

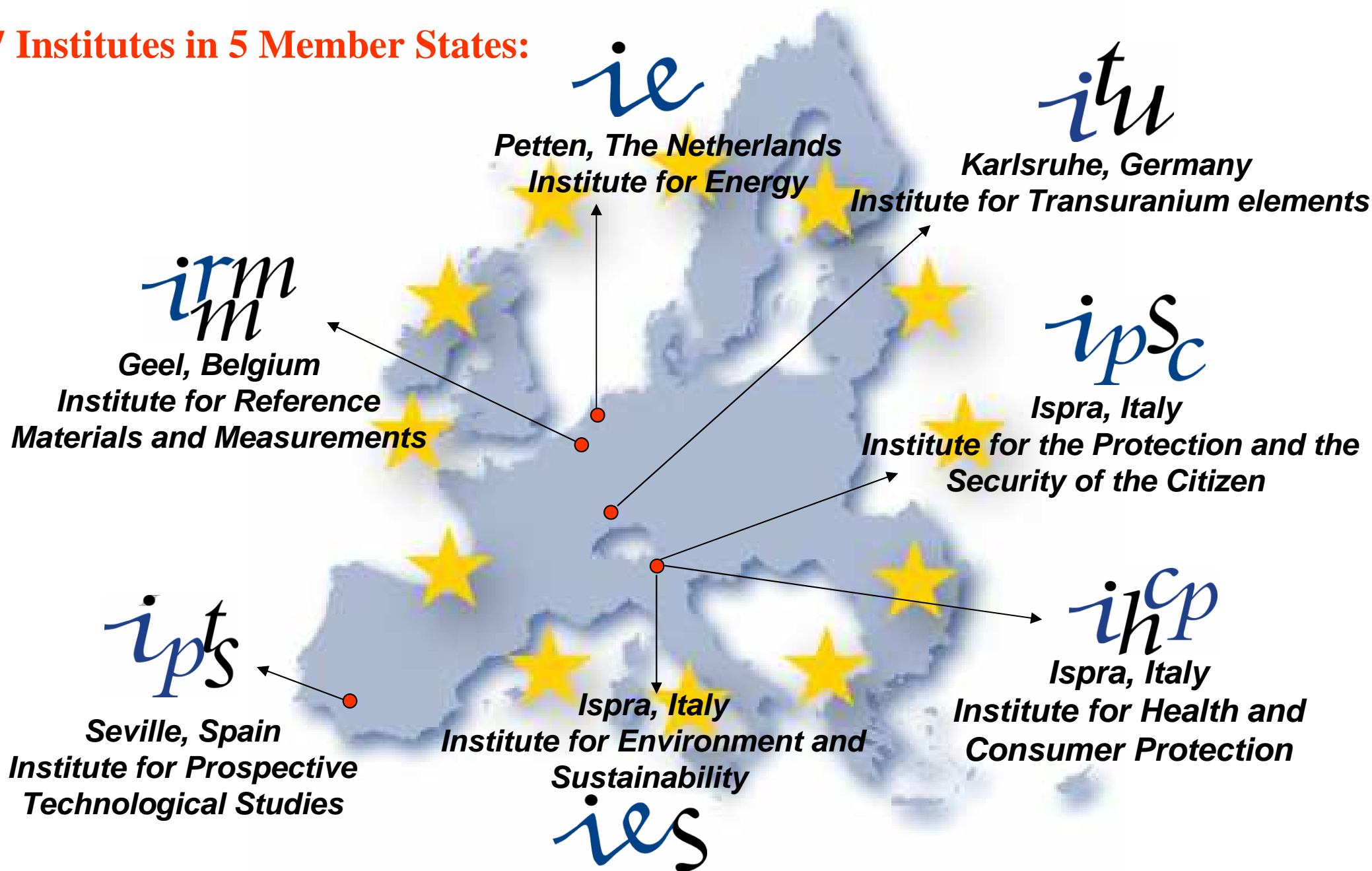
# Towards DNA copy number ratios as GM quantification units



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## 7 Institutes in 5 Member States:



# Genetically Modified Organism

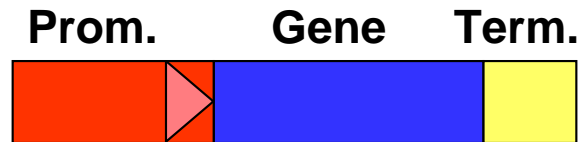
## Maize Wild type



## Maize Transgenic plant



**+ foreign DNA**



# European legislation

## European Regulation (EC) No 1829/2003

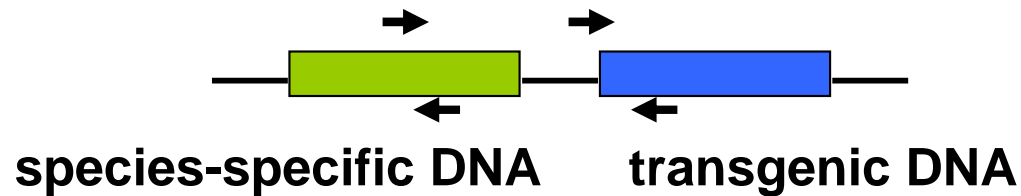
- Labelling threshold for technically unavoidable presence of GM material: 0.9 %
- Mass fraction:

$$\text{GM content/species [\%]} = \frac{\text{mass GM [g]}}{\text{mass GM [g] + mass non-GM [g]}} \times 100$$

