Detection of genetically modified organisms - GMO

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

Abed Kagundu
George Ngundo
KEPHIS

Outline

- Institutional Capacity
- Laboratory capacity
- Regional Capacity
- Capacity in Universities
- Bio-Containment and Confinement capacity
- Legal and administrative capacity
- Institutional Collaboration

SEMIS UON
Small Enterprise Management Institute
University of Nairobi

Legal and administrative capacity

International

- Codex Alimentarius (FAO/WHO): Principles of risk analysis and guidelines for food safety assessment – NBA could use the principles for Food safety assessment
- Cartagena Protocol on Biosafety (CBD/UNEP): Safe transfer, handling, transboundary movement – KEPHIS participates in implementation
- International Plant Protection Convention (FAO): Standards for risk analysis – KEPHIS is a signatory to this Convention

National

- Kenya Standards on Food safety for Modern Biotechnology
- Effective border control and monitoring system – KEPHIS has inspectors at all major port of entry.
- The Kenya Standing Technical Committee on Imports and Export.
- A National Policy on Biotechnology approved by Cabinet in 2006
- The Biosafety Act was gazetted in February 2009 – NBA implements the Act together with regulatory agencies
- Regulations of the Biosafety Act

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

Institutional Capacity

KEPHIS

- Release of plant varieties. GM Varieties are not exceptional
- Participation in Institutional Biosafety committees.
- Participation in decision making at the Board of the National Biosafety Authority
- Competent staff: MSc, PhD

National Biosafety Authority

- Competent staff: MSc, PhD
- Coordination structure for regulatory agencies in place

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

Laboratory capacity

SEMI
Seed Enterprises Management Institute
University of Nairobi

OMESA Referral Laboratory at the Plant Quarantine and Biosecurity Station
ted with modern equipment and capacity for:

- **Real time PCR**
- **Conventional PCR**
- **ELISA**
- **Rapid Strips**

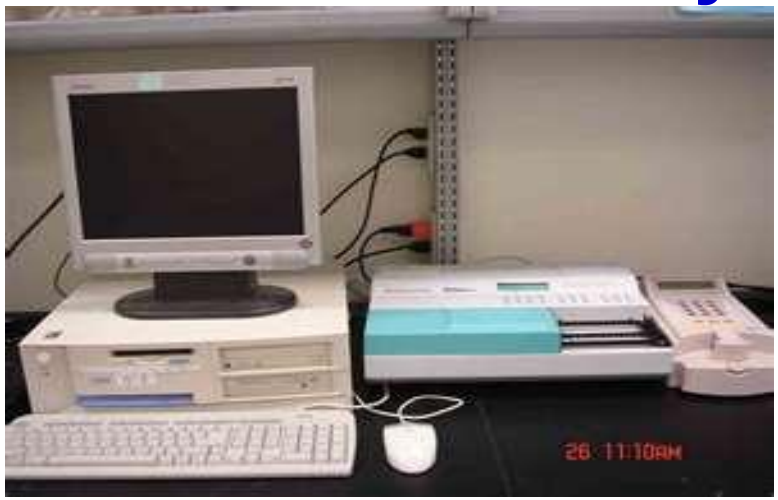
Laboratory capacity

SEMIS UON
Seed Enterprises Management Institute
University of Nairobi

World Class laboratory at KEPHIS Headquarters housing

- **Molecular laboratory (Realtime PCR, Conventional PCR, ELISA, Rapid Kits)**
- **Plant Health Laboratory**
- **Food microbiology laboratory**
- **Analytical Chemistry Laboratory**

