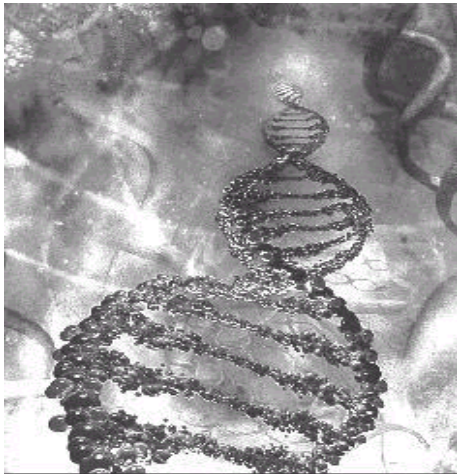


Testing for biotechnology products – Why, how, and are the results really that straightforward?

Frank Spiegelhalter
Eurofins GeneScan, Inc.



ISO/IEC 17025



Testing Cert 1940.01

www.gmotesting.com

Eurofins Laboratories Worldwide



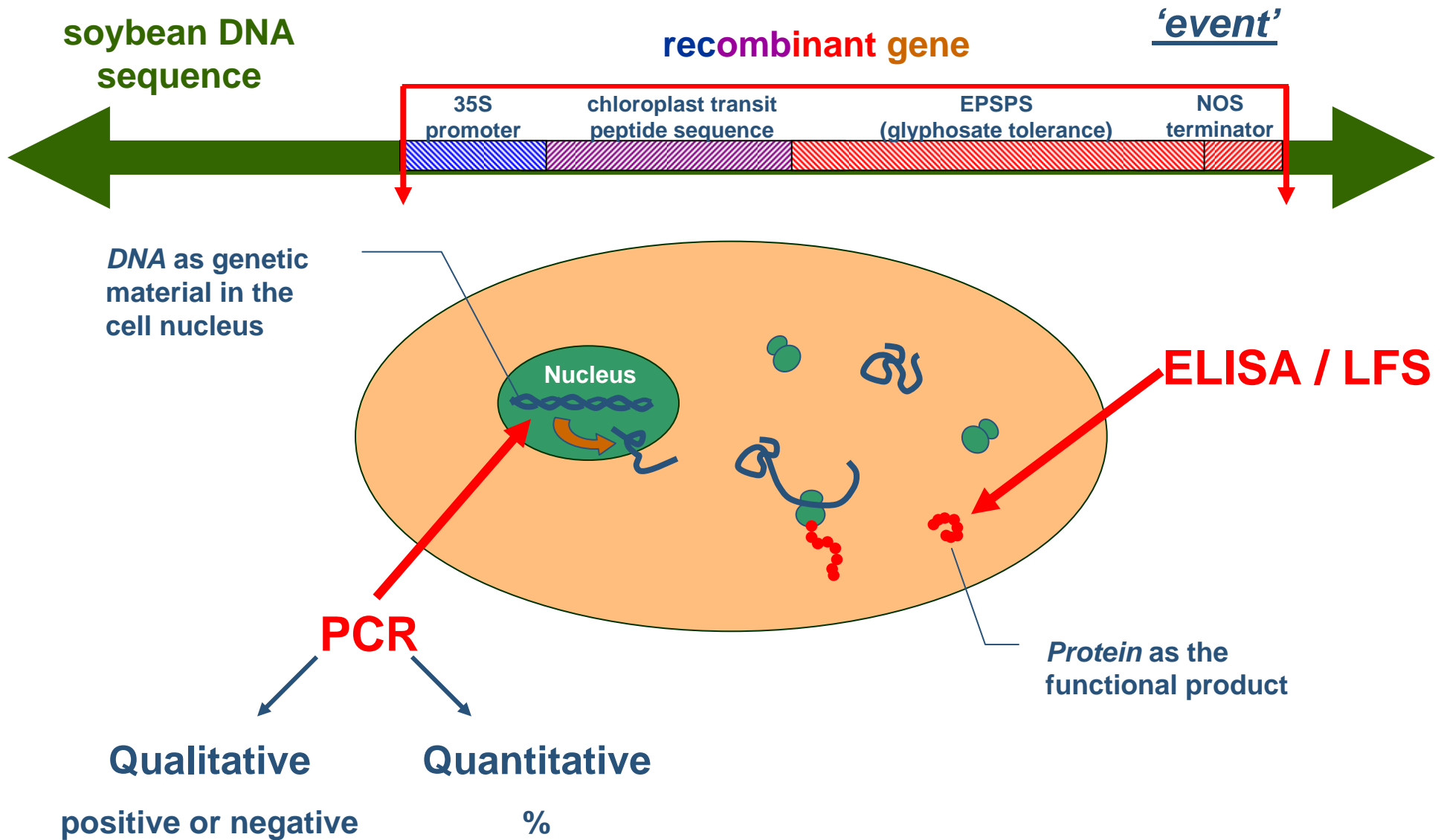
Biotech Testing 101

- How?
- Why??

Same sample - Same method - Same data - Different results?

AP of 'stacked' biotech corn events in non-GMO IP corn

Testing for 'GMO' - DNA and Protein Tests



Polymerase Chain Reaction

Laboratory Test

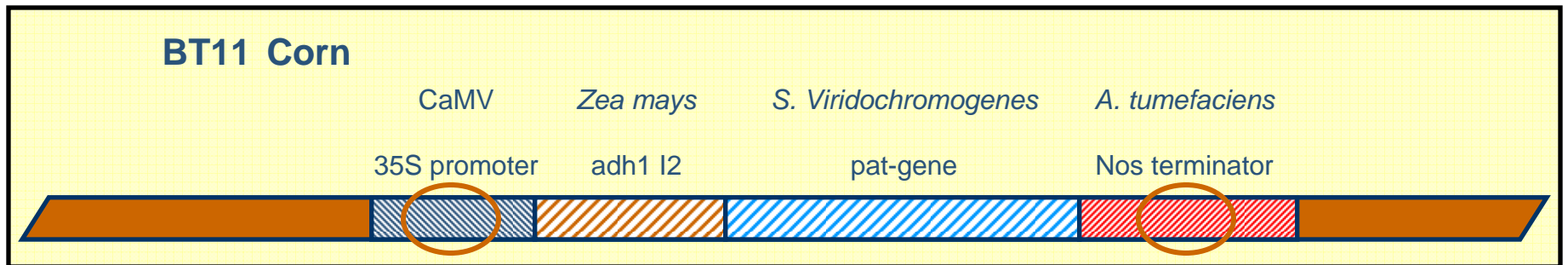
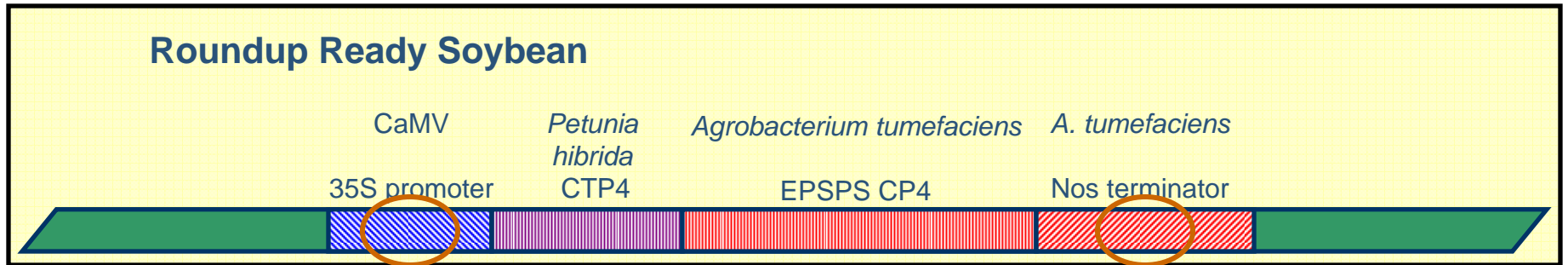
Enzyme-Linked Immunosorbent Assay