# European legislation (2)

European Recommendation (EC) No 787/2004

$$\text{GM content/species [\%]} = \frac{\text{transgenic DNA copy numbers [cp]}}{\text{species-specific DNA copy numbers [cp]}} \times 100$$



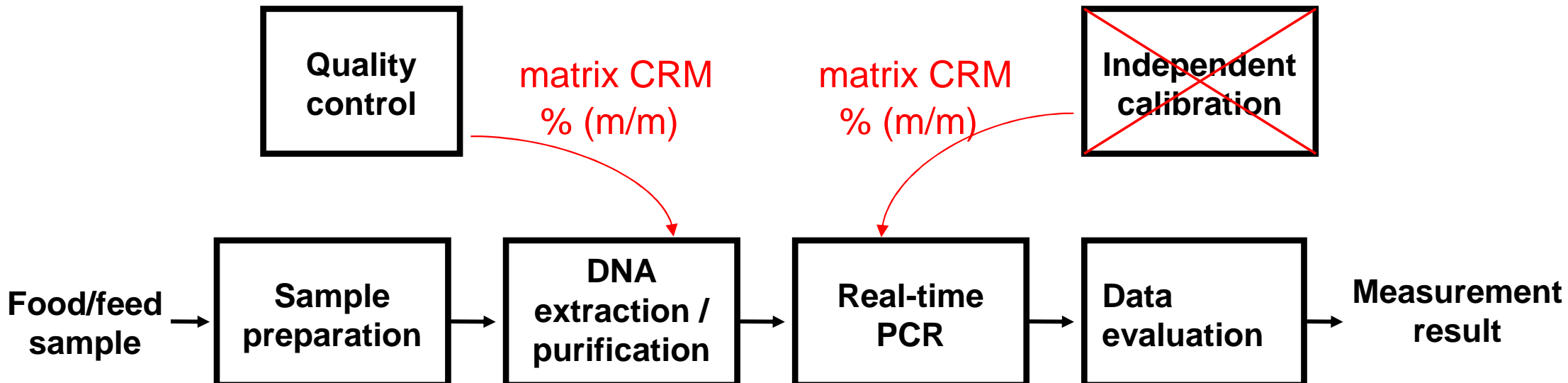
**Copy number = number  
of molecules**

# GM quantification: mass fraction m/m [%]

*Measurement principle*

*Analytical method*

*GM Analytical procedure*



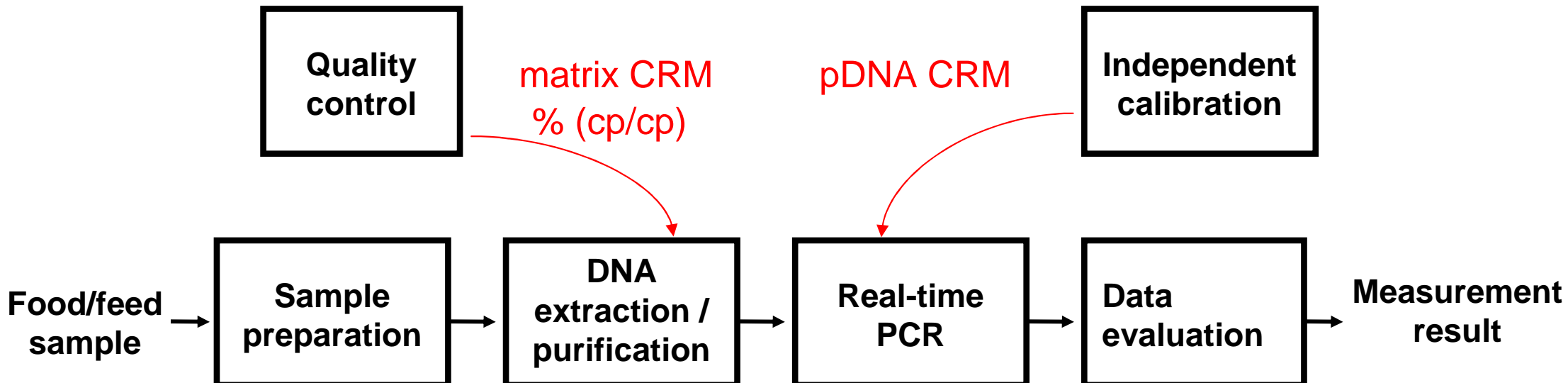
Ct values plotted vs ng DNA: % (m/m)

