

Detection of Cauliflower mosaic virus

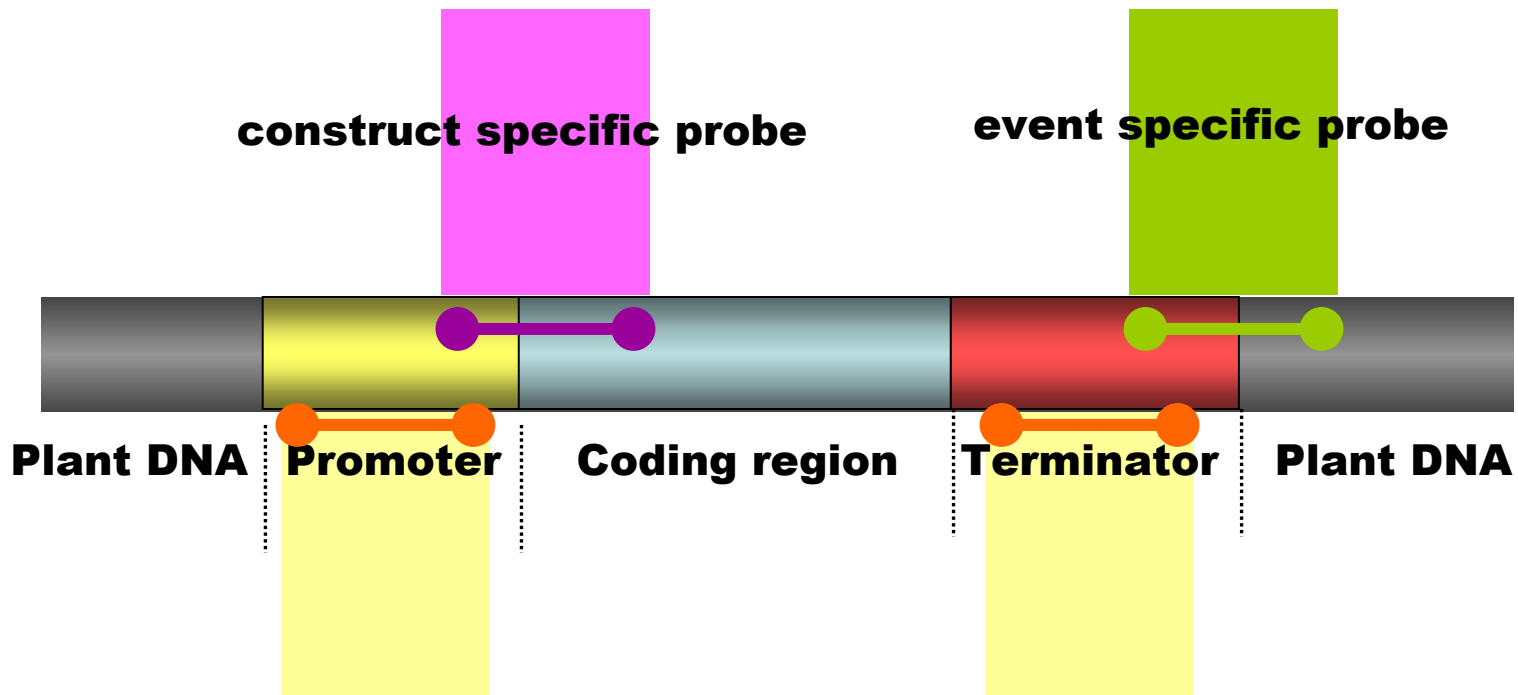
Identification of false positive results
of 35S promoter screening



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2. GMO identification and quantification