Laboratory Test

**Lateral Flow Strip / Lateral Flow Device /
Strip Test ...**

Rapid Field Test

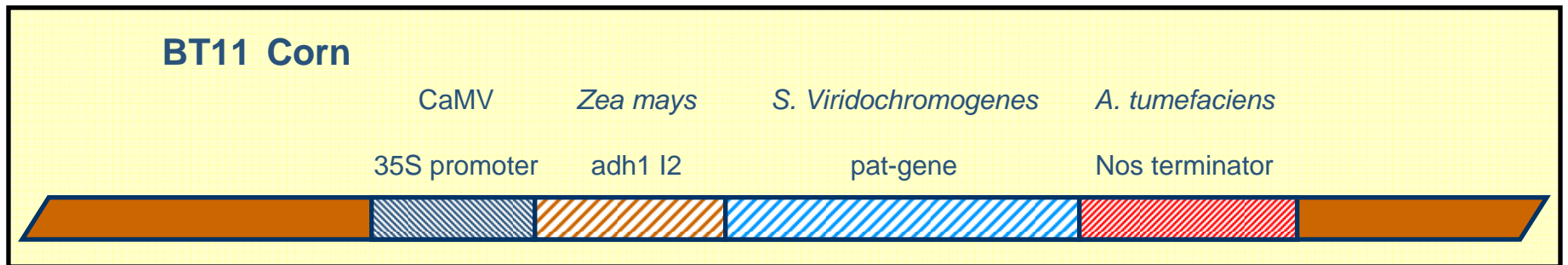
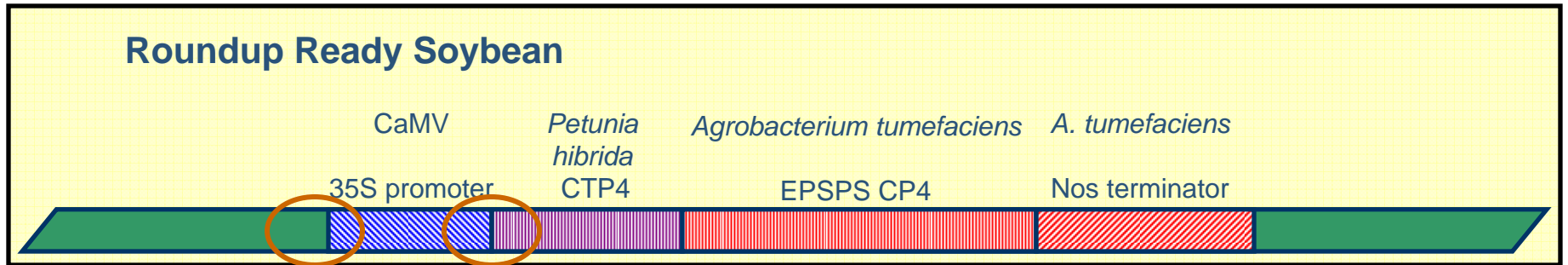
Screening Tests



35S promoter, nos terminator and other screening tests help determine the presence of biotech DNA in general

- Inclusive for a broad range of biotech crops
- Negative results quite informative
- Positive results are not very informative

Specific Tests



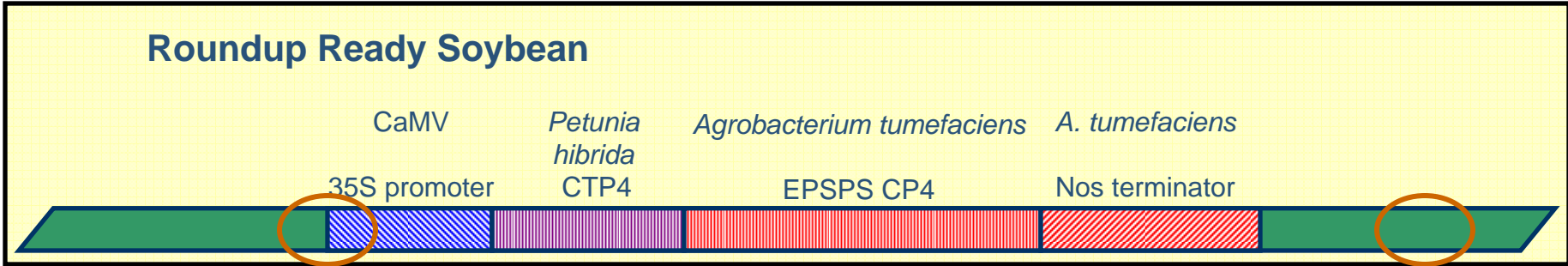
‘Construct’ specific tests

‘Event’ specific tests – the only analytical proof for presence of a particular event

Examples of Tests for Biotech Corn

event	trait	PCR			LFS detection in grain
		35S	nos	event or construct specific	
176	IR	+		+	
MON810	IR	+		+	Cry1Ab
Bt11	IR	+	+	+	Cry1Ab
TC1507	IR	+		+	Cry1F
MON863	IR	+	+	+	Cry3Bb
NK603	HT	+	+	+	EPSPS
GA21	HT		+	+	
T25	HT	+		+	Bar
DAS-59122-7	IR / HT	+		+	Cry34/35Ab1
MIR604	IR		+	+	Cry3Aa2
MON88017	IR / HT	+	+	+	EPSPS Cry3Bb1
MON89034	IR	+	+	+	Cry1A105/Cry2Ab2