# GM quantification: DNA copy number ratio [%]

*Measurement principle*

*Analytical method*

*GM Analytical procedure*



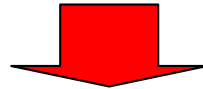
Ct values plotted vs cp DNA: % (cp/cp)

# Genetically Modified Certified Reference Materials (CRMs)




# Scientific challenge

- No scientific basis
- Outline strategy: different factors



measurement results

- Large number of measurement results
- Publication of call for expression of interest  
 **Interlaboratory comparison**

# Interlaboratory comparison

- Maize event MON 810
- Participation of 43 laboratories
- 67 independent data sets
  - 1 data set: (analyses were spread over at least 2 days)
    - DNA extraction from 4 unknown powder samples
    - DNA quantification
    - Preparation of dilutions from unknown samples, genomic and plasmid DNA calibrants
    - Two real-time PCR measurements
- 6 method combinations
- 268 real-time PCR measurements

# Investigation of different factors

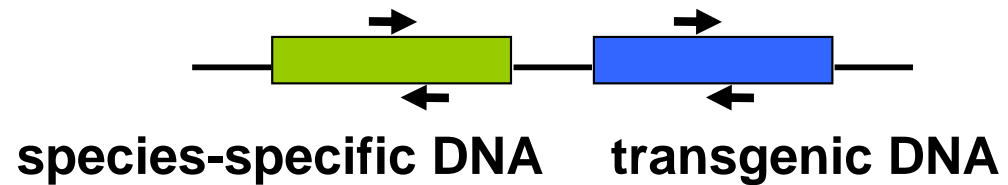
- Particle size of the unknown powder samples from maize seeds:
  - four unknown powder samples of which two coarse and two fine materials
- DNA calibrant:
  - plasmid DNA
  - genomic DNA from leaves of maize MON 810 plants
- DNA extraction methods: CTAB, Wizard, GENE *Spin*
- Real-time PCR detection method:
  - construct-specific or event-specific