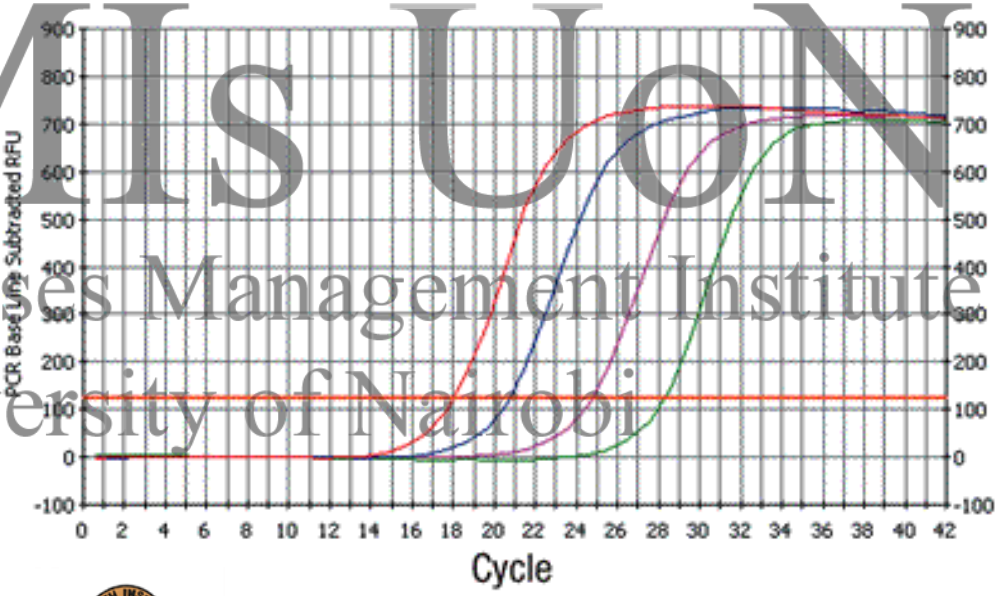
Laboratory testing Equipment



ELISA Reader

-80 Freezer

Real time PCR



Real time PCR Curve

SEMIS UoN
Seed Enterprises Management Institute
University of Nairobi

Regional Capacity at BeCA - ILRI

Bioinformatics, DNA sequencing, Genotyping and Oligonucleotide synthesis
Modern biosciences training laboratory

SEEMIS UoN
Seed Enterprises Management Institute
University of Nairobi

University of Nairobi and Kenyatta University

SEMIS UON
Seed Enterprises Management Institute
University of Nairobi

Biotechnology Centers available

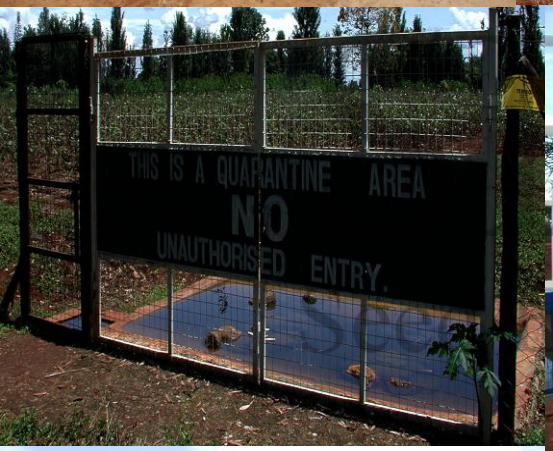
Cartagena Protocol Based Bio-Containment facility at KARI Biotechnology Centre

SEMIS.UON
Seed Enterprises Management Institute
University of Nairobi

Biosafety level 2 facility at ILRI - BecA

SEMIS UoN
Seed Enterprises Management Institute
University of Nairobi

Confined Field Facilities in KARI Stations



- **Confined Field Trials (CFT) conducted for a number of GM crops since 1991 when the first CFT (sweet potato) was initiated.**
- **Experience has been gained by researchers & regulators.**
- **Now there is a specific law and regulations as well as Standard Operating Procedures**
  - ***Isolation maintained***
  - ***Protection from animals, man, environment during trials***

EMIs UoN
Environmental Management Institute
University of Nairobi

Institutional Collaboration

- The capacities in the country are in different government and regional agencies
- It is therefore critical for institutional collaboration between NBA, relevant agencies and research institutions to utilise the available capacity.

