#) and [Regulation \(EU\) No 619/2011](#) requires determining the concentration of GMOs in food and feed products – an obligation that can only be fulfilled using certified reference materials.

Since 1997, GMO certified reference materials for various GM events in maize, soya, potato, sugar beet, and cotton have been developed and produced at IRMM. Research investigations have resulted in major improvements, for instance a dry-mixing technique to avoid DNA degradation in ground GMO and non-GMO powders produced from seeds and cryo-grinding techniques to allow the milling of material with a high fat content.



### News archive

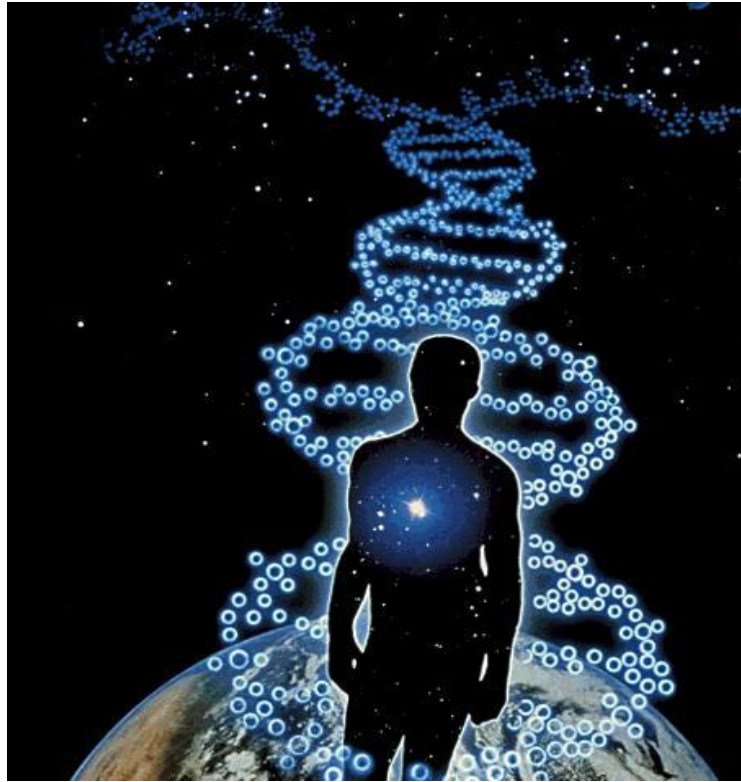
- [Environmental analysis](#)
- [Nuclear research](#)
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JRC-IRMM  
video

YouTube

ERM JRC IRMM  
catalogue

[http://irmm.jrc.ec.europa.eu/activities/GMO\\_reference\\_materials](http://irmm.jrc.ec.europa.eu/activities/GMO_reference_materials)



# The End!

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