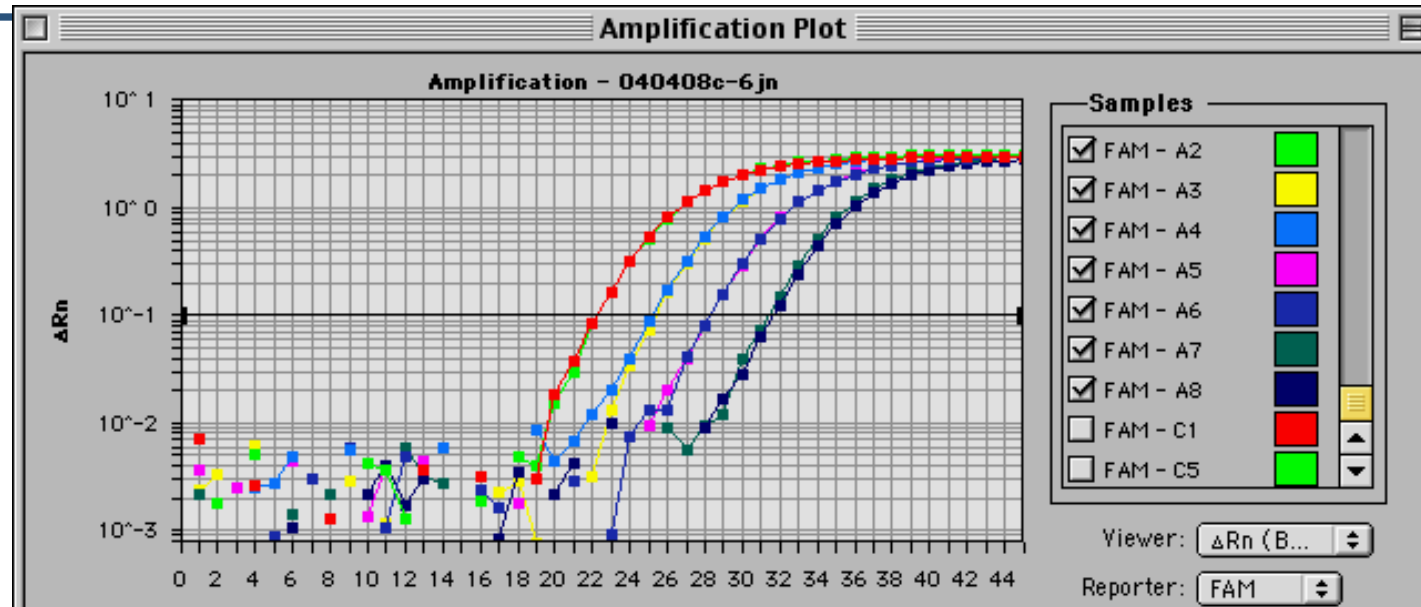
Quantitative PCR Tests



Biotech DNA Test

Plant Species DNA Test

Quantitative PCR Tests



Threshold Cycle Ca

Threshold

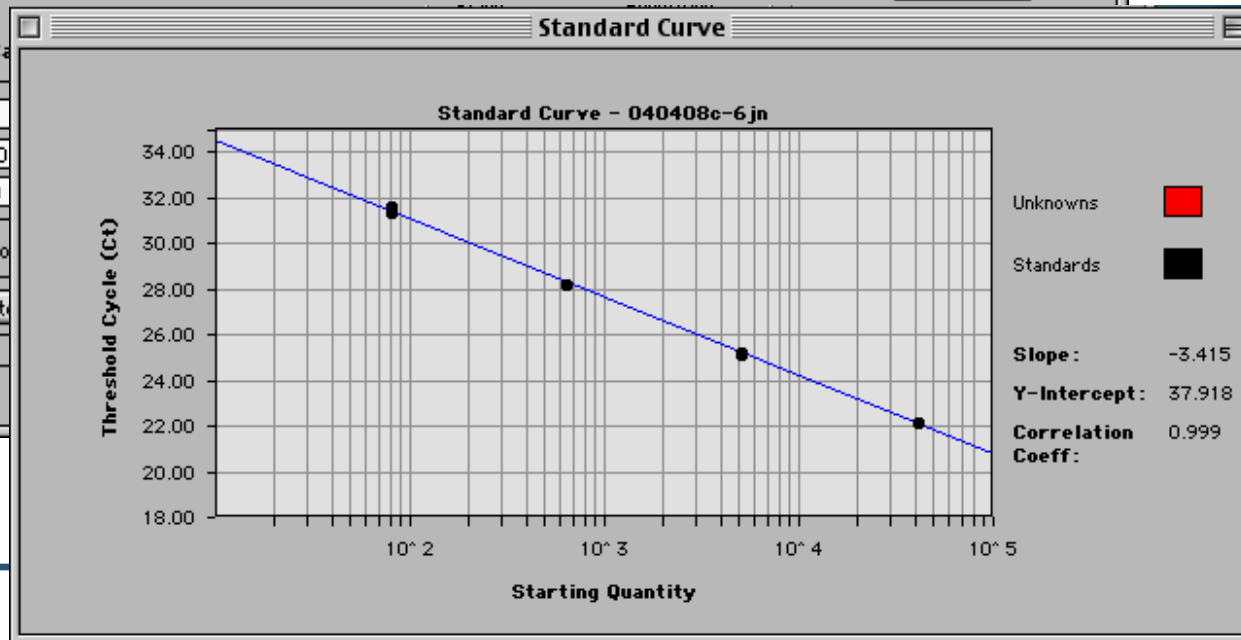
Use Threshold:

Mult. * Stddev:

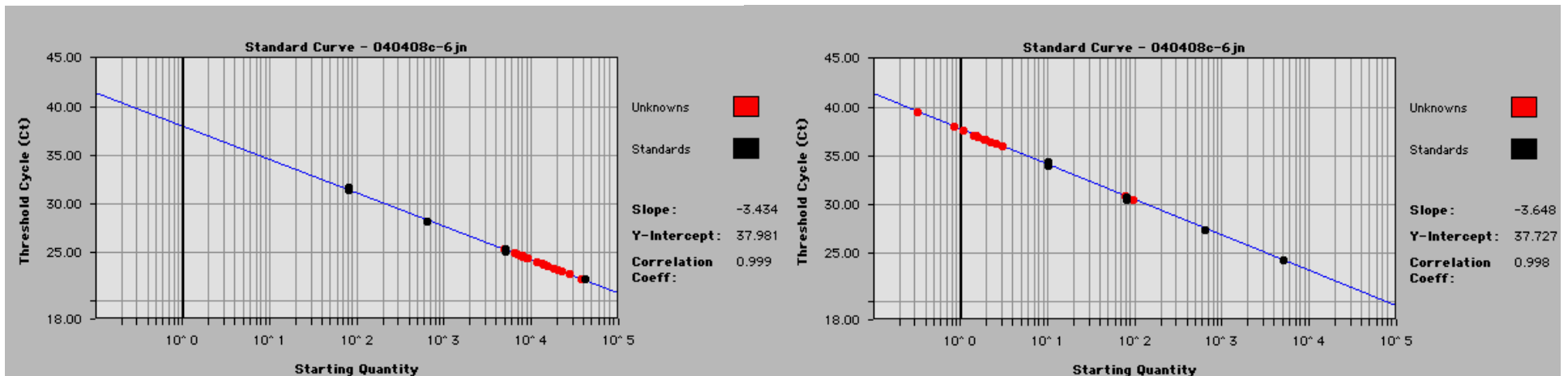
Omit Threshold:

Baseline

Start: Sto



Quantitative PCR Tests



$$\frac{[\text{biotech DNA}] \times 100}{[\text{species DNA}]} = \% \text{ biotech DNA}$$

relative quantification

Why test for biotech crops and derived food and feed products??

In the US and number of other countries,
a good rule of thumb is:

*Generally, testing for 'GMO'
is not required...*

...because biotech derived crops and their products are
not considered substantially different from the
conventional counterparts.

Exceptions to the rule – 1 Reasons for ‘GMO’ Tests

Seed Quality Control

- Confirm Biotech Traits

grow-out

ELISA

PCR

LFS



- Check against unintended admixtures of biotech seeds other than the intended product
- Check conventional seeds for unintended presence of biotech seeds

Exceptions to the rule – 2

Catering to consumer preferences in select domestic or export markets



Labeling of food products:

Mandatory labeling of GM products

Voluntary labeling of non-GM products

LFS

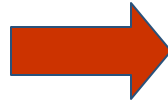
PCR

ELISA

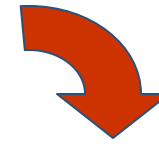
Testing in a non-GMO IP Export Grain Handling System



Trucks
unload
LFS Test



Storage
Silos



Panamax
vessels loaded
PCR Analysis



Barge loading

- LFS Test
- Send samples to PCR lab



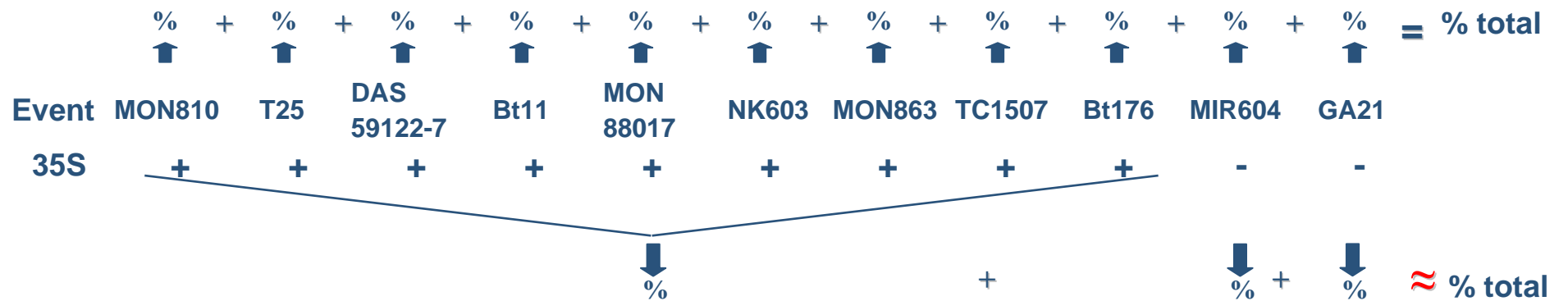
Examples of Tests for Biotech Corn

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T25	HT	+		+	Bar
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MON88017	IR / HT	+	+	+	EPSPS Cry3Bb1
MON89034	IR	+	+	+	Cry1A105/Cry2Ab2

Common DNA Testing Strategy for Non-GMO Corn

Most accurate: Test each biotech corn event separately, add individual results

Cost prohibitive in a commercial situation



More cost effective

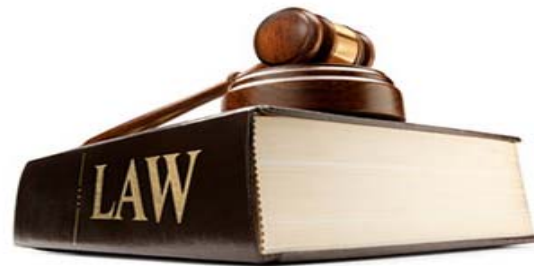
Which 'non-GMO' soy products are tested? | GeneScan

What do PCR results tell us?

	Sensitivity or 'LOD'	Qualitative tests	Quantitative tests
Whole soybeans	Typically around 0.1% limited by sample	LFS tests work well too	ELISA has been used too
Soy flour, meal, flakes protein isolates 'normal' DNA concentrations	Can be very sensitive, as low as 0.01%	due to low level presence likely Test not necessarily useful	More reproducible than sampling & testing grain
Crude lecithin or oil limited DNA concentration	sensitivity of % 'GMO' with...	Test raw materials Identity Preservation Systems Traceability	
Refined soy lecithin or oil very little or no DNA	'GMO' not detectable		
Finished products with multiple ingredients	Sensitivity of % 'GMO' and is often unknown	challenging	especially from screening tests challenging

Exceptions to the rule – 3

Lack of approval



Both, biotech and non-biotech crops are subject to testing
Testing is not related to 'GMO' labeling

PCR

Not Approved?

Many countries have different regulations to approve biotech crop lines ('events') for environment, food and feed.

A particular event that is approved and in commercial use in country A can sometimes still await approval in country B.

