



# Simultaneous detection and relative quantification of GMO by ligation-dependent probe amplification



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# Outline

## 1. Background:

- Principles of the ligation-dependent probe amplification (LPA)
- Advantages of the LPA technique for the routine testing

## 2. Development of an LPA system for the analysis of GMO:

- Criteria for the design of LPA probes
- Specificity and sensitivity testing
- Assessment of the quantitative properties

## 3. Summary



## European Network on Safety Assessment of Genetically Modified Food Crops (ENTRANSFOOD)

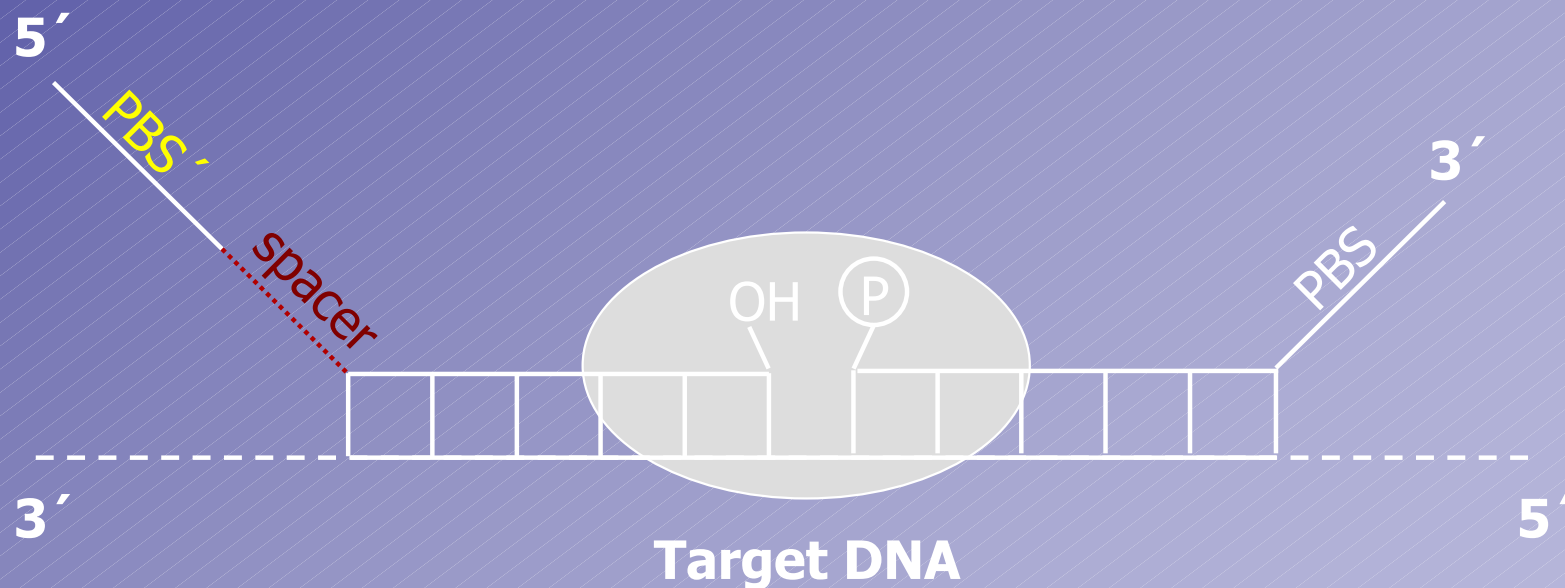
**“Multiplexing of qualitative (quantitative) analyses is a necessity, because the number of GMO’s to be tested for is already high and steadily increasing...”**