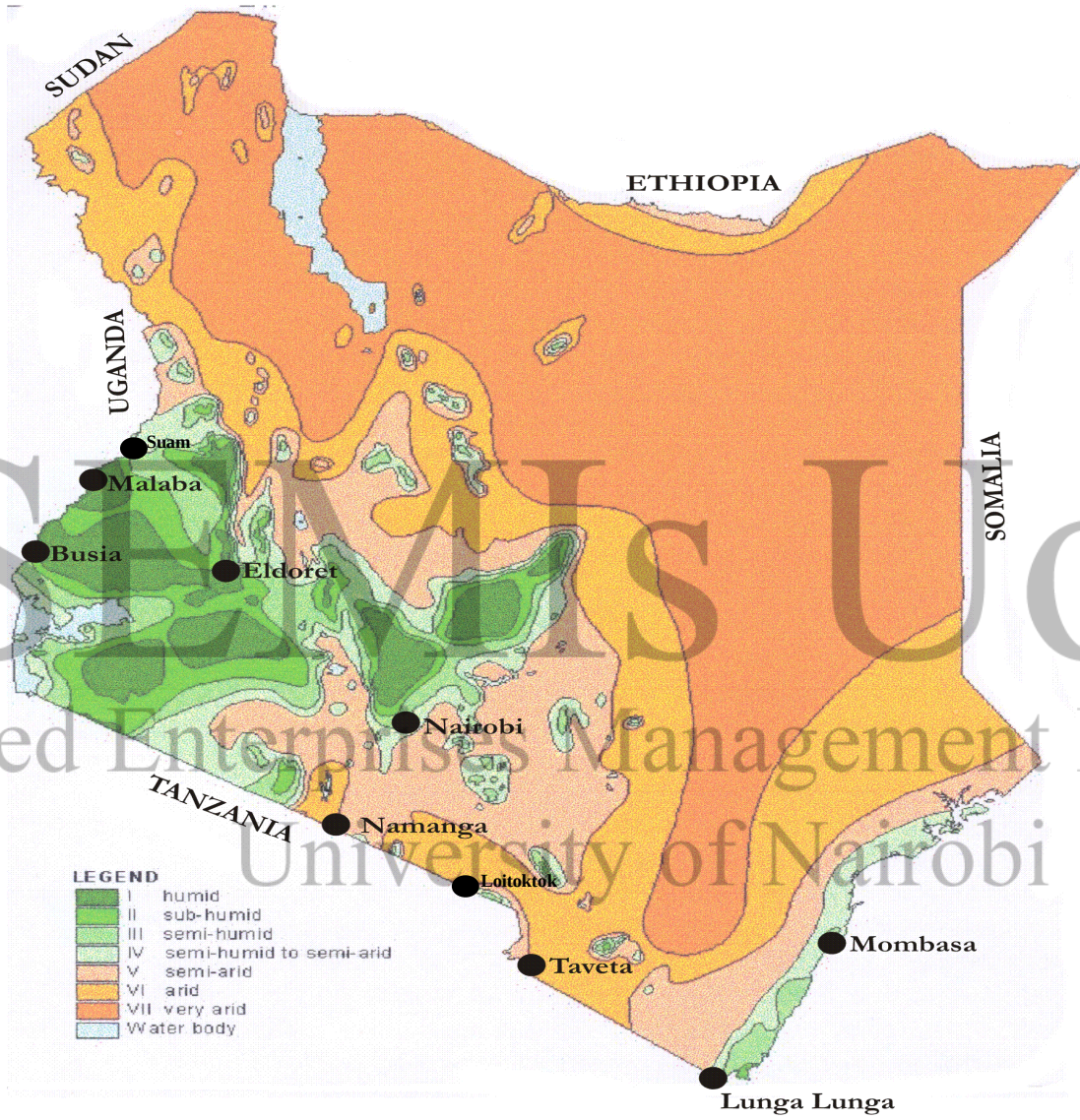
SEMIS UoN
Seed Enterprises Management Institute
University of Nairobi

IBCs

1. Prepare applications and refer them to the NBA for approval
2. Advise the research institutions on Biosafety matters
3. Assist their institutions in the establishment of the appropriate monitoring mechanisms for risk assessment and management
4. Ensure that the conditions in the approval are adhered to
5. Review and ascertain the suitability of both physical and biological containment
6. Control procedures appropriate to the level of assessed risk
7. Advice their relevant Institutions and Principal investigators on mitigations measures to be undertaken in case of an accident.

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

Points of Entry into Kenya



SEMIS UoN
Seed Enterprises Management Institute
University of Nairobi

Figure 1: Map of Kenya Showing agro climatic zones and points of entry where phytosanitary inspection is carried out

Detection and Surveillance Capacity

1. All importation and use of GM plants has to meet Phytosanitary and Biosafety standards

2. All GMO activities have to first be approved by the **National Biosafety Authority (NBA)**, followed by Phytosanitary compliance.