'Asynchronous Approval'

There have been cases of unintentional admixture of regulated events in trace amounts into commercial seed supply.

Some discontinued events that were marketed for only a few years, have never been authorized for certain markets.

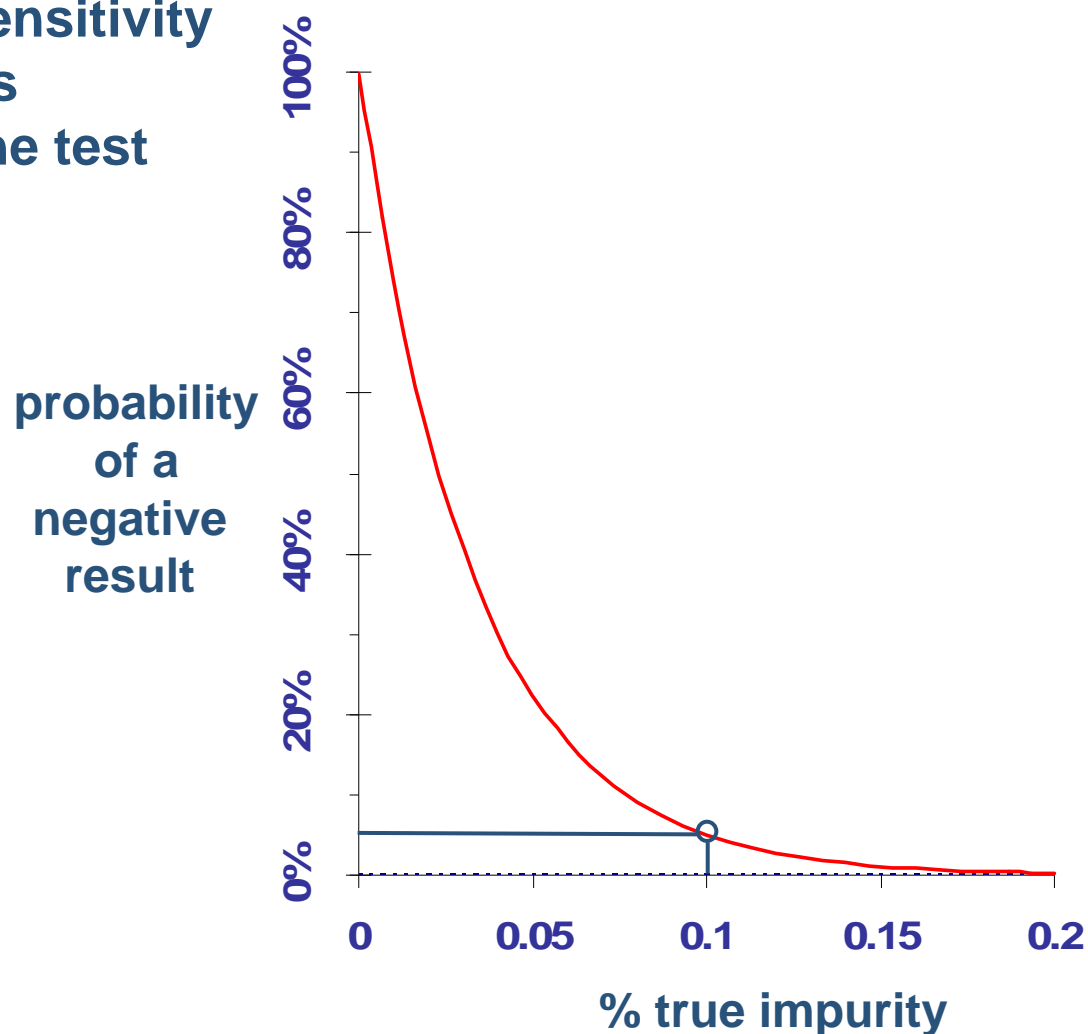
In some countries even smallest detectable amounts of unauthorized biotech events can be considered violations. This lack of tolerance levels can pose risks for trade.

Sample Size and Detection of Impurities in Trace Amounts

For seed and grain, the sensitivity of the overall procedure is typically determined by the test sample size.

Example: 3000 seeds

One negative sample of
3000 seeds means:
<0.1 %
with 95% confidence

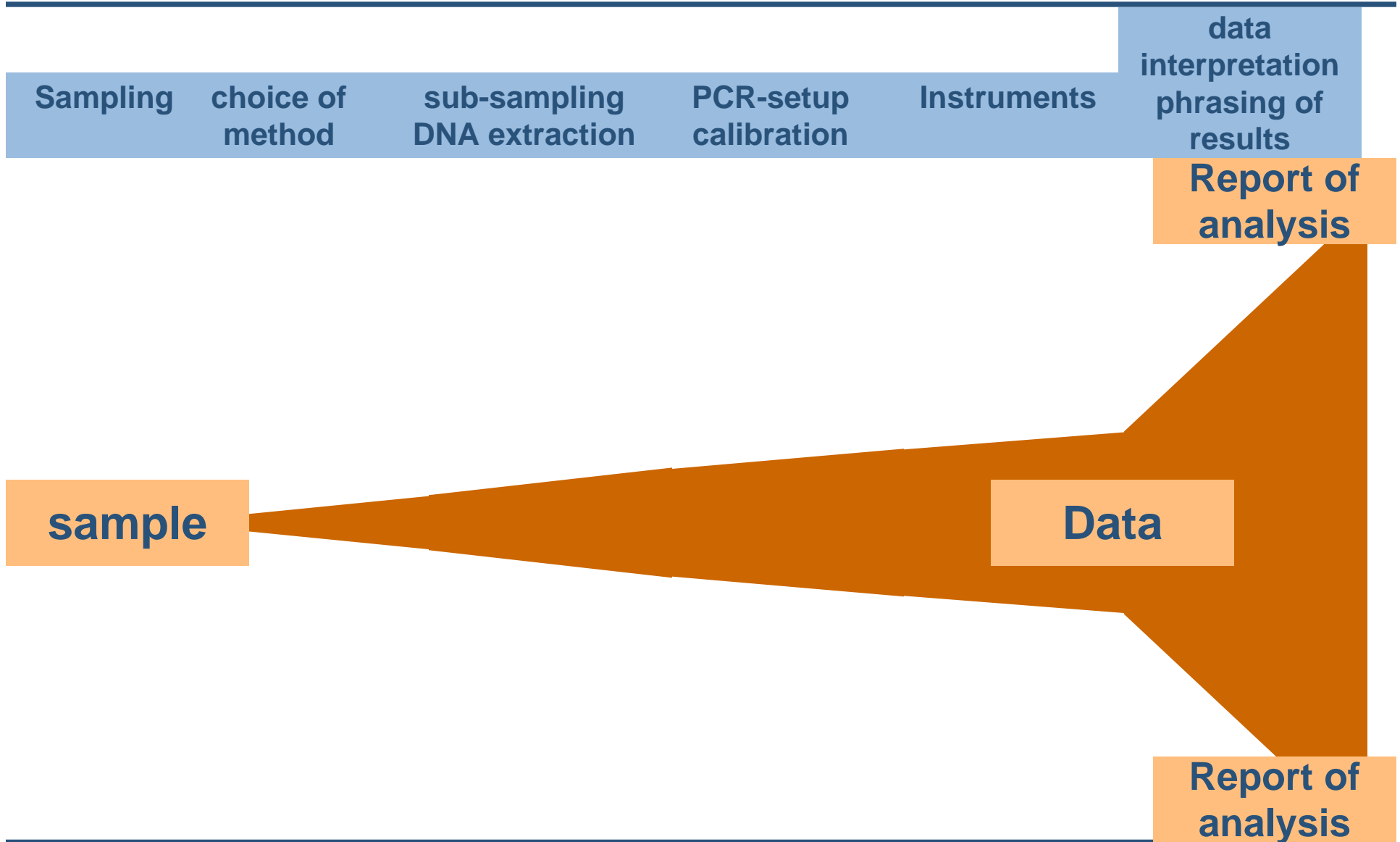


Approximate Sample Sizes and Genome Sizes in Different Crop Plants

Crop	3000 Seeds	Seeds / 1 kg	Genome (bp)	Genome (pg)	Genomes / 200 ng DNA
Wheat	110 g	27,000	1.6×10^{10}	17.2	11,500
Corn	850 g	3,500	2.5×10^9	2.7	73,000
Canola	12 g	250,000	1.2×10^9	1.3	151,500
Soybeans	600 g	5,000	1.1×10^9	1.2	165,500
Flax	20g	170,000	7.0×10^8	0.7	285,000
Rice	90 g	33,000	4.0×10^8	0.4	455,000

- **Same sample - Same method - Same data - Different results?**

Potential Variability from Sample to Result



Positive or Negative?