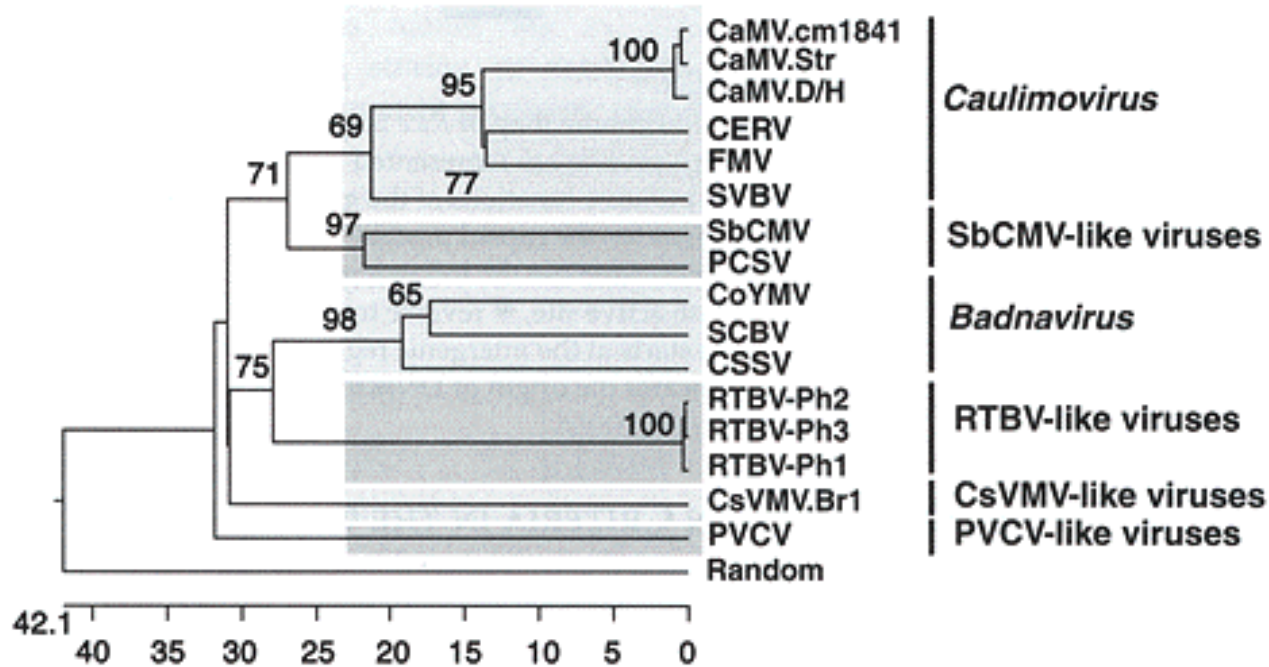
1. GMO Screening

35S screening

	Transformed with 35S	Other promoter or unknown	Total of approved lines worldwide
Maize	17	1 (GA21)	18
Rapeseed	5	9	14
Cotton	5	2	7
Soybean	6	0	6
Tomato	5	1	6
Potato	2	2	4
Sugar beet	2	0	2
Rice	1	0	1
Papaya	1	0	1