3. Research activities involving GMOs undertaken in the country have to be monitored to for compliance.

SEMIS UON
Seed Enterprises Management Institute
University of Nairobi

Surveillance of GMOs

1. **Surveillance of plant imports** to ensure that ordinary plant permits are not used to import GMOs
2. **Seed Quality compliance** to ensure unapproved genetic elements are not released to farmers
3. **Variety release** are tested for their genetic purity and conformance to biosafety guidelines
4. **Border surveillance** is undertaken in collaboration with NBA

SEMIS UoN
Seed Enterprises Management Institute
University of Nairobi

Elements targeted in detection programs

1. Selectable Marker
2. Transgene
3. Promoter
4. Terminator

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

GMO Detection

GMOs can be identified by detecting the inserted genetic material at several levels

- DNA level,
- the mRNA transcribed from the newly introduced gene,
- The resulting protein,
- Metabolite or phenotype

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

Frequently tested traits

- **Herbicide tolerance**
- **Bt-derived insect resistance**
- Virus resistance
- Fungal resistance
- Male sterility/fertility restoration
- Starch Biosynthesis alteration.

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

Available Analytical Methods For GMO Detection

1. **Detection** – objective is to determine if a product **contains GMO or not**.
   - The result is a **positive/negative** statement.

2. **Identification** – purpose is to find out **which** GMO is present and if they are authorized or not.

3. **Quantification** – **determination of amount** of the GMO and assess compliance with the threshold regulation

SEMI UoN
Seed Enterprises Management Institute
University of Nairobi

Analytical Methods, Cont'

- PCR tests can be designed to detect any of the inserted genetic material: promoter, transgene, terminator or selectable marker gene.
- The exact design of any particular test depends on the objective.
- For a **general detection of GMOs** using primers that recognize commonly used promoters like ***Cauliflower Mosaic Virus (CaMV***) 35S promoter or
- *Agrobacterium tumefasciens* ***nos*** promoter, or the terminator (***nos)***.

SEMI's UoN
Seed Enterprises Management Institute
University of Nairobi

Protein-Based GMO Detection

This methods give a **present / absent** result

I) Immunoassays

a) ELISA

b) Lateral Flow Strip

II) Western Blot

Lateral Flow Strips

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

ELISA, Lateral-flow device

- Protein based
- Easy to perform, cheap and reliable
- Test results within few minutes
- Best for raw agricultural commodities
- Identification of a specific event not possible

SEMIS UoN
Seed Enterprises Management Institute
University of Nairobi

Lateral-flow device, Cont'

- Lateral flow sticks can be used to detect GMOs in leaves, seeds and grains.
- One single step is enough for performing the assay.
- Antibodies specific to the foreign protein are coupled to a color reagent and incorporated into the lateral flow strip.
- Lateral flow techniques are qualitative or semi-quantitative.
- By following appropriate sampling procedures, it is possible to obtain a 99% confidence level of less than 0.15% GMO for a given lot.

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

DNA-Based GMO Detection

PCR-Based Detection

Qualitative PCR (tests for 35S promoter, nos, etc)