*Miraglia et al. / Food Chem. Tox. 42 (2004) 1157–1180*



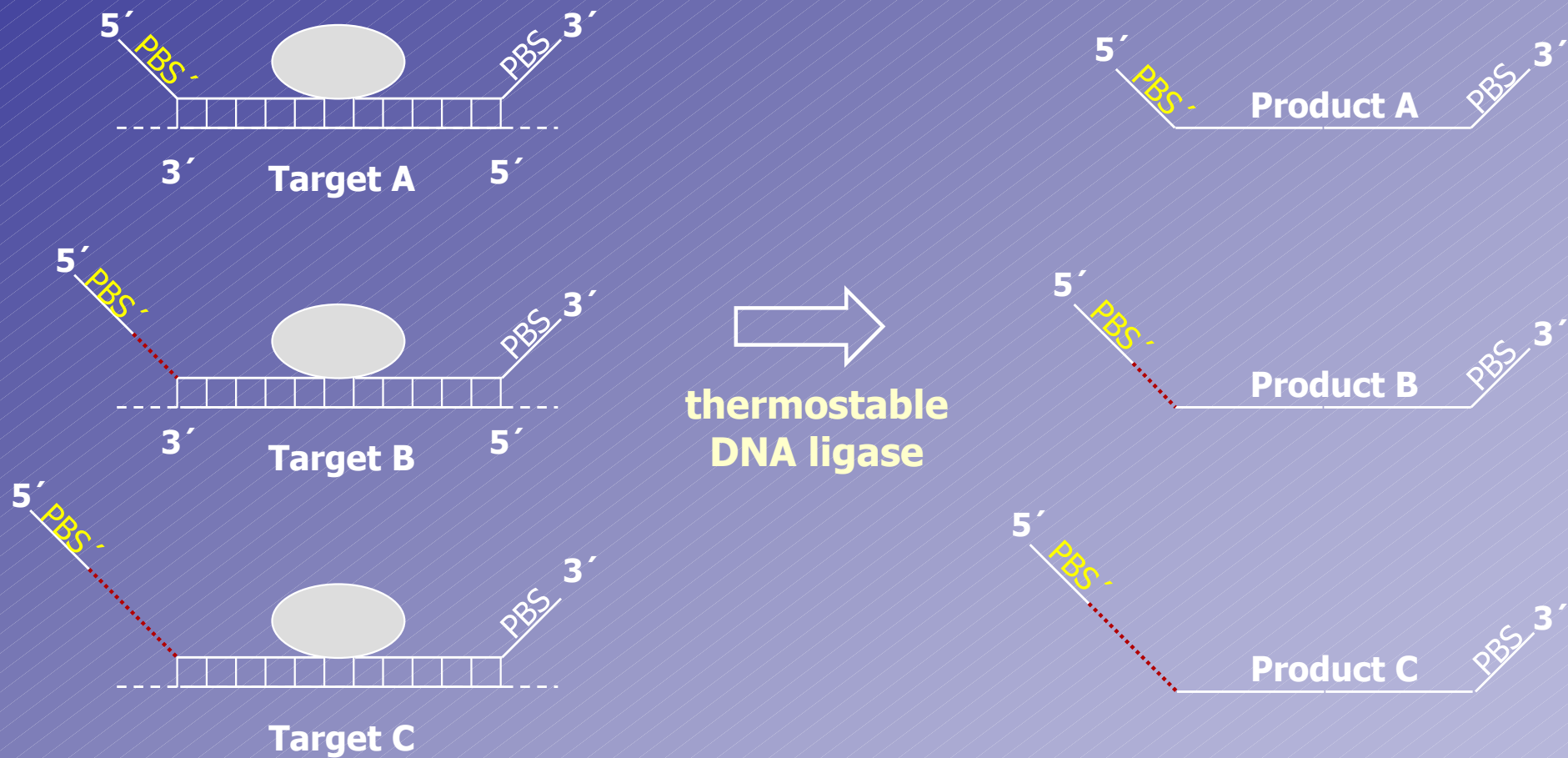
# Ligation-dependent probe amplification; LPA

Simultaneous detection and relative quantification  
of various DNA target sequences