<http://www.agbios.com>

CaMV virus

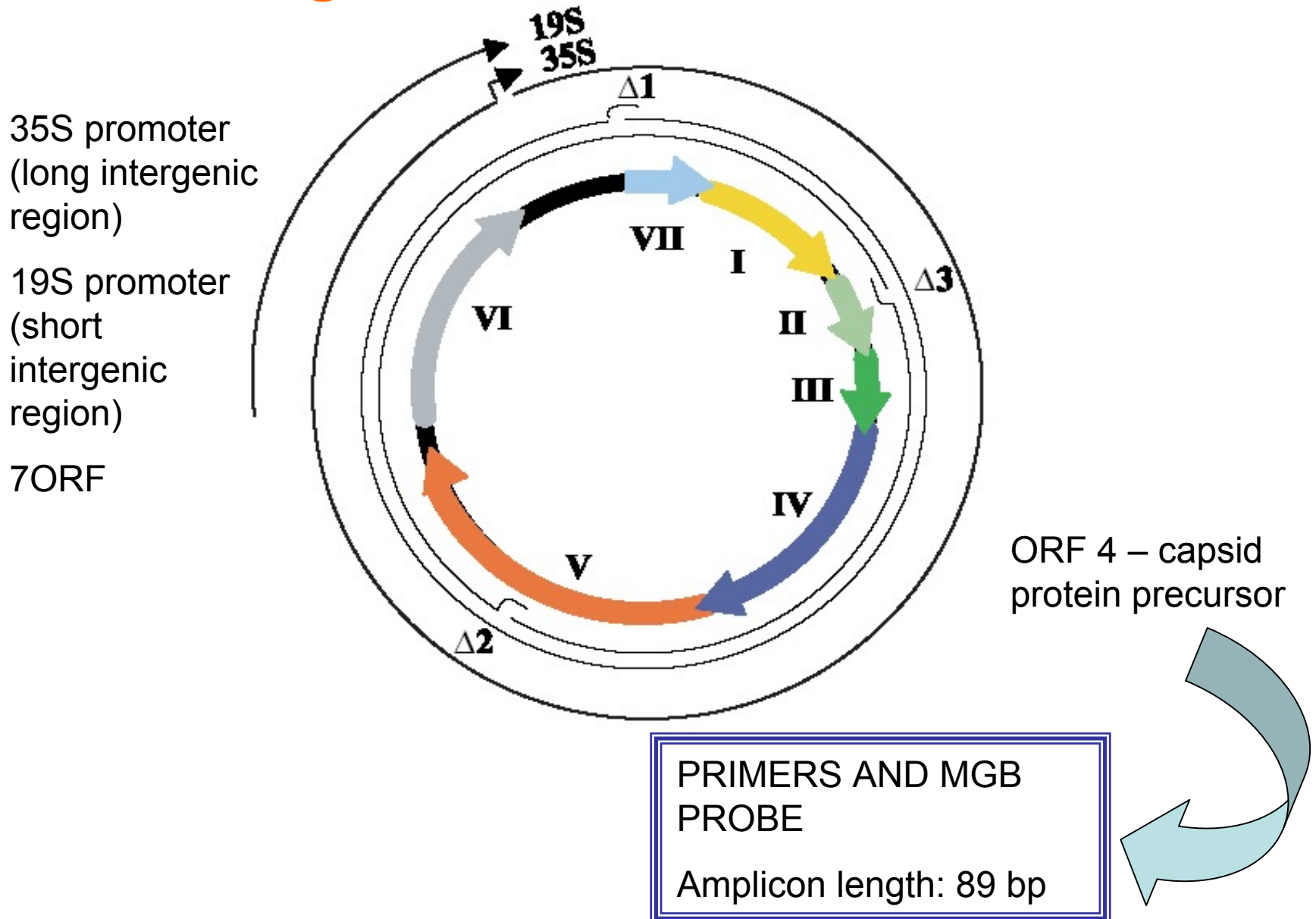


-family *Caulimoviridae*

-Closely related viruses: FMV, CERV, SbCMV

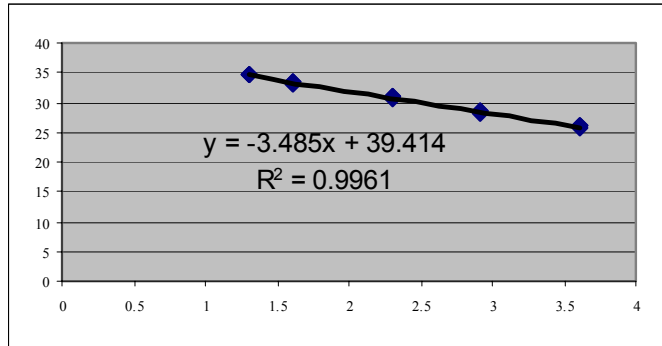
-Host range: *Brassicaceae*, *Resedaceae*,
Solanaceae