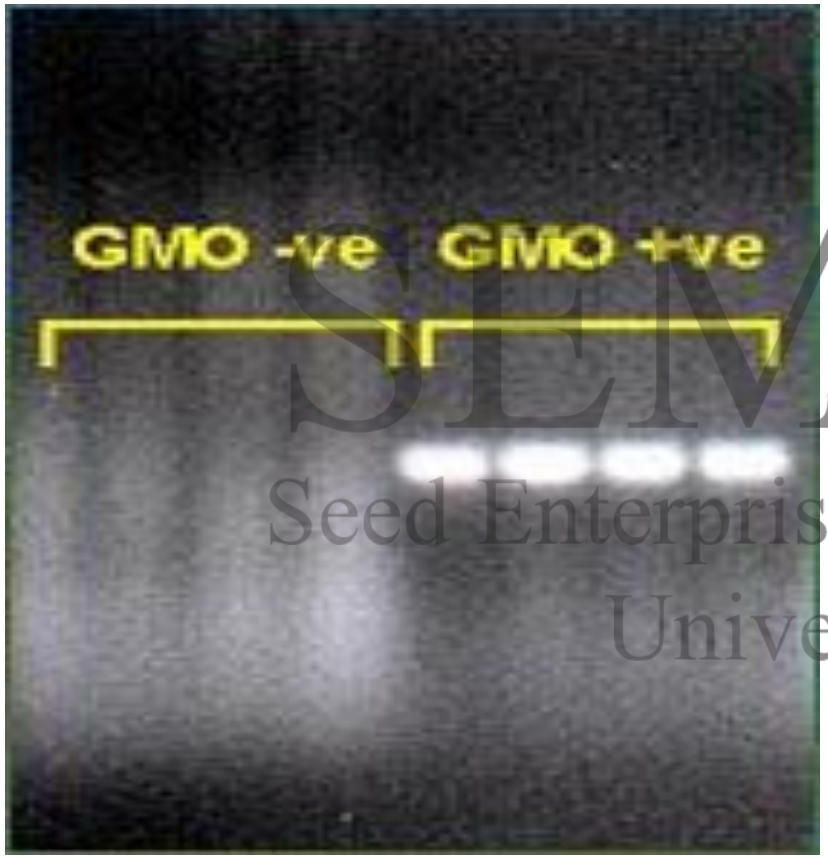
- This methods give a **present / absent** result
- Conventional PCR measures the products of the PCR reaction at a single point in the reaction profile.
- Relationship between DNA concentration and PCR signal is not linear
- The precision for quantification using conventional PCR is limited.

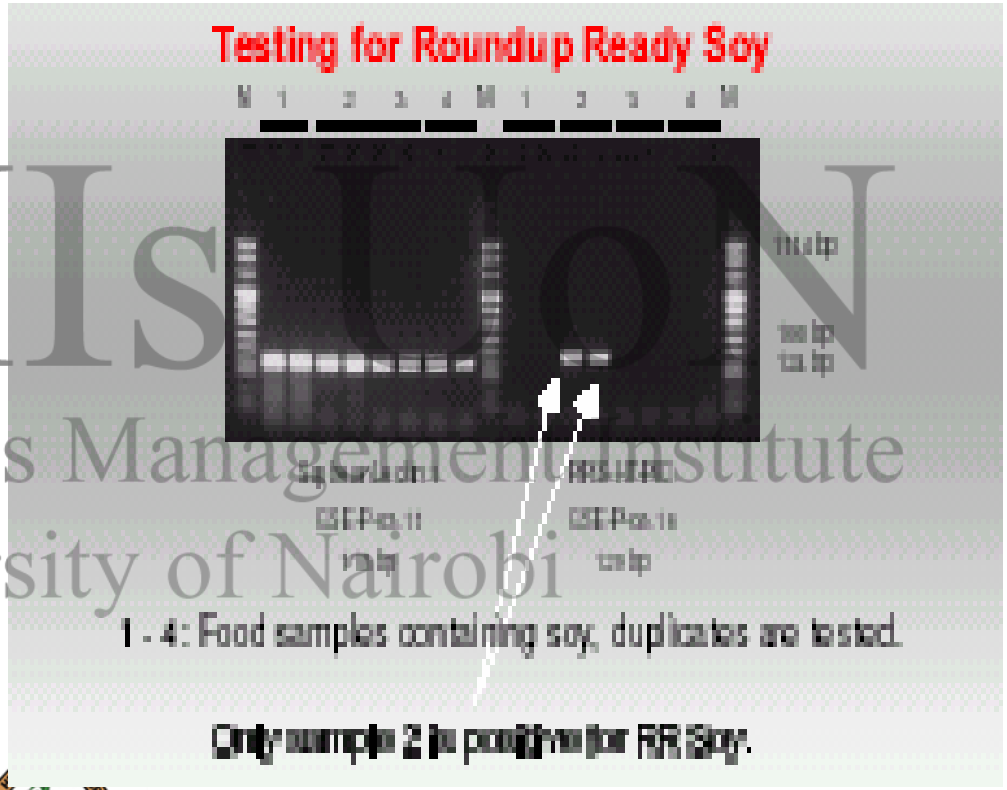
SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi

Confirmatory Assays

Restriction, Hybridization, sequencing

Qualitative PCR for GMO Detection -results





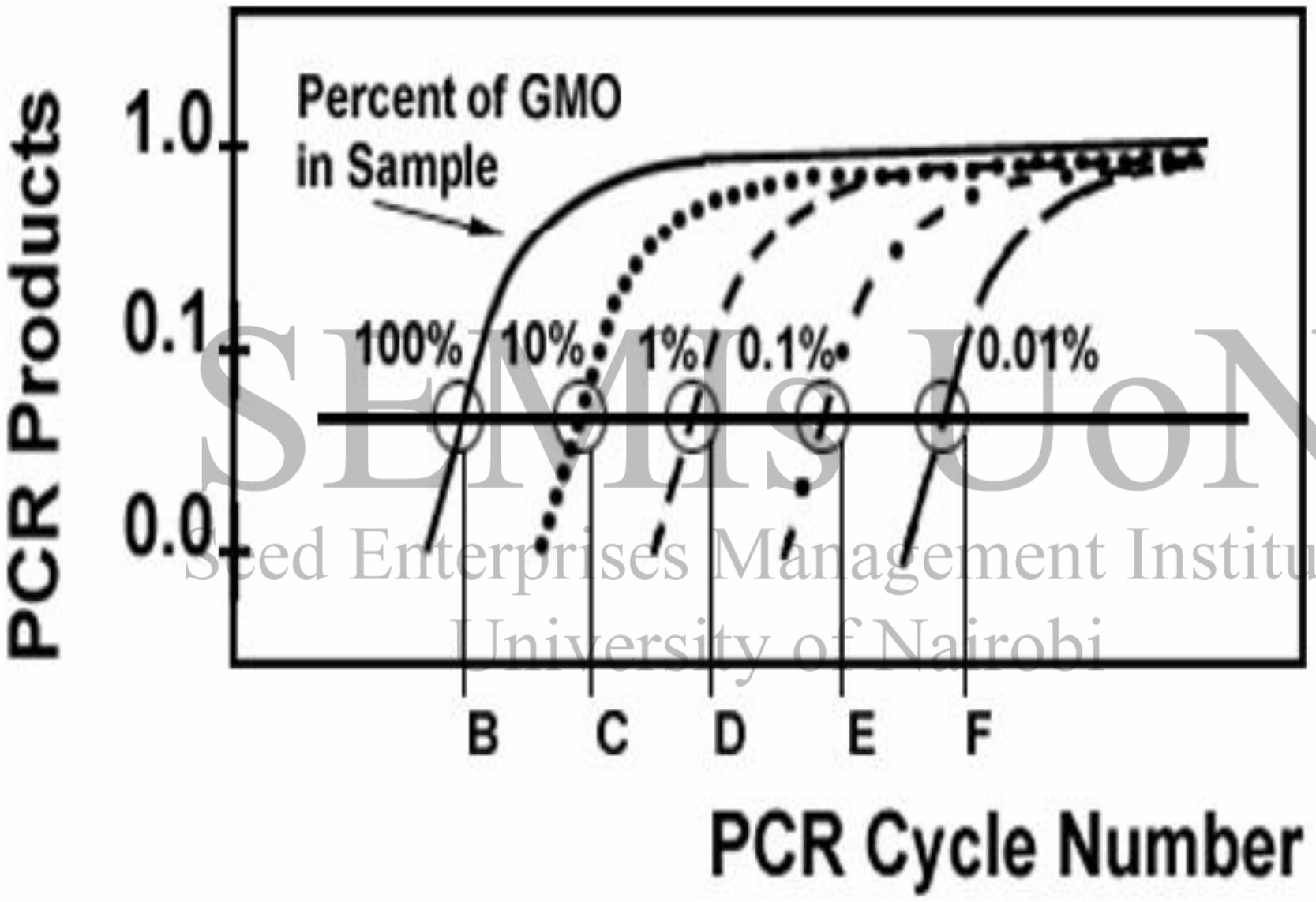
SEMIS.ON
Seed Enterprises Management Institute
University of Nairobi

Real Time PCR

This method provides precise quantification of the GMO content of agricultural products.