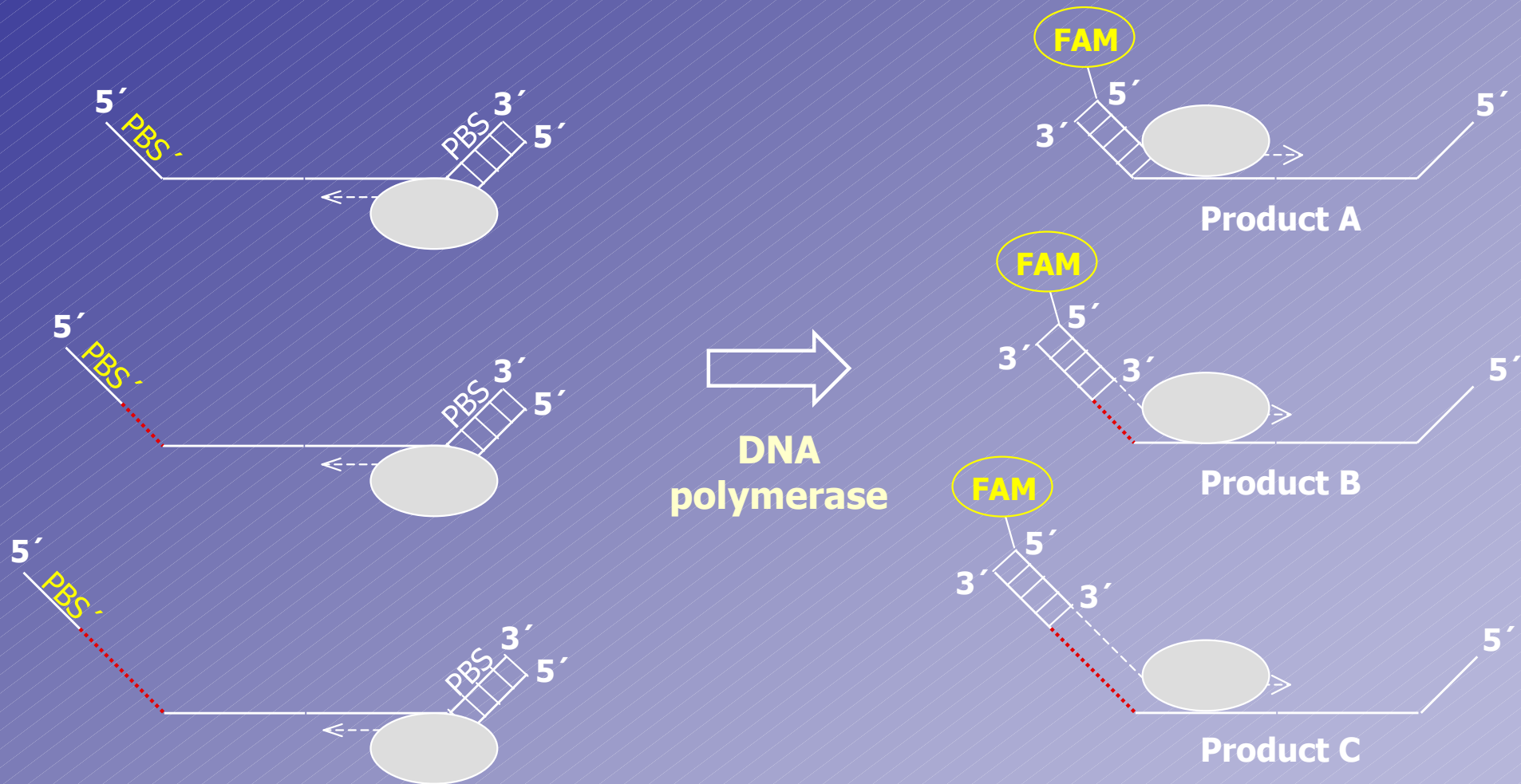


# LPA Reaction: Ligation