CaMV genome



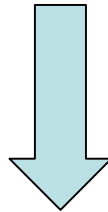
Quantification of the virus, LOD, LOQ

35S standard curve



DNA isolated from virus infected plant tissue

RoundUp Ready soybean certified reference material



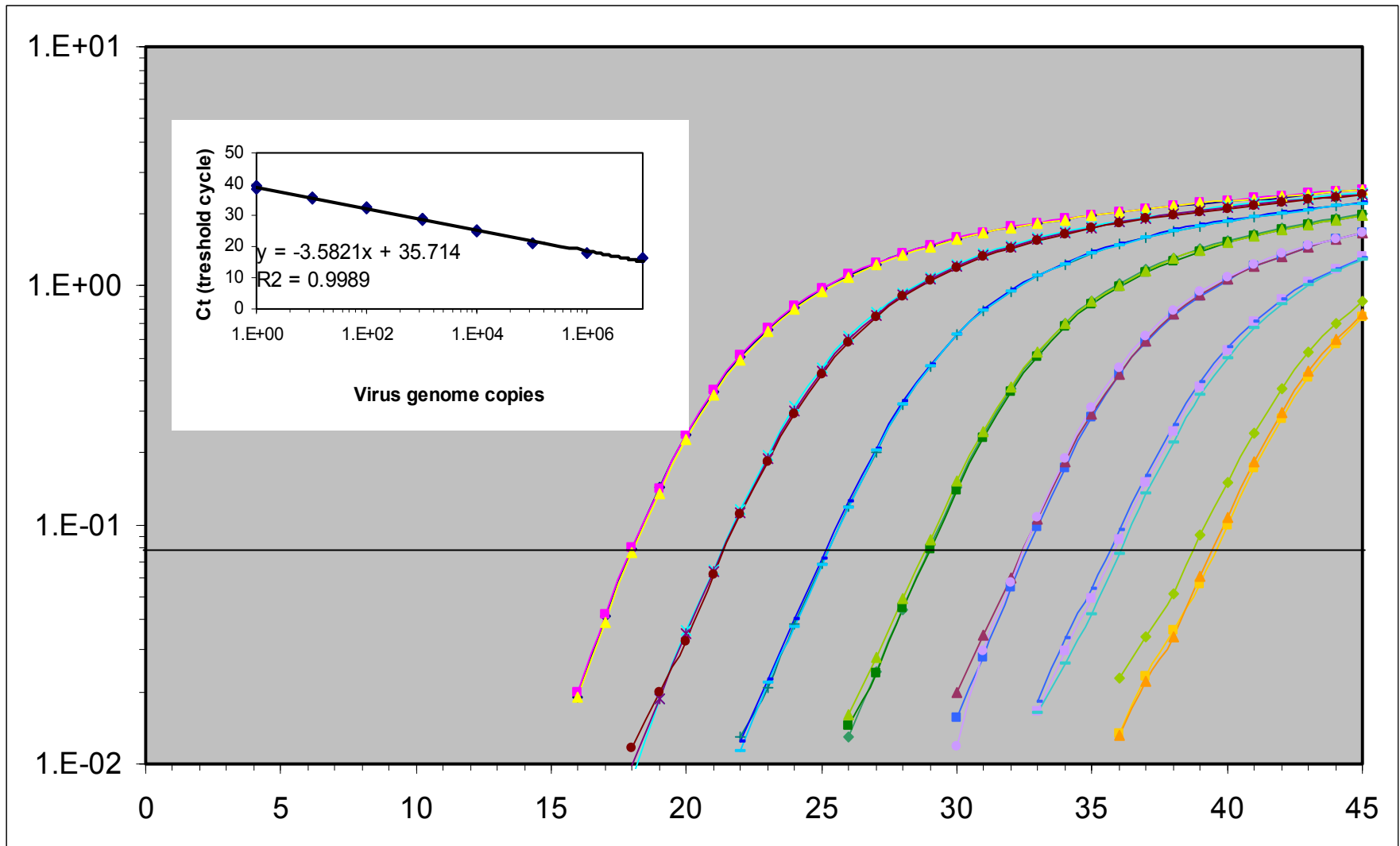
Known virus concentration



LOD = 1-5 copies

LOQ = 10 -15 copies

Range of linearity



Real-time PCR efficiency

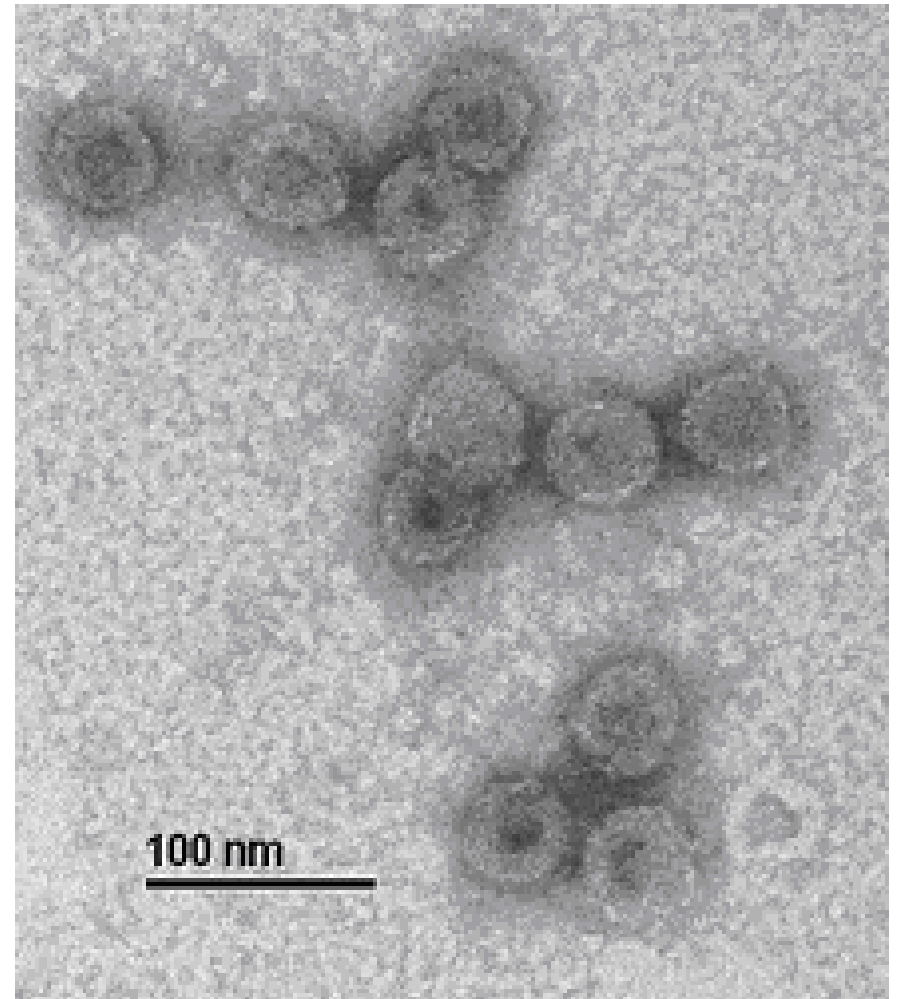
Run	Slope	Efficiency (%)
1	3.5366	91,7
2	3.3686	98,1
3	3.2168	104,5
4	3.5122	92,6
5	3.5821	90,2
6	3.8371	82,2

Average PCR
efficiency:
93%

Assay specificity

VIRUS	Strain	Source	CaMV assay
CaMV	W260	University of Missouri, Columbia, USA	+
	D4	University of Missouri, Columbia, USA	+
	Cm1841	University of Missouri, Columbia, USA	+
	CabbageB	University of Missouri, Columbia, USA	+
	Unknown	Central Science Laboratory, York, UK	+
	Unknown	Plant Protection Service, Wageningen, Netherlands	+
	Unknown	Horticulture Research International, Warwick, UK	+
	Unknown	Academy of Sciences of the Czech Republic	+
CERV	Unknown	Federal Biological Research Centre for Agriculture and Forestry Braunschweig, Germany	-
	Unknown	Federal Biological Research Centre for Agriculture and Forestry Braunschweig, Germany	-
	Unknown	Federal Biological Research Centre for Agriculture and Forestry Braunschweig, Germany	-