Threshold oriented approach
(e.g. $\Delta\Delta\text{Ct}$, number of seeds,
LOD, 'semi quantitative')

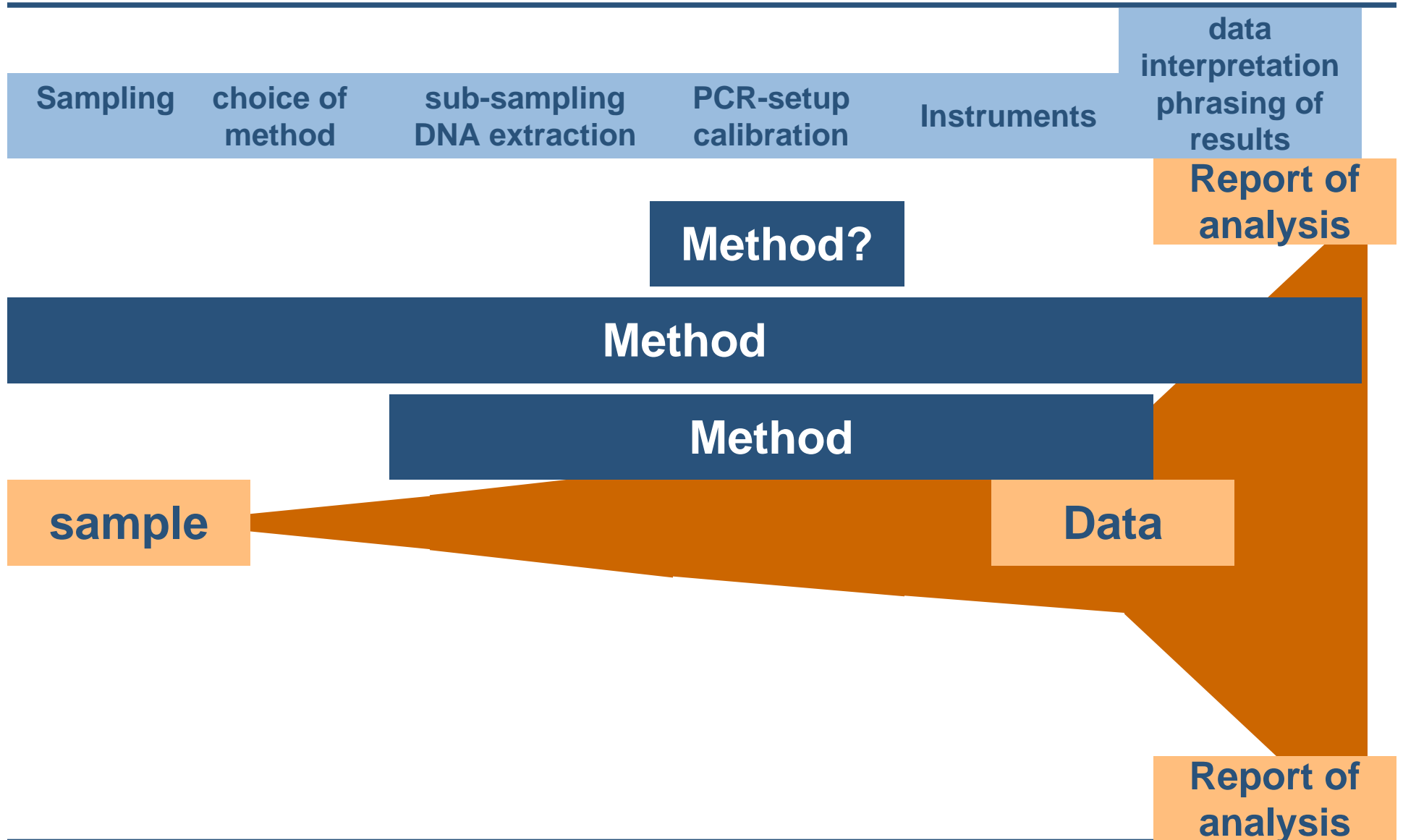
'Negative'
'Not detected'
'Less than X%'

one set of data that
shows a weak signal
(e.g. Ct values)

Qualitative without threshold
(concentration of analyte
potentially below LOD)

'Positive'
'Detected'
'Less than X%'

What does the Method cover?



Feed additive containing a small amount of corn meal

Lab A:

Some biotech corn events detected >> 10 % relative to corn DNA

Lab B:

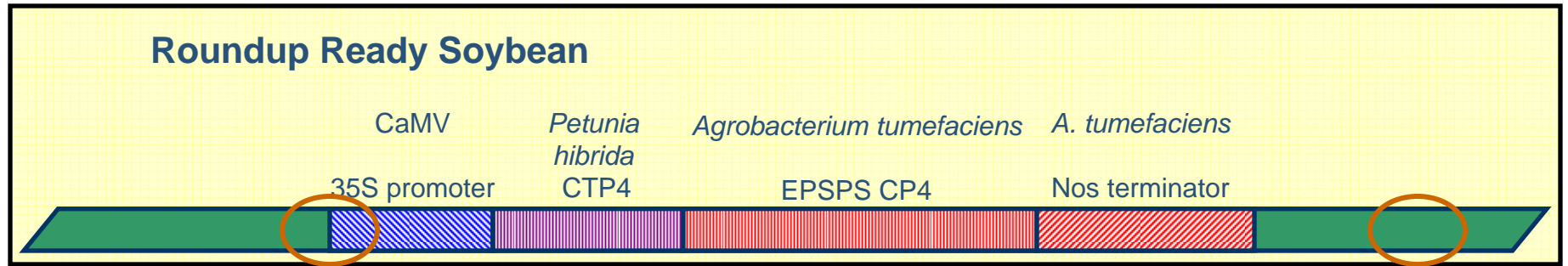
Some biotech corn events detected >> 10 % relative to corn DNA

35S promoter DNA relative to corn DNA >100 %

Lab C:

35S DNA detected, <0.1%

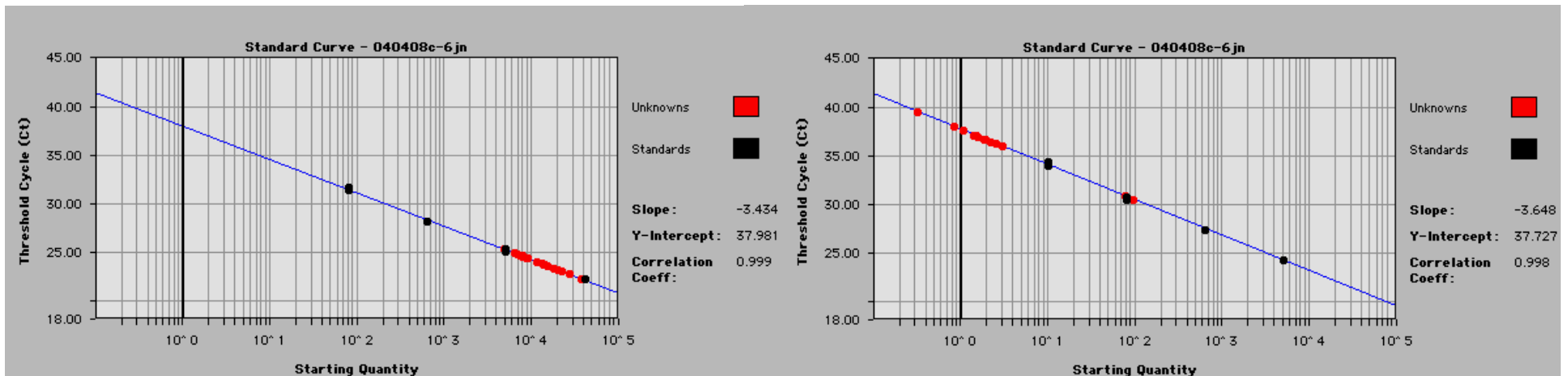
Quantitative PCR Tests



Biotech DNA Test

Plant Species DNA Test

Quantitative PCR Tests



$$\frac{[\text{biotech DNA}] \times 100}{[\text{species DNA}]} = \% \text{ biotech DNA}$$

relative quantification

'GMO' Quantification by PCR - A Ratio, not an Absolute Amount

	Corn DNA*	'GMO' DNA*	Result	LOD
Corn	100,000	1,000	1 %	0.1% **
Corn flour	100,000	1,000	1 %	0.01%
Corn gluten	10,000	100	1 %	0.1%
Corn starch	1,000	10 ?	Not quantifiable	1%
Maltodextrin	100	1 ??	Not quantifiable	10%
Glucose syrup	0 ?	???	Not quantifiable	100%

* approximate relative numbers of target DNA molecules that can typically be extracted from a sample and analyzed in quantitative PCR reactions

** with a 3000 kernel sample

Relative composition of the DNA solution extracted from a sample.