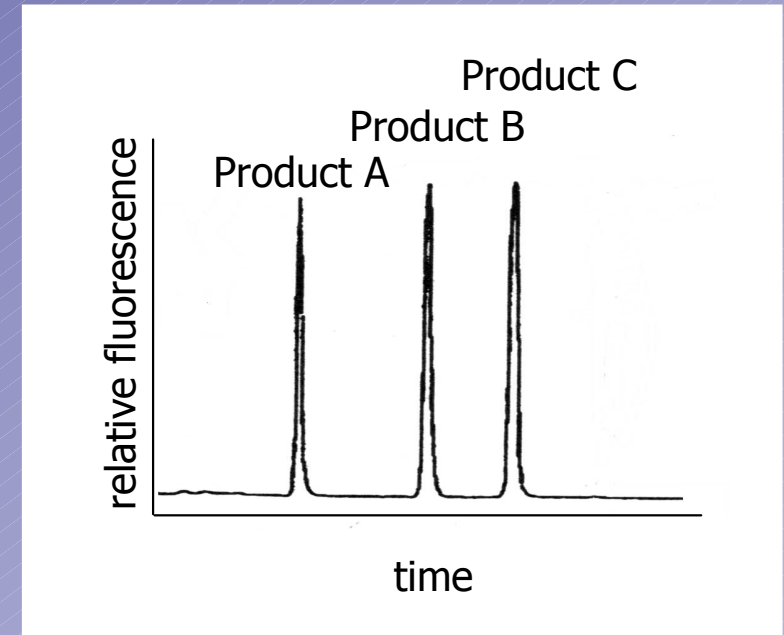
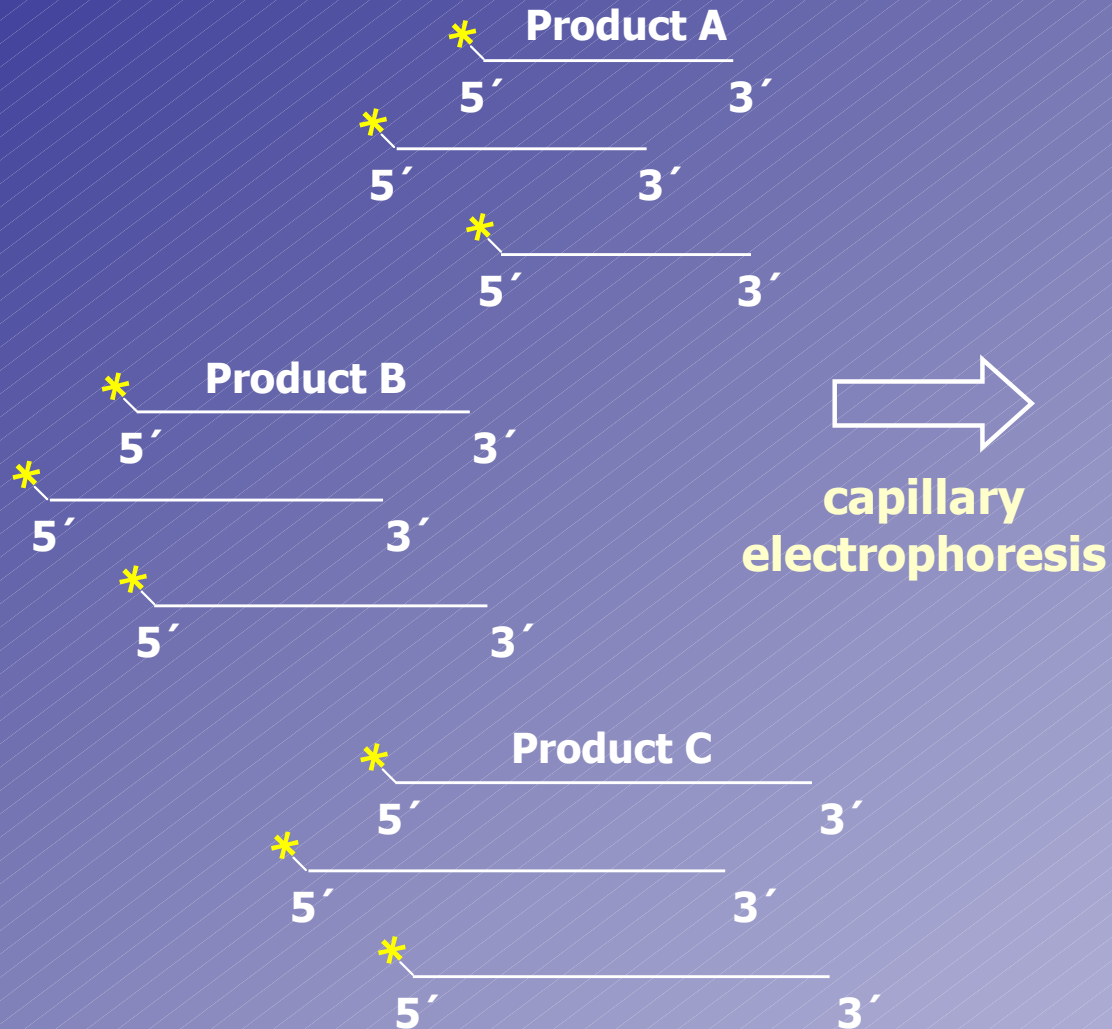