# Real-time PCR detection method

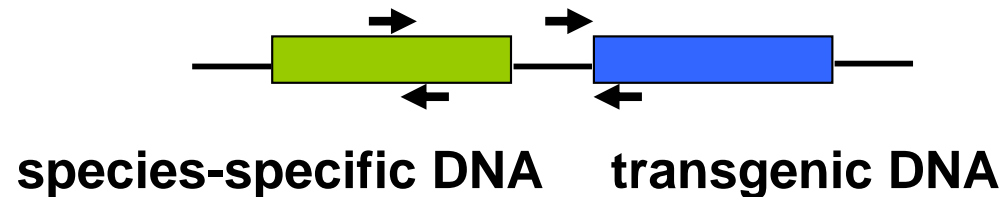
## Maize Transgenic plant



### Construct-specific detection method

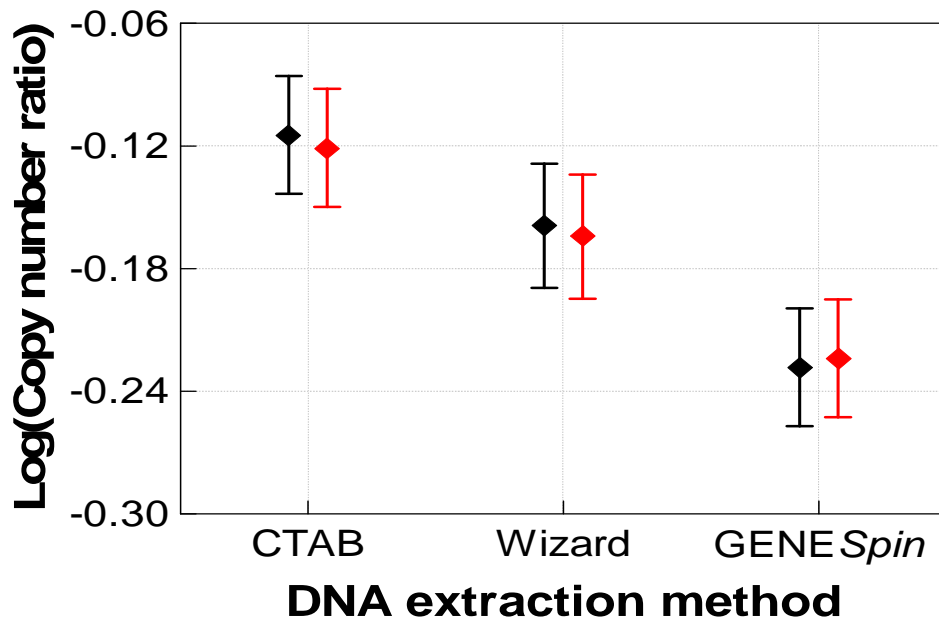


### Event-specific detection method

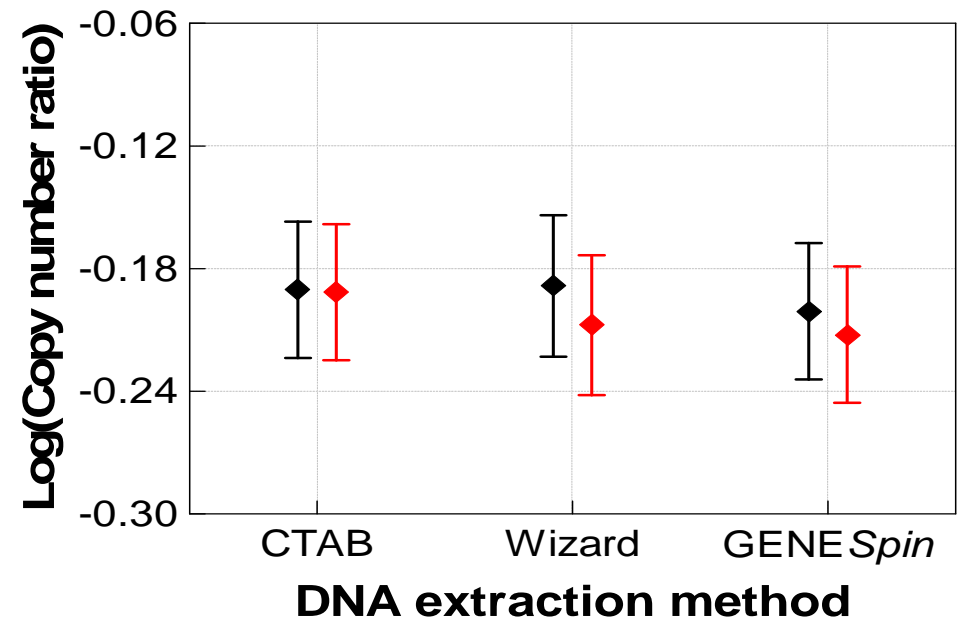