This percentage is **not** a measure of the **absolute** amount of biotech DNA in a sample.

Back to the Case Study

Feed additive containing a small amount of corn meal

Lab A:

Some biotech corn events detected >> 10 % relative to corn DNA

Relative quantification

Lab B:

Some biotech corn events detected >> 10 % relative to corn DNA

35S promoter DNA relative to corn DNA >100 %

Relative quantification

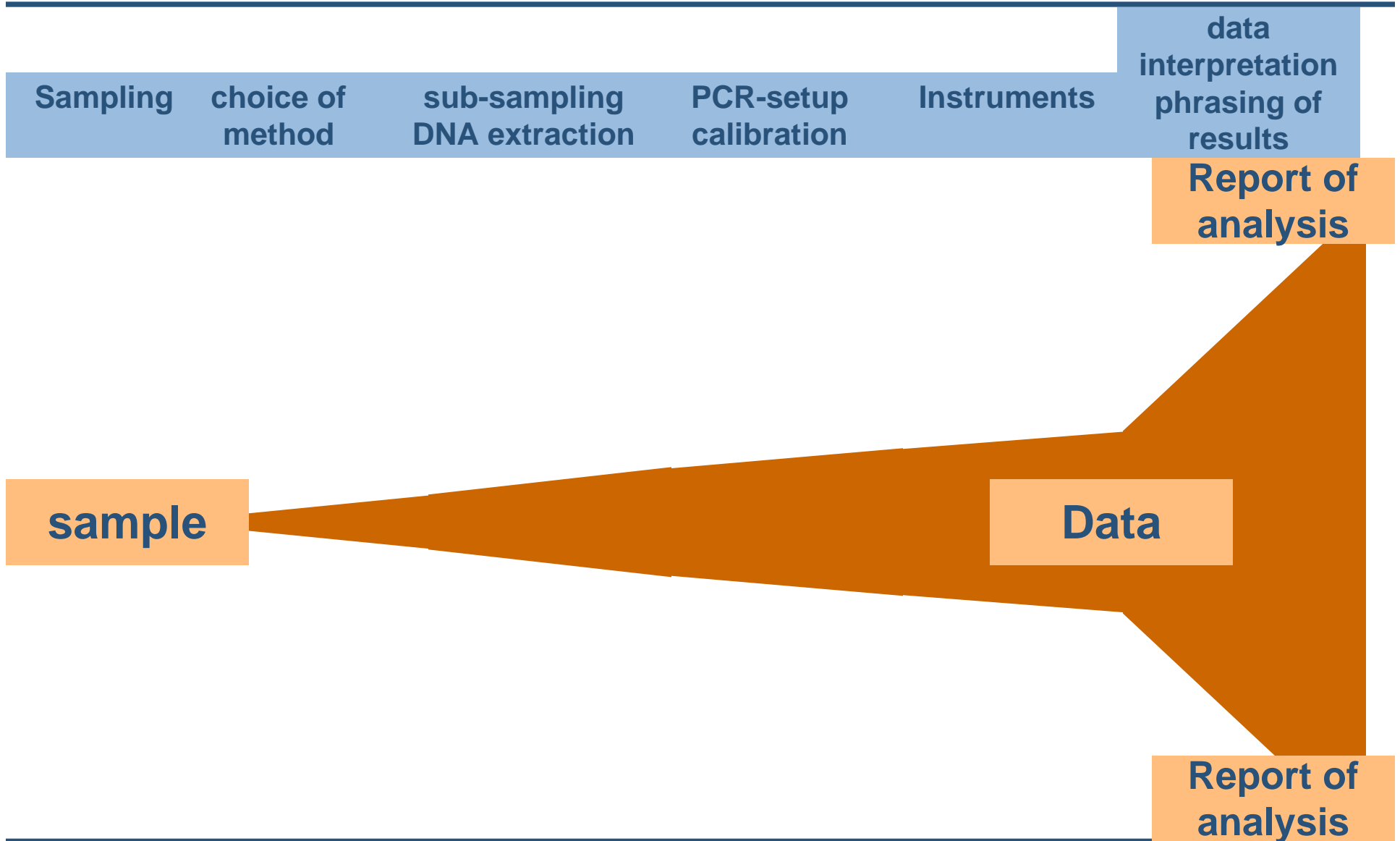
Lab C:

35S promoter DNA detected, <0.1%

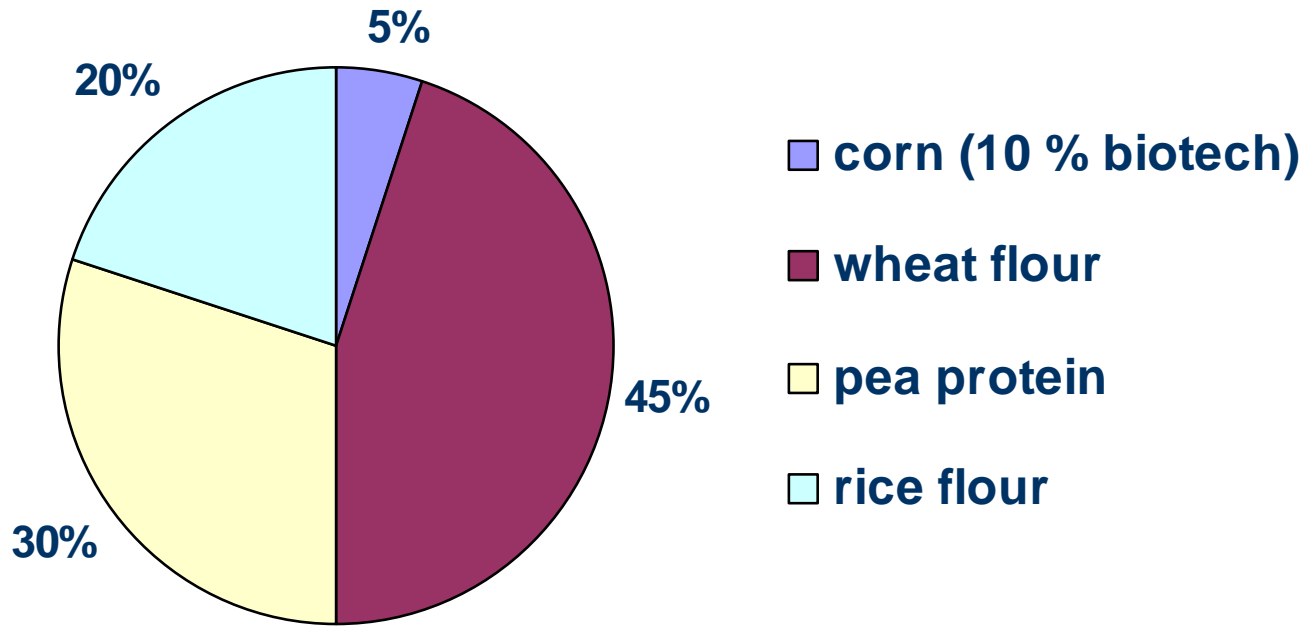
Absolute measurement of 35S DNA?

35S DNA relative to total DNA?