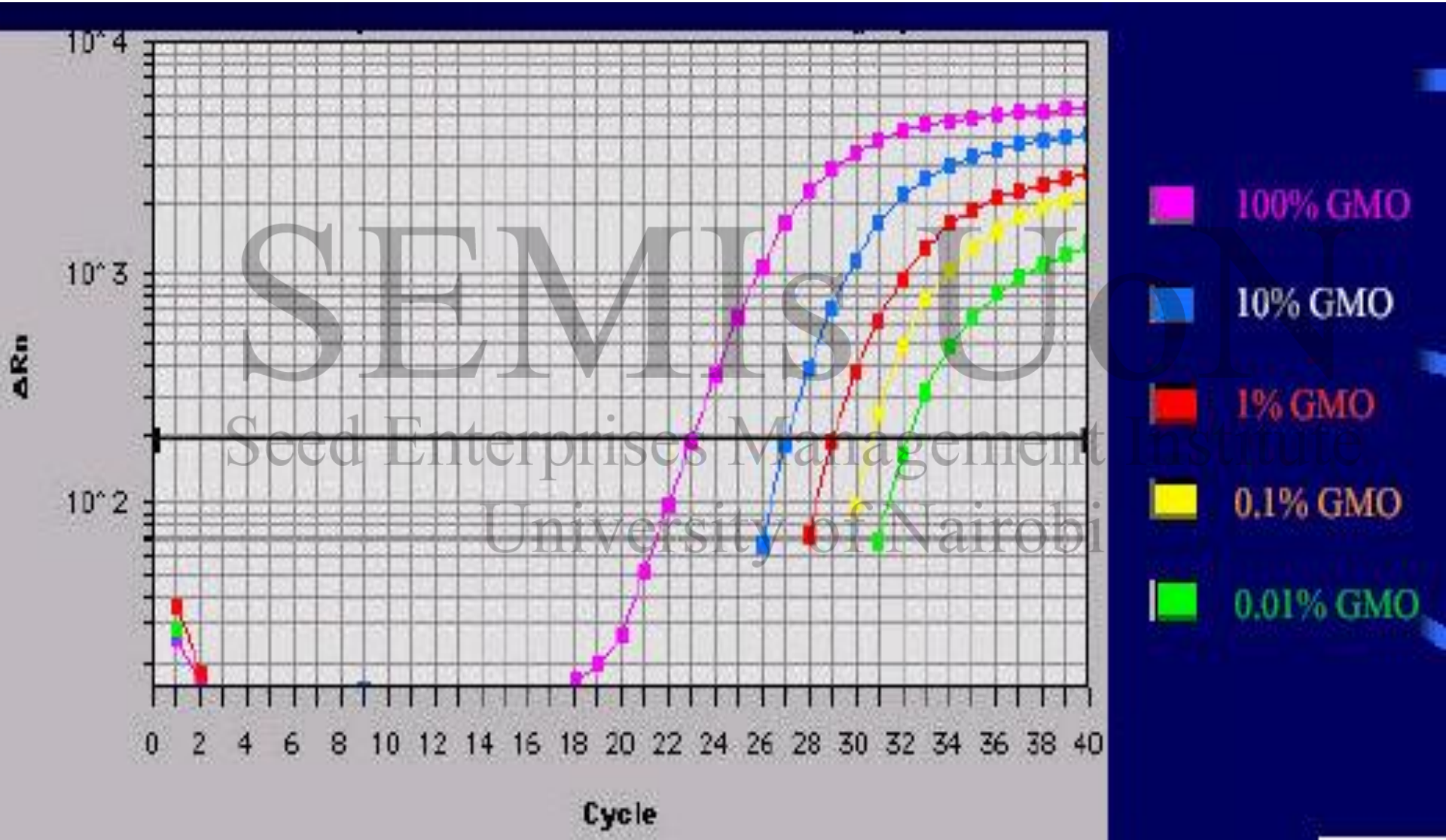
- Each series of analyses includes the analysis of a full set of standards, giving rise to a standard curve.
- The results obtained for individual unknown samples are compared to the standard curve to determine the GMO content of those unknowns.
- Most real-time systems are automated

SEMIs UoN
Seed Enterprises Management Institute
University of Nairobi



SEMIS UoN
Seed Enterprises Management Institute
University of Nairobi

Real Time PCR - GMOs Detection Thresholds



SEMIS
Seed Enterprises Management Institute
University of Nairobi

Contact
KEPHIS
P.O. Box 49592-00100, Nairobi, Kenya
Tel.: +254-20-3536171/2
Fax: + 254-20-3536175/
Email: director@kephis.org
Website: www.kephis.org

Thank you

SEMIS Training
Seed Enterprises Management
University of Nairobi