# Selected results

## Construct-specific detection method

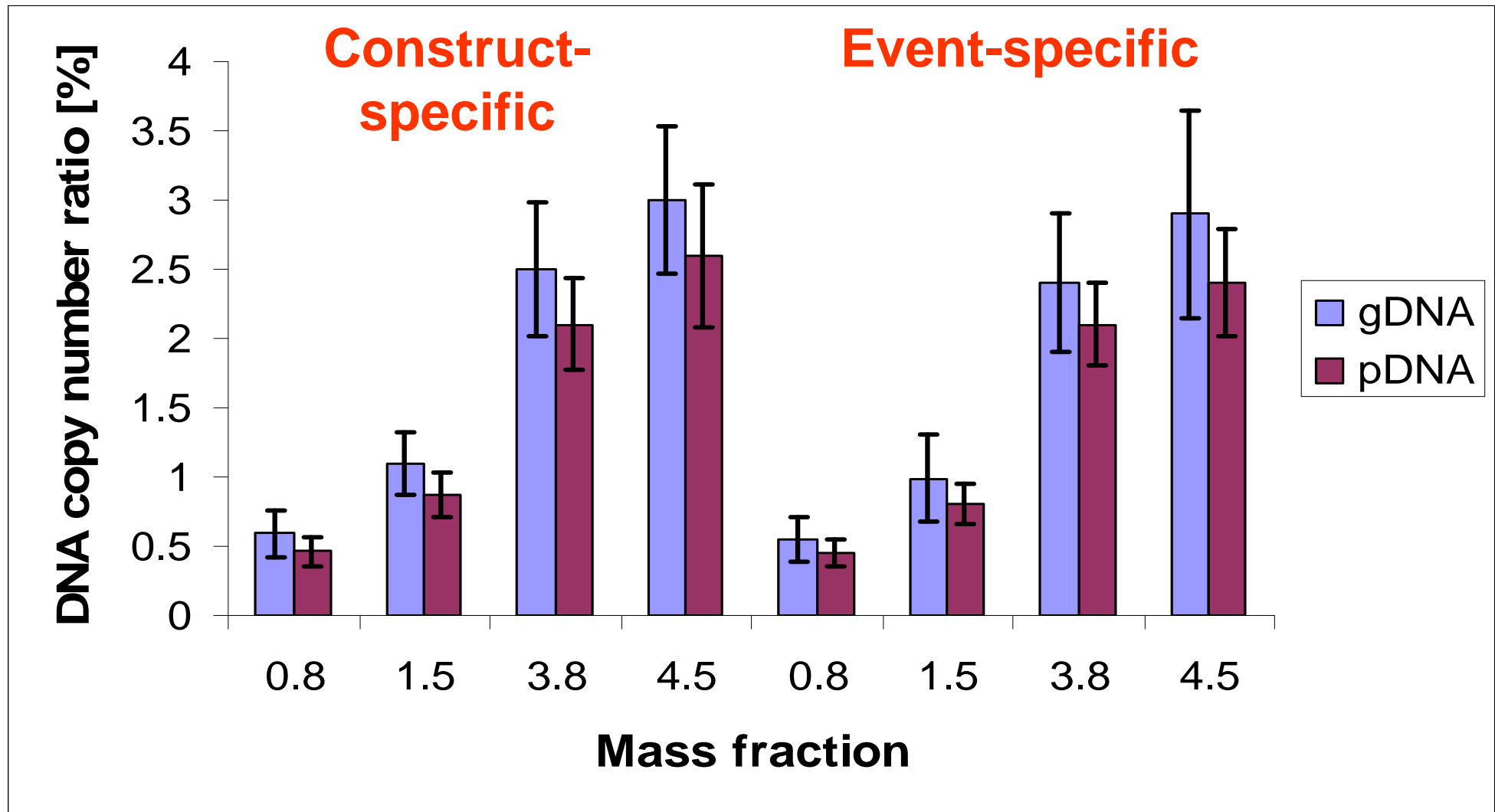


## Event-specific detection method

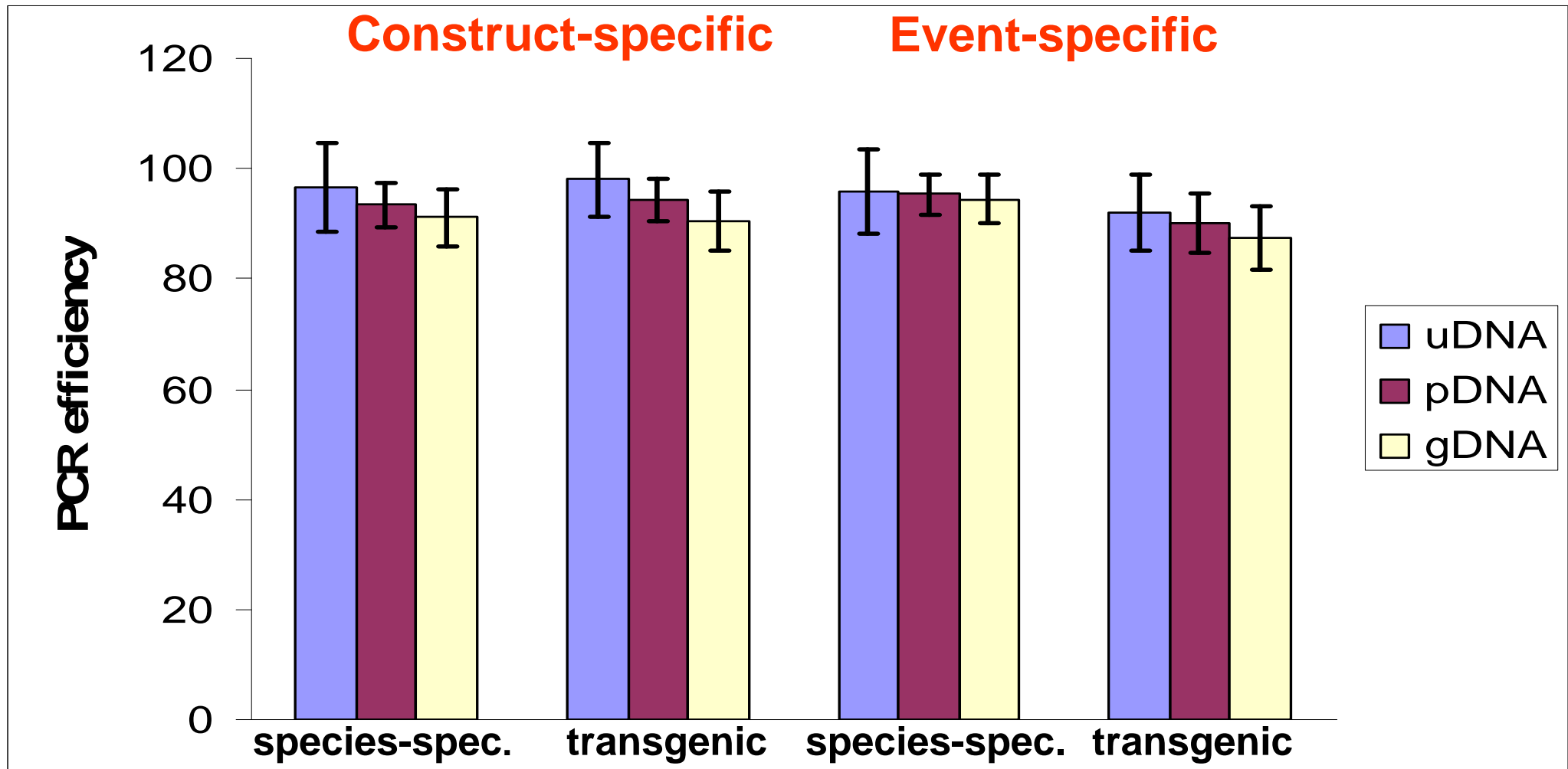


- ◆ fine
  - ◆ coarse
- unknown powder material

# Effect of the DNA calibrant



# Effect of the PCR efficiency

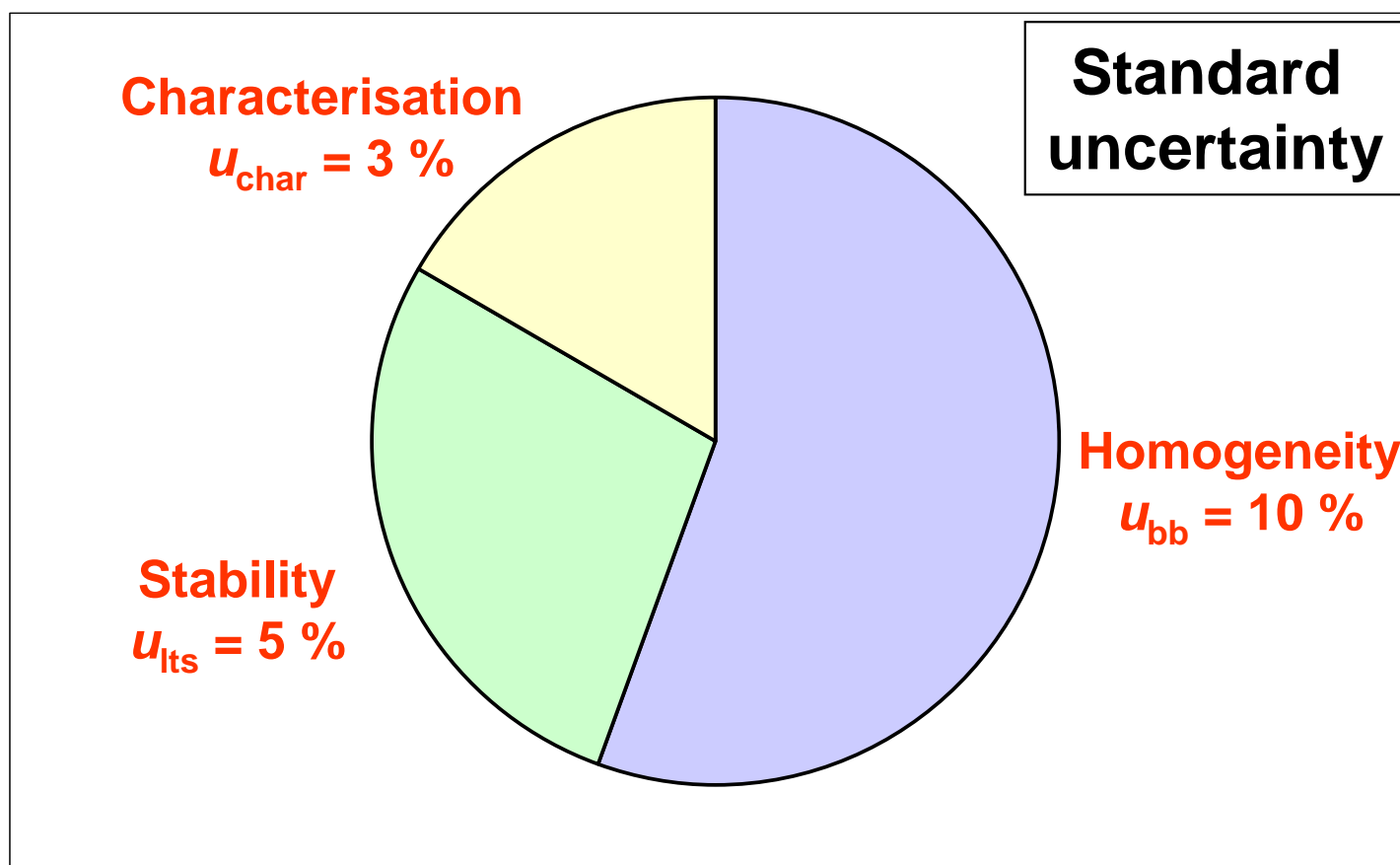


# Value and uncertainties

Mass fraction of 1.5 % ~ Copy Number Ratio 0.81 %

$$U_{\text{CRM, rel}} = 22 \%$$

$$U_{\text{CRM}} = 0.2$$



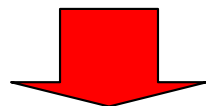
$$U_{\text{CRM, rel}} = k(u_{\text{char}}^2 + u_{\text{bb}}^2 + u_{\text{Its}}^2)^{1/2}$$