# LPA Reaction: Amplification



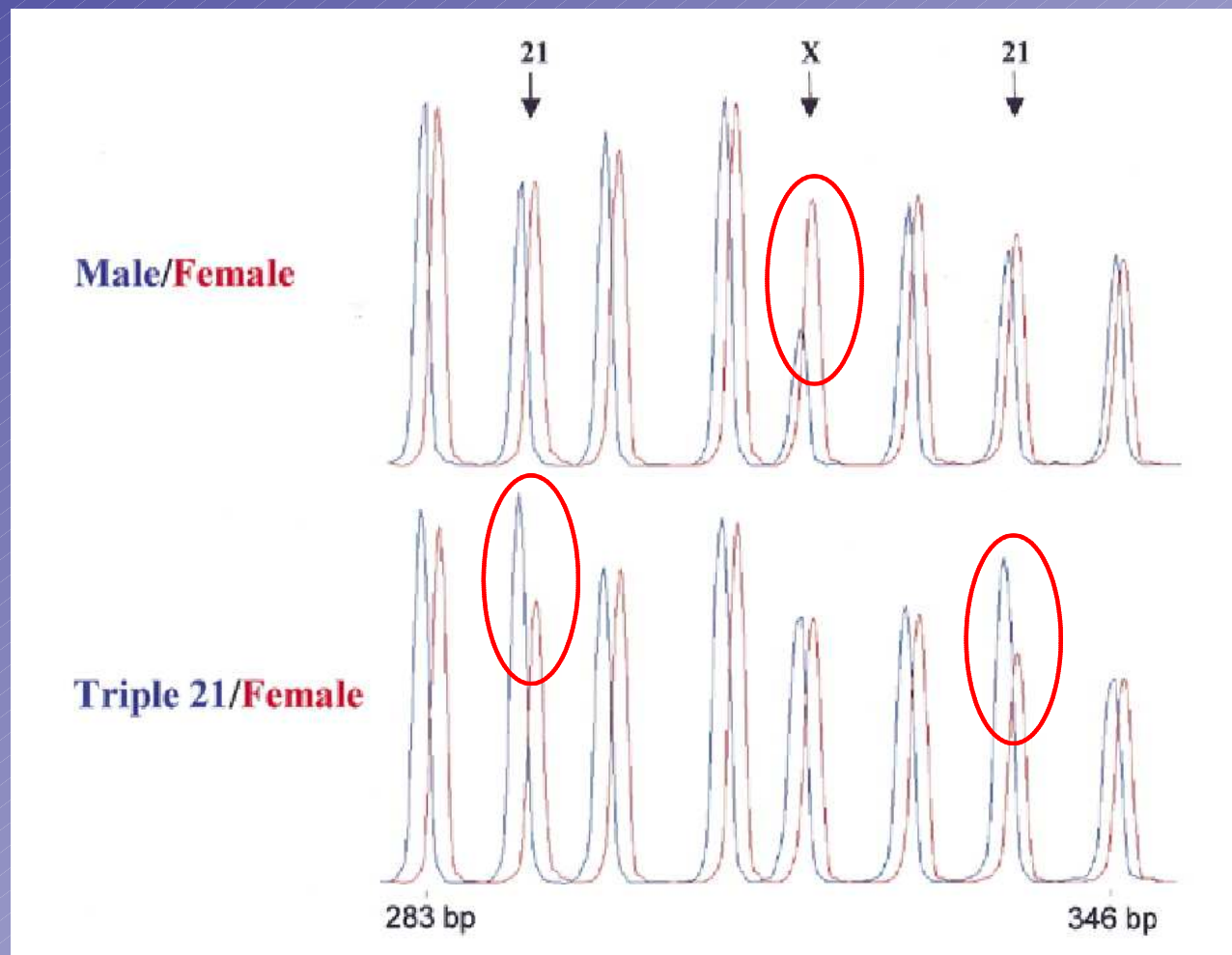


# LPA Reaction: Detection



# Applications: Clinical diagnostics

Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Schouten et al. (2002)





# Advantages for the routine testing

- **Suitable for the simultaneous detection and relative quantification of multiple DNA targets**
  - Detection of GMO-specific sequences and taxon-specific reference genes
  - Normalization of signal intensities
- **Modularity**
  - Design of further probes to broaden the spectrum of DNA targets
  - Forthcoming GMO authorizations / appropriate screening targets
- **Detection *via* capillary electrophoresis**
  - Requires no further DNA preparation
  - Robust, automated sample injection and analysis

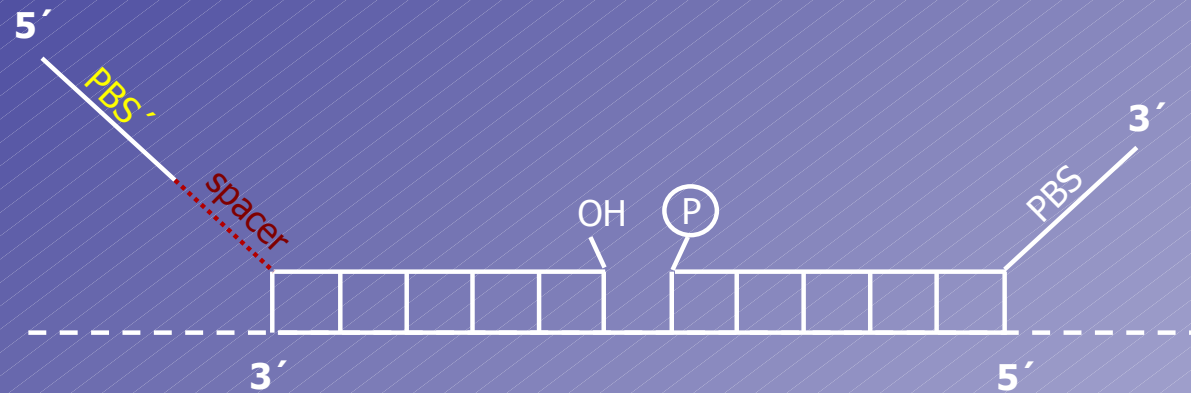


# Comparison to alternative approaches

- **Application of biosensors or DNA-Arrays**
  - Complex detection technologies
  - Difficult automation
- **Detection of previously amplified (multiplex) PCR products**
  - No modularity, cannot be arbitrarily complemented with further primers
  - Analyses are not quantitative
- **Application of hybridization probes / bipartite Primers**
  - Requires prior amplification of target sequences
  - Competitive amplified probes have been shown to allow relative quantifications in relation to a reference gene



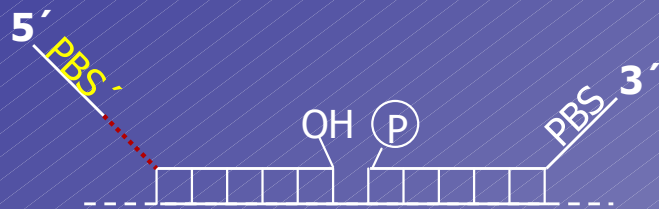
# Design of LPA probes



- **Application of synthetic probes**
- **Selection of appropriate PBS and spacer sequences**
- **Selection of appropriate hybridization sites:**
  - Length and thermodynamic properties of the targeted sites
  - Analogies with non-targeted sequences (data bases)
  - Intra- and/or intermolecular interactions
  - Range of quantification