Confirmation of virus by electron microscopy



Cross-reactivity with GMOs

			CaMV Real-time	Amplifiability control	
	Source	Conc.	rezultat	sistem	rezultat
GM maize T25	Aventis	100%	-	invertaza	+
GM maize Mon 810	IRMM	5%	-	invertaza	+
GM maize Bt 176	IRMM	5%	-	invertaza	+
GM maize Bt11	IRMM	2%	-	invertaza	+
GM maize NK603	Fluka	1%	-	invertaza	+
GM maize CHB351	Fluka	1%	-	invertaza	+
GM maize GA21	Fluka	1%	-	invertaza	+
GM RoundUp Ready soybean	IRMM	5%	-	lektin	+

Cross-reactivity with plant DNA

	CaMV Real-time	Amplifiability control	
<i>Nicotiana tabacum</i> (White Barley)	-	actin	+
<i>Solanum melangena</i> (Black beauty)	-	actin	+
<i>Lycopersicon esculentum</i> (Money maker)	-	actin	+
<i>N. clevelandii</i>	-	actin	+
<i>N. benthamiana</i>	-	actin	+
<i>Capsicum anuum</i>	-	actin	+
<i>B. oleracea botrytis</i>	-	actin	+
<i>B. napus</i> spp <i>rapifera</i>	-	actin	+
<i>Brassica oleracea capitata</i> (Brunswick)	-	actin	+
non-GM rapeseed	-	actin	+
<i>Brasica campestris</i> Just Right	-	actin	+
non-GM maize	-	invertase	+
non-GM soybean	-	lectin	+
<i>Triticum aestivum</i> (Siossons)	-	actin	+
<i>Dianthus caryophyllus</i>	-	actin	+
<i>Dianthus barbatus</i>	-	actin	+

DNA spiking

Successful amplification of viruses at as low as 10 copies

Reduced efficiency

		Slope	Efficiency
Maize	flour	-3.56	91%
	grain	-3.69	86%
	animal feed	-3.47	94%
	tortilla chips	-3.70	86%
Soybean	flour	-3.48	94%
	grain	-3.79	83%
	animal feed	-3.79	83%
	soybean drink	-3.65	88%

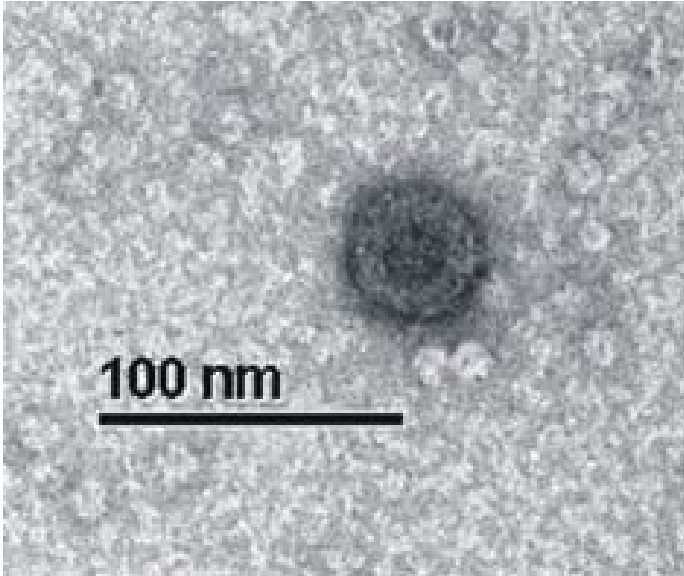
Applicability of the assay in routine screening

- positive rapeseed samples
- Other reports: soybean (coarse meal), broccoli, frozen vegetable flakes, frozen vegetable burger (Wolf et al., 2000)

RECCOMENDED FOR

- GM plants from families of *Brassicaceae* and *Solanaceae*
- Mixed plant samples
- Samples that may be infected during transport or storage





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- Academy of Sciences of the Czech Republic
- Federal Biological Research Centre for Agriculture and Forestry Braunschweig, Germany

Thank you