# Conclusions

- Dependence of real-time PCR results on DNA extraction method applied
- Importance of validating DNA extraction methods in conjunction with real-time PCR methods
- No influence of the particle size
- Effect of DNA calibrant
- pDNA suitable calibrant for maize MON 810
- Results published in Journal of Agriculture and Food Chemistry

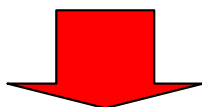
# Outlook

- Establish certification strategy for other GM events



will be applied to 1 % mass CRMs

- Interlaboratory comparisons with large number of data sets to validate the certification strategy



- matrix CRMs certified for mass fraction and DNA copy number ratio
- independent DNA calibrant

# CRM Certificate

Mass fraction  
Copy number Ratio

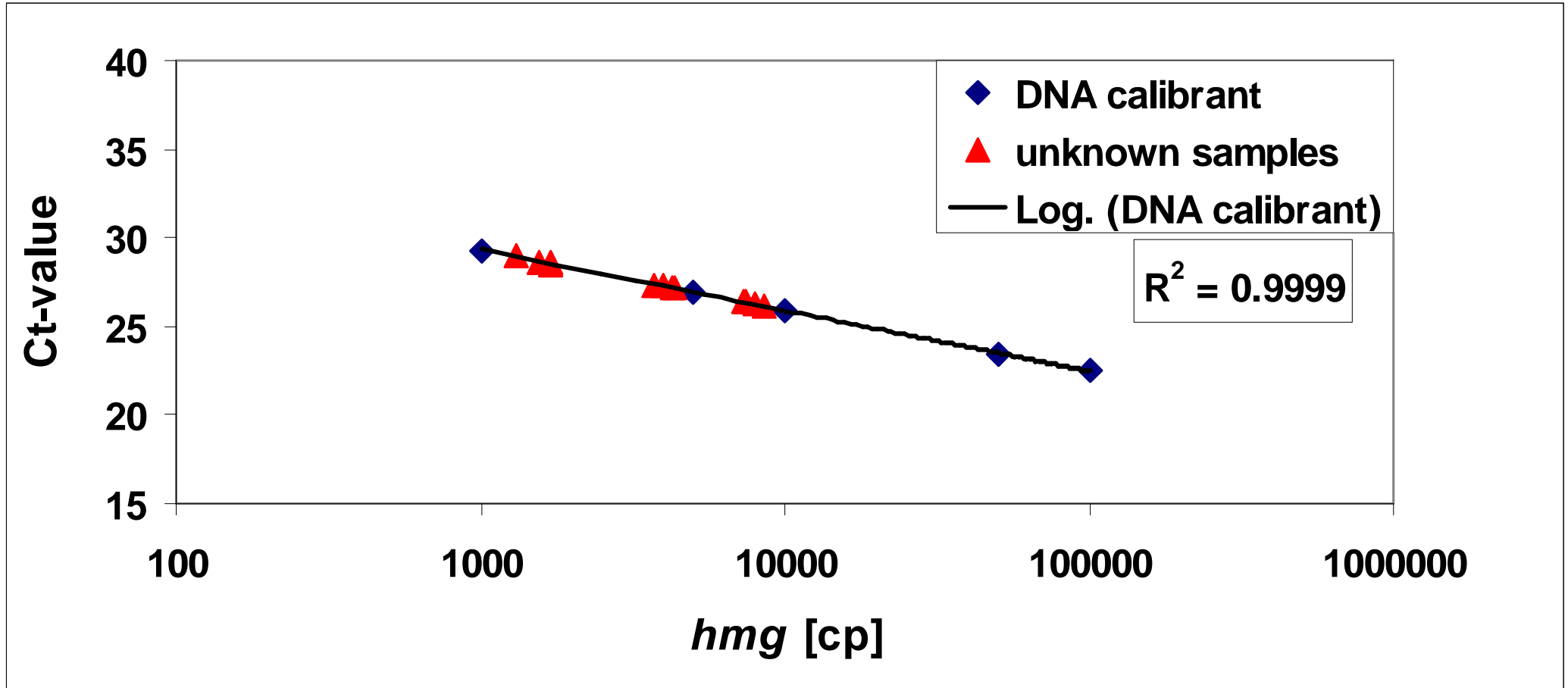
MAIZE SEED POWDER		
	Certified value	Uncertainty <sup>3)</sup>
MON 810 maize mass fraction	10.0 g/kg <sup>1)</sup>	0.5 g/kg
MON 810 maize DNA copy number ratio	0.57 % <sup>2)</sup>	0.17 %
<p><sup>1)</sup> The certified value is based on the masses of mixed dried genetically modified MON 810 maize seed powder and of dried non-modified maize seed powder, taking into account their respective purity with regard to MON 810 maize and their water content. The value is traceable to the International System of Units (SI).</p>		
<p><sup>2)</sup> The certified value is the DNA copy number ratio of the event-specific plant/P35S junction region and a single copy target of the high mobility group gene (<i>hmg</i>) measured by real-time Polymerase Chain Reaction, expressed in percent. The certified value is traceable to the real-time Polymerase Chain Reaction detection method ISO 21570:2005, Annex D2 calibrated with the MON 810 maize plasmid DNA Certified Reference Material ERM<sup>®</sup>-AD413.</p>		
<p><sup>3)</sup> The certified uncertainty is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) with a coverage factor <math>k = 2</math>, corresponding to a level of confidence of about 95 %.</p>		