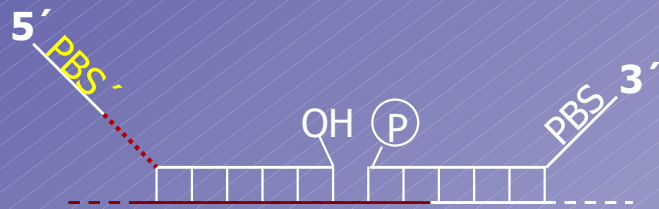
# GMO detection system



reference gene

## Reference genes

- Soya: *Le1*- gene 100 nt
- Maize: *HMGa*- gene 105 nt



insert DNA

plant DNA

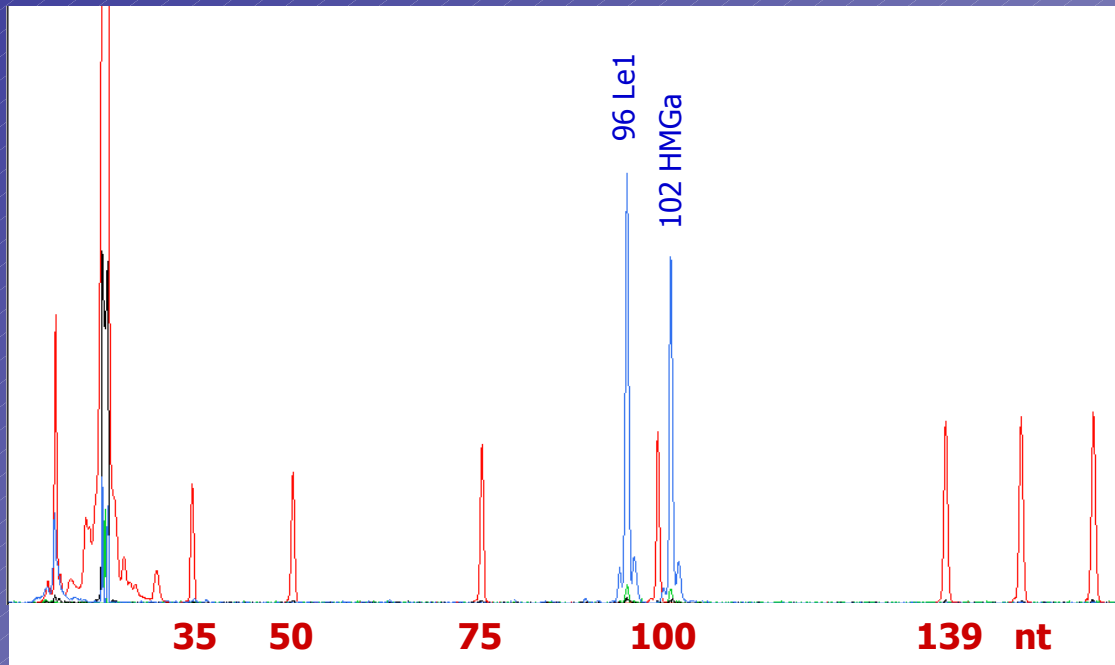
event-specific  
junction region

## Event-specific junction regions

- RR soya DNA/CaMV 35S 109 nt
- MON810 maize DNA/CaMV 35S 115 nt