Potential Variability from Sample to Result

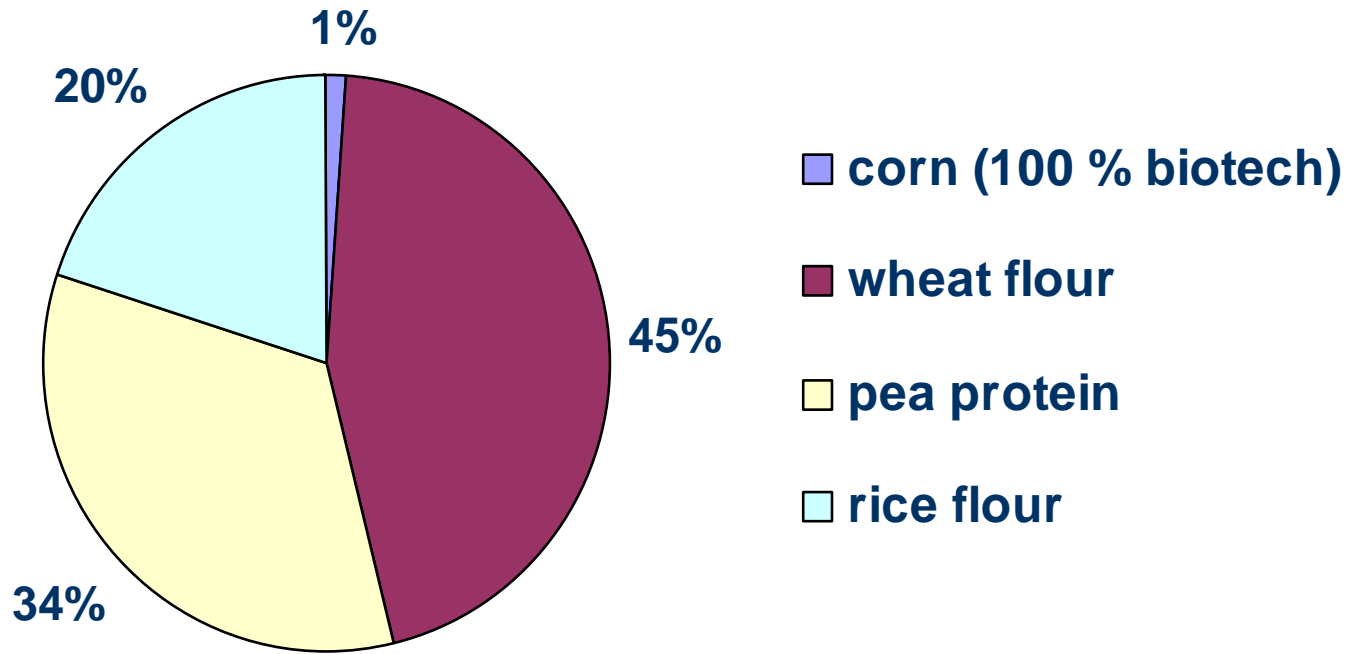


How much 'GMO'??



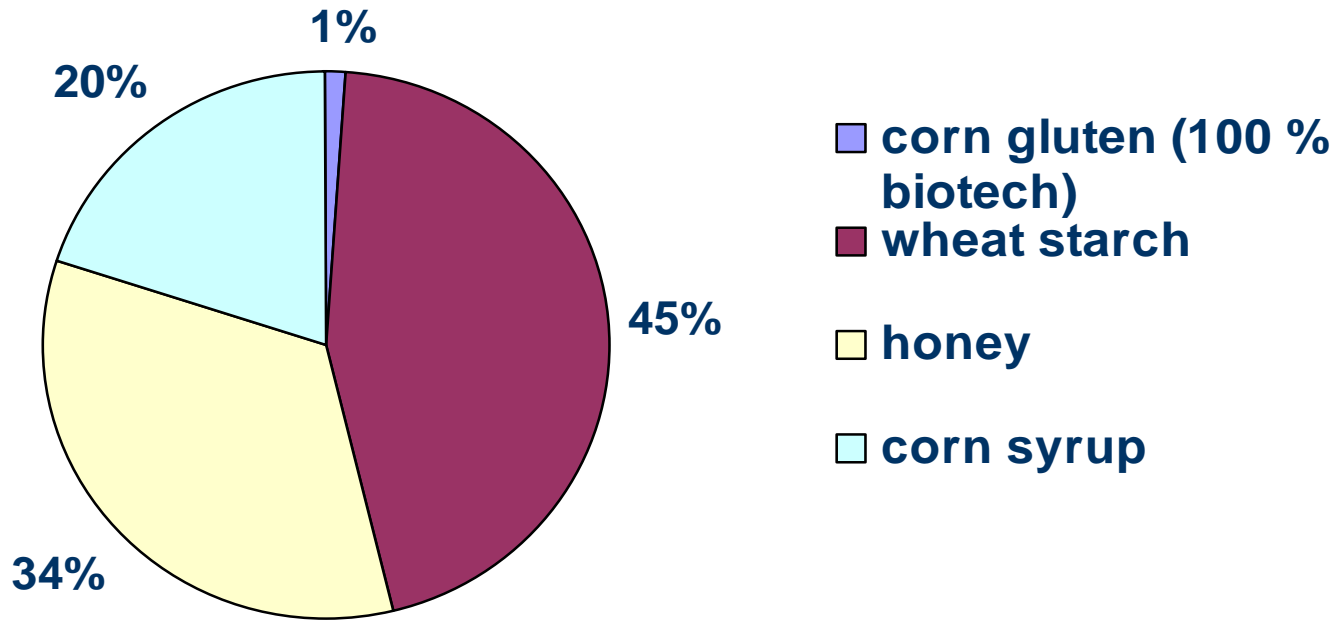
Corn DNA	5000 copies	
35S DNA	500 copies	0.5%?
"other" DNA	?? ng	10%?
total DNA	200 ng	

How much 'GMO'??



Corn DNA	1000 copies	
35S DNA	1000 copies	1%?
"other" DNA	?? ng	100%?
total DNA	200 ng	

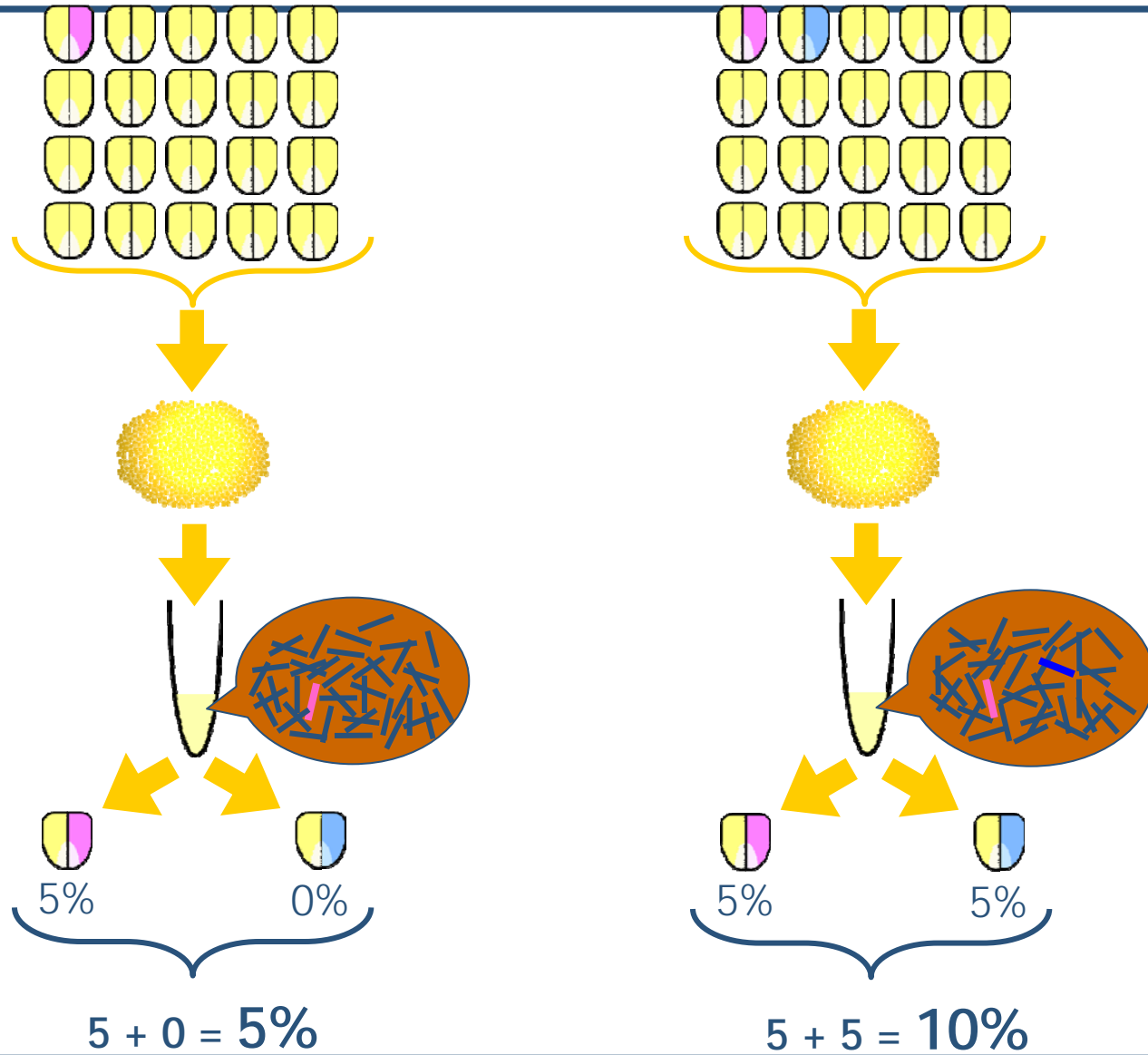
How much 'GMO'??