# Thank you for listening!



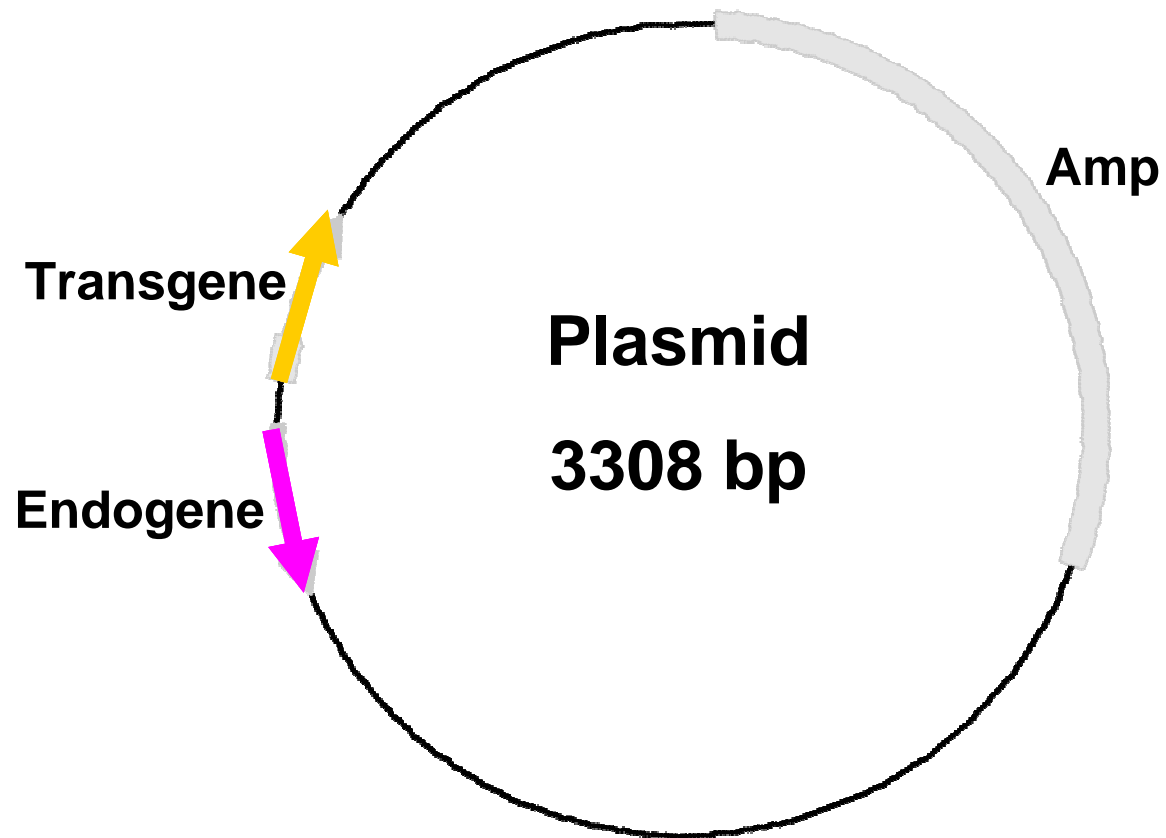
## Questions?

# PCR efficiency



**Slope = -3.43  $\Rightarrow$  PCR efficiency = 96 %**  
**PCR efficiency  $\varepsilon = 10^{-1/\text{slope}} - 1$**

# Plasmid DNA



# Calculation of copy number

## Genomic DNA of maize

Nuclear DNA content [Mbp]	# cp in 50 ng	Reference
2292	21357	Arumuganathan
2716	18023	Arumuganathan
5000	9790	Janckiewicz
2425	20186	used at IRMM

$$1 \text{ ng} = 0.979 \cdot 10^{12} \text{ bp}$$

$$\# \text{ copies} = (50 \text{ ng} * 0.97 \cdot 10^{12} \text{ bp}) / \text{nuclear DNA content}$$