# LPA system: Specificity

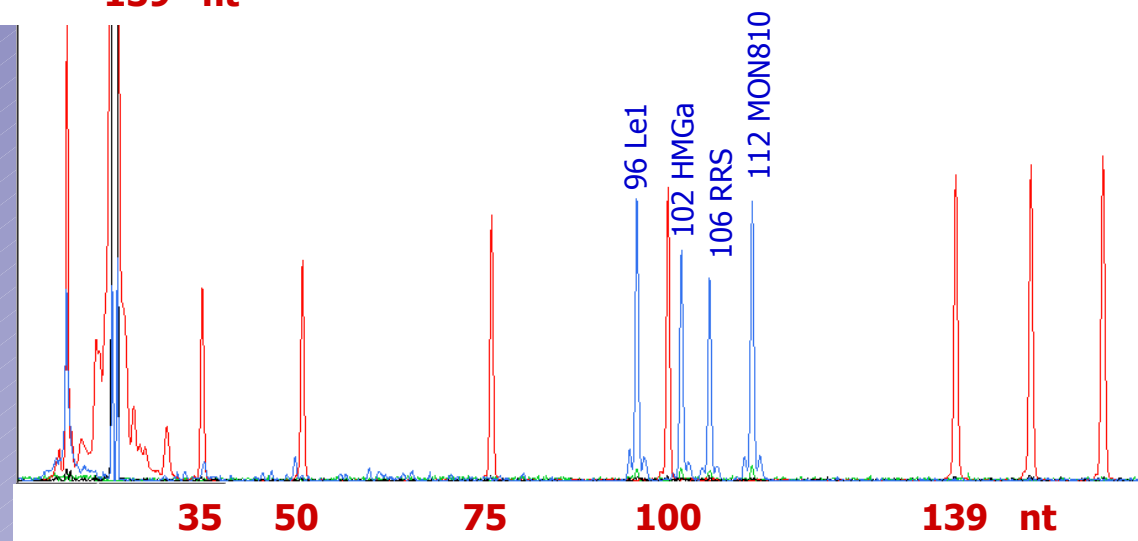


## Sample 1:

- Conventional soya DNA
- Conventional maize DNA

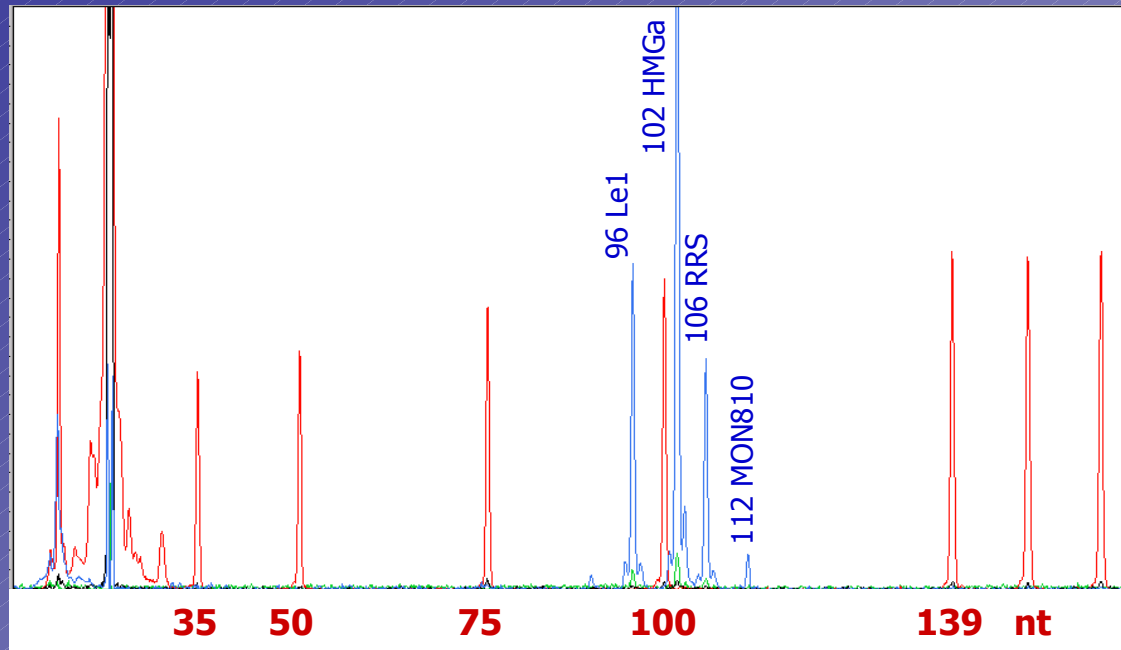
## Sample 2:

- Roundup ready soya (2%)
- Maize MON810 (2%)





# LPA system: Sensitivity

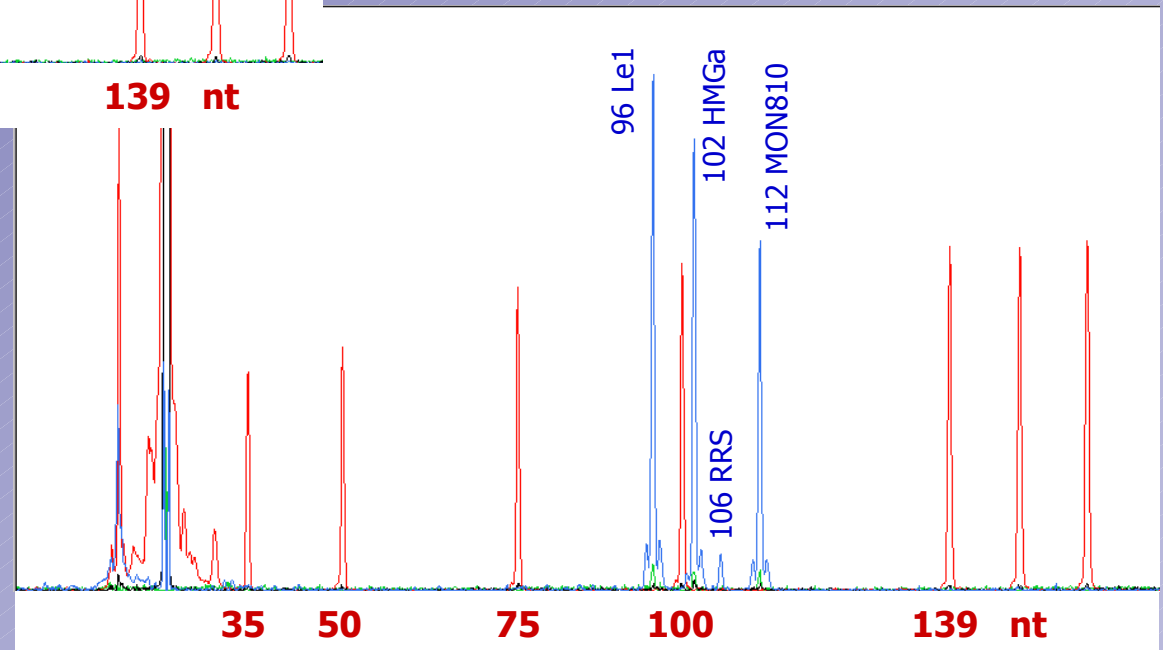


## Probe 3:

- Roundup ready soya (5%)
- Maize MON810 (0.1%)