Corn DNA	100 copies	
35S DNA	100 copies	1%?
"other" DNA	?? ng	100%?
total DNA	2 ng	

- **AP of ‘stacked’ biotech corn events in non-GMO IP corn**

Adventitious presence of single events



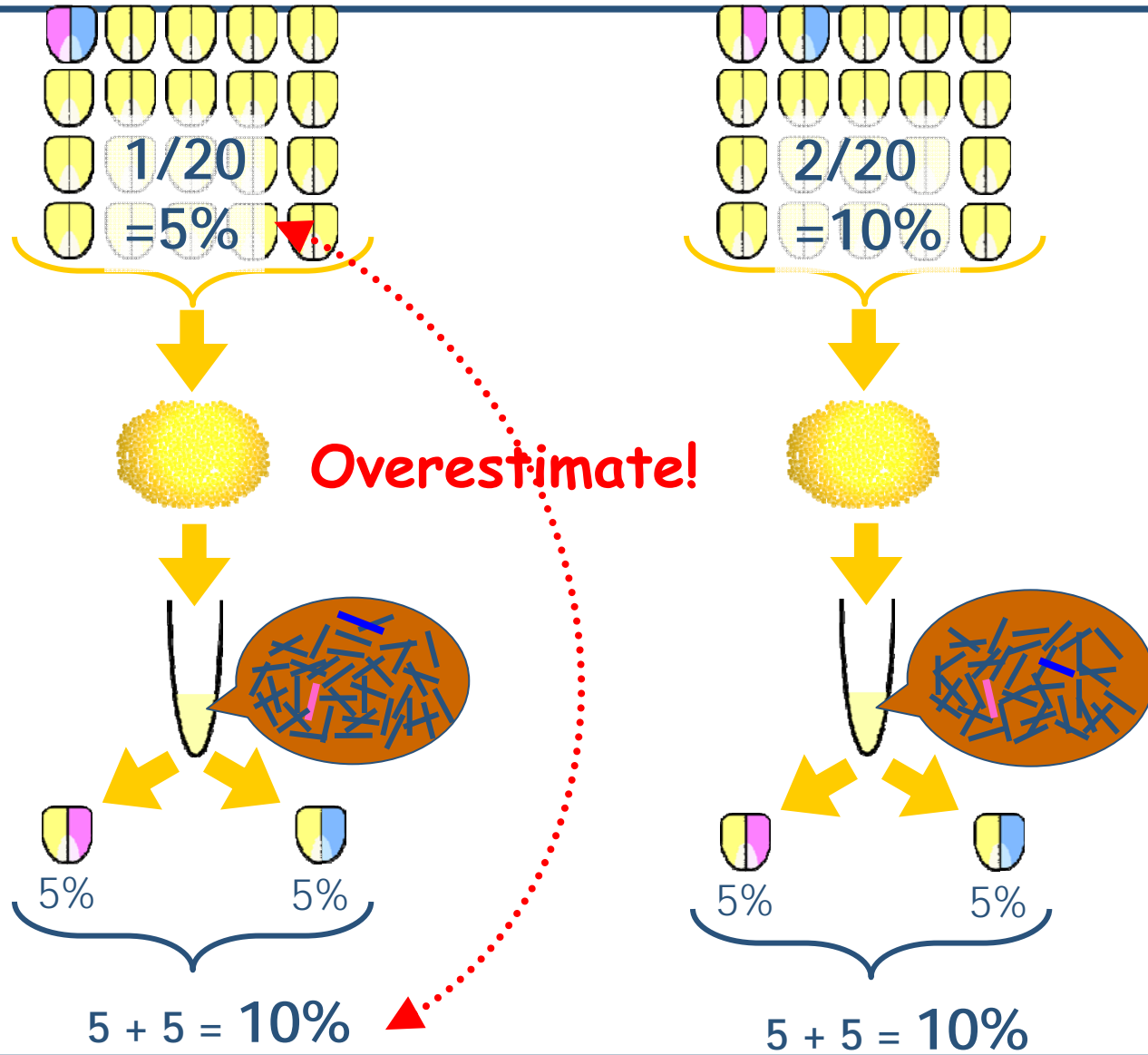
Bulk samples

Homogenization

DNA Extraction

PCR tests

Adventitious presence of stacked events



Bulk samples

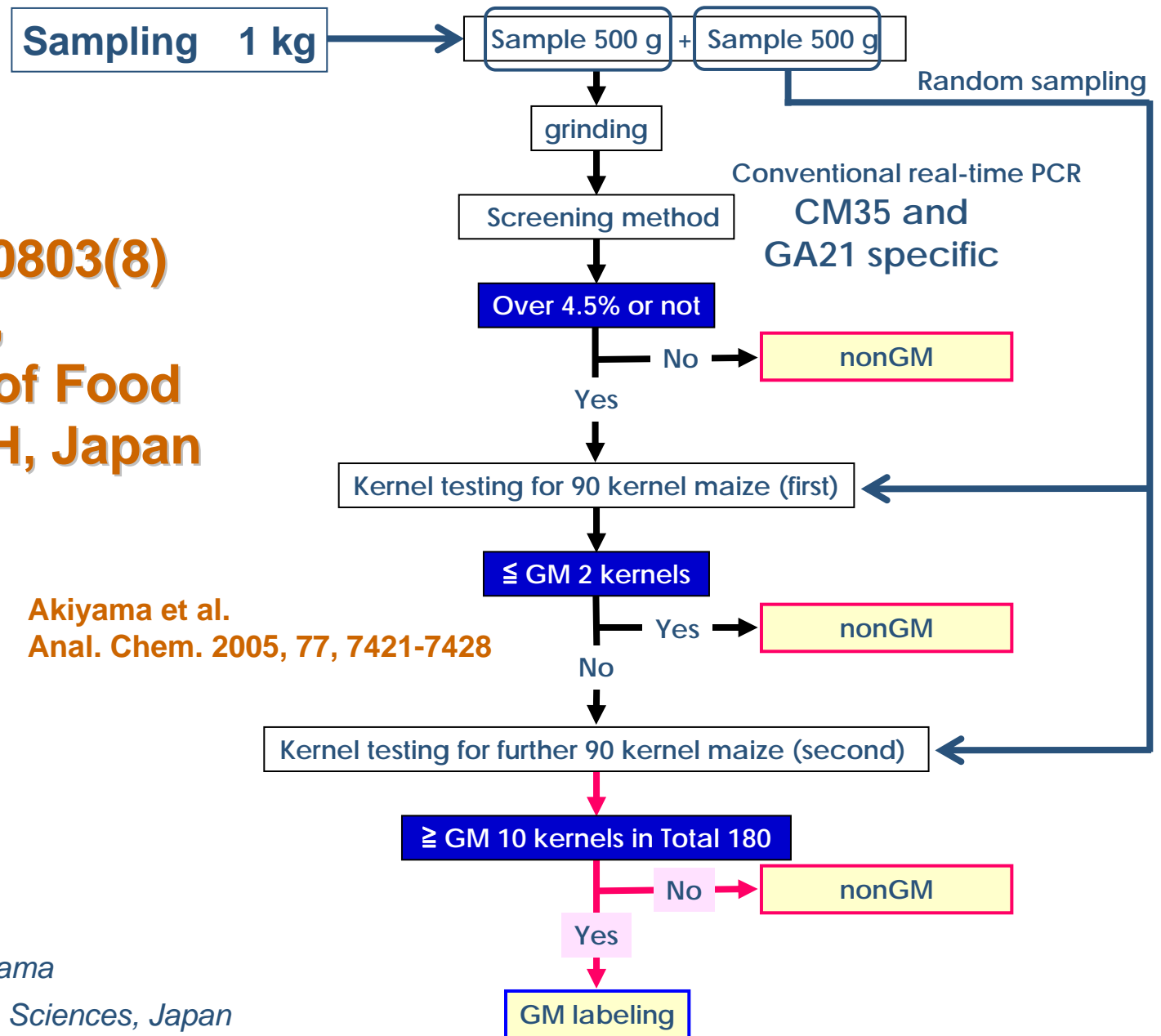
~~Homogenization~~

DNA Extraction

PCR tests

*Adapted from Hiroshi Akiyama
NIHSS, Japan*

**Notification 0803(8)
Aug. 3, 2009,
Department of Food
Safety, MHLH, Japan**



Akiyama et al.
Anal. Chem. 2005, 77, 7421-7428

Adapted from Hiroshi Akiyama
National Institute of Health Sciences, Japan

Thank you for your attention!

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