African Regional Training of Trainers workshop on the Identification and Documentation of LMOs

Item 6:

SAMPLING AND DETECTION OF LMOs

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African Regional Training of Trainers workshop on the Identification and Documentation of LMOs

Introduction And Overview

DETECTION METHODS

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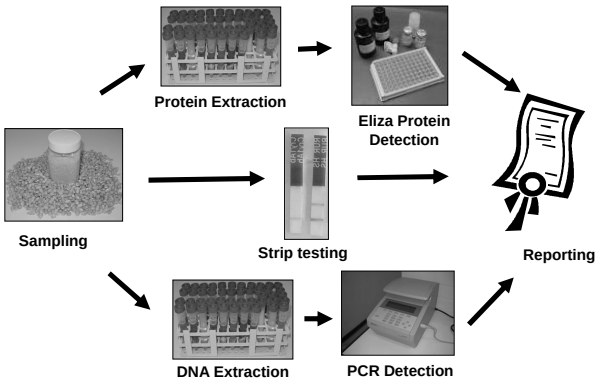
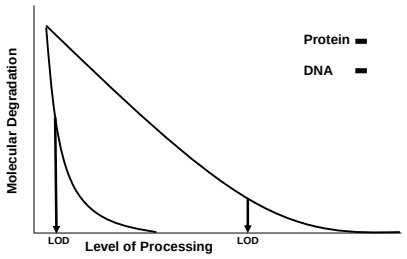
Principle Steps in GMO Detection



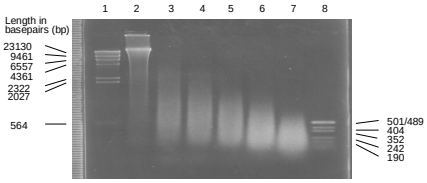
GMO Testing Methods

- Protein Detection (Antibody Recognition)
  - Transgene Product Specific Strip Test
  - ELISA Quantification
- DNA Detection (DNA Sequence Recognition)
  - PCR Detection
  - Real-time Quantification

Protein and DNA Degradation during Food Processing



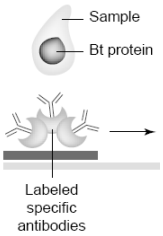
DNA Stability during Processing



Lane 1) Size Marker (Phage-Lambda-DNA, Hind III digested)
Lane 2) Bovine-DNA, fresh, 2 µg
Lane 3) Bovine-DNA, 5 min 100°C, 2 µg
Lane 4) Bovine-DNA, 15 min 100°C, 2 µg
Lane 5) Bovine-DNA, 30 min 100°C, 2 µg
Lane 6) Bovine-DNA, 5 min 121°C, 2 µg
Lane 7) Bovine-DNA, 13 min 121°C, 2 µg
Lane 8) Size Marker (pUC18-DNA, Hpa II digested)

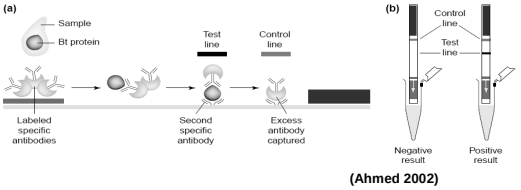
GMO Testing Methods

- **Protein Detection (Antibody Recognition)**
  - **Protein Specific**
    - **Strip Test**
    - **ELISA**



Protein Testing

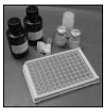
- **Lateral Flow Strip Test**
  - **Qualitative (Yes/No)**
  - **Limit of Detection**
    - **± 1% BT11**
    - **± 2% Mon810**
    - **± 0.12% RR Soybean**



(Ahmed 2002)

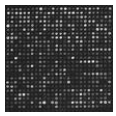
Protein Testing

- **ELISA**
  - **Same Principle as Strip testing in a Microwell Plate Format**
  - **Qualitative and Quantitative (%)**
  - **Limit of Quantification**
    - **± 0.1-0.2% GM**

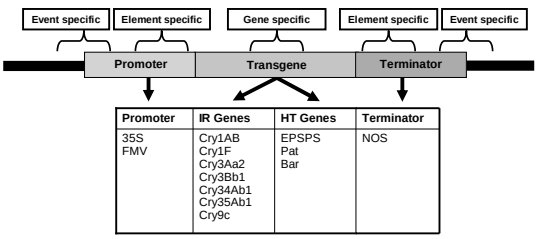


Protein Testing

- **Advantages**
  - **Quick and Easy (Strips)**
  - **Low Technology Input (Strips)**
  - **Quantitative (ELISA)**
- **Disadvantages**
  - **Specific for a Particular Transgene Protein**
  - **Cannot Detect All GMOs**
  - **Rely on Commercial Availability**
  - **Not Applicable for Processed Products**



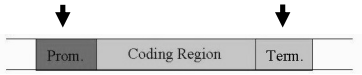
The Transgene Construct



Promoter	IR Genes	HT Genes	Terminator
35S FMV	Cry1AB Cry1F Cry3Aa2 Cry3Bb1 Cry34Ab1 Cry35Ab1 Cry9c	EPSPS Pat Bar	NOS

Element Specific Detection

- **GMO Screening based on Regulatory Sequences**
- **Common to Different GMOs**

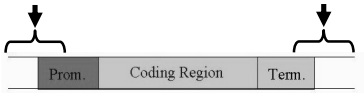


Gene Specific Detection

- Detection of the Transgene
- Cry1Ab is Common to Mon810 and BT11

Event Specific Detection

- Specific to each Event
- Combination of Native and Transgenic DNA

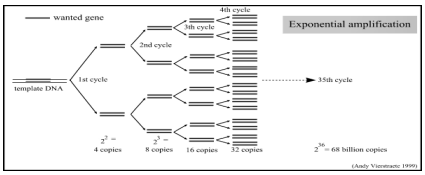


GMO Testing Methods

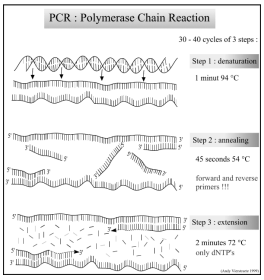
- DNA Detection (DNA Sequence Recognition)
  - Qualitative PCR
    - GMO Screening
    - Gene Detection
    - Event Detection
  - Real-time Quantitative PCR
    - Event Specific Quantification
    - Total GM Quantification

Polymerase Chain Reaction

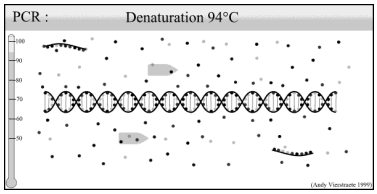
- PCR Reaction
  - Primers (For target sequence)
  - Polymerase
  - Nucleotides
  - Reaction Buffer
  - DNA Template (Extracted DNA)
- PCR Primers Specifically Determine the Target Sequence to be amplified



PCR Cycling



PCR Cycling



DNA Testing

- **Advantages**
  - **GMO Screening, Trait or Event Detection**
  - **Applicable for Unprocessed and Processed Foods**
- **Disadvantages**
  - **Technology Intensive**
  - **Requires Specialized Methods**