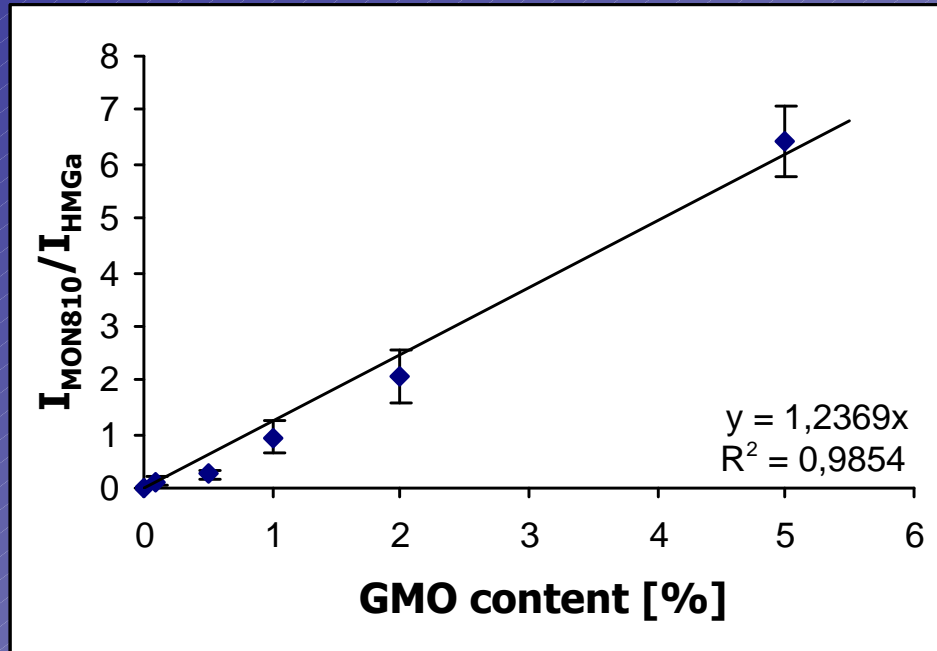
## Probe 4:

- Roundup ready soya (0.1%)
- Maize MON810 (5%)

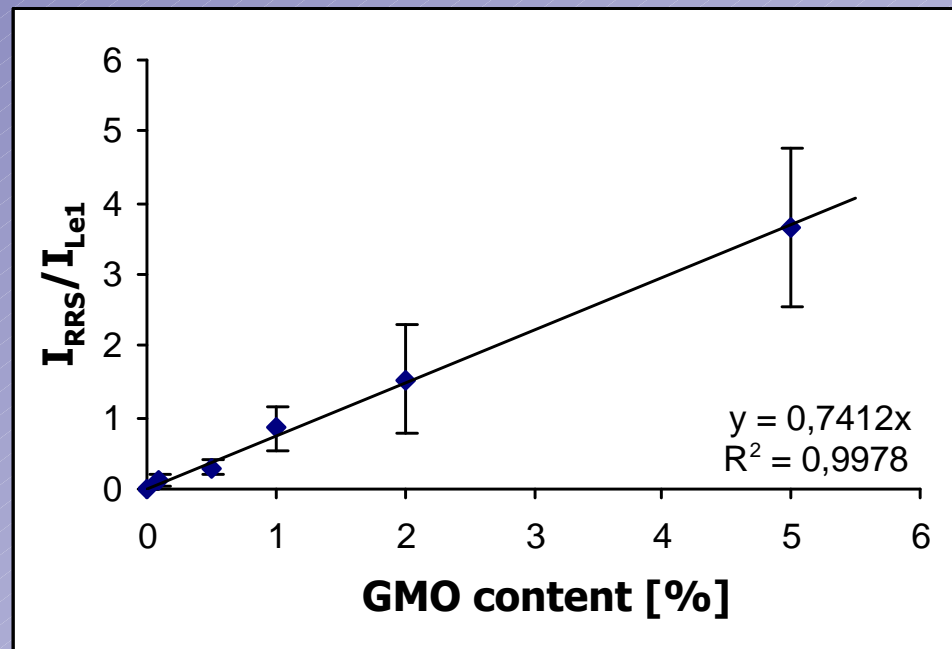




# LPA system: Quantitative properties



Calibration line:  
Roundup ready soya





# Summary

- **Design and synthesis of LPA probes**

- Criteria for the design were widely defined
- Suitability of chemically synthesized LPA probes was tested

- **Modularity**

- Avoidance of interactions between probes through targeted design
- Extension with additional probes to broaden the spectrum of analysis

- **Detection**

- Qualitative detection of various DNA sequences in a single reaction
- Further studies are required for the accurate assessment of the quantitative properties (LOD, LOQ)



**Thank you for your attention!**

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