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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Fifteenth Session

Moscow, Russian Federation, May 24 to 27, 2016

FAST SINGLE-STEP DETECTION AND IDENTIFICATION OF MILTIPLE PHYTOPATHOGENS AND GMO WITH REAL-TIME PCR-MATRIX TECHNIQUE

Document prepared by an expert from Russian Federation

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation “Fast Single-step Detection and Identification of Multiple Phytopathogens and GMO with real-time PCR-matrix Technique” to be made at its fifteenth session of the Working Group on Biochemical and Molecular Techniques and DNS-Profiling in particular (BMT).

Alexander Golikov, Science Director, GenBit LLC, Russian Federation

[Annex follows]

FAST SINGLE-STEP DETECTION AND IDENTIFICATION OF MILTIPLE PHYTOPATHOGENS AND GMO WITH REAL-TIME PCR-MATRIX TECHNIQUE

Alexander Golikov

for

UPOV TECHNICAL WORKING PARTY FOR BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND DNA-PROFILING IN PARTICULAR (BMT)

Fifteenth Session, Moscow, Russia, May from 24 to 27, 2016

(with Preparatory Workshop on May 23, 2016)

FAST SINGLE-STEP DETECTION AND IDENTIFICATION OF MILTIPLE PHYTOPATHOGENS AND GMO WITH REAL-TIME PCR-MATRIX TECHNIQUE

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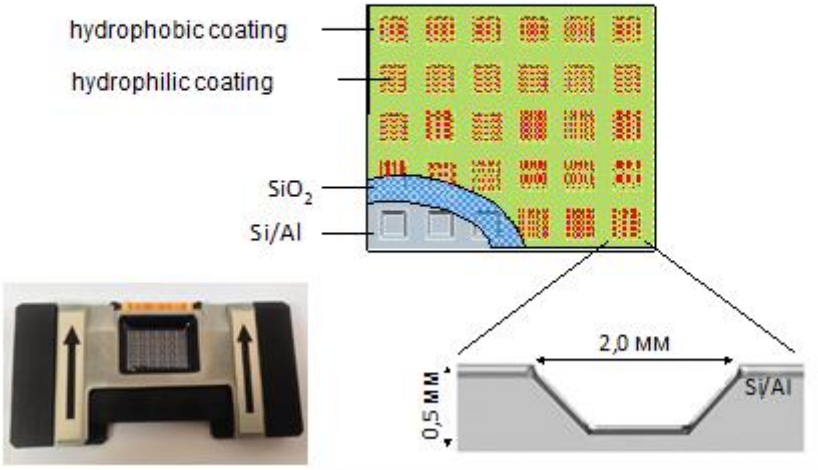
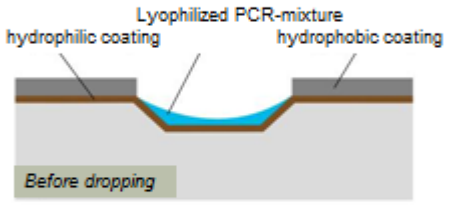
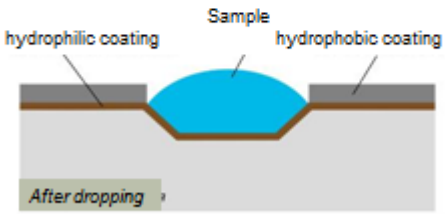
PCR-matrix



PCR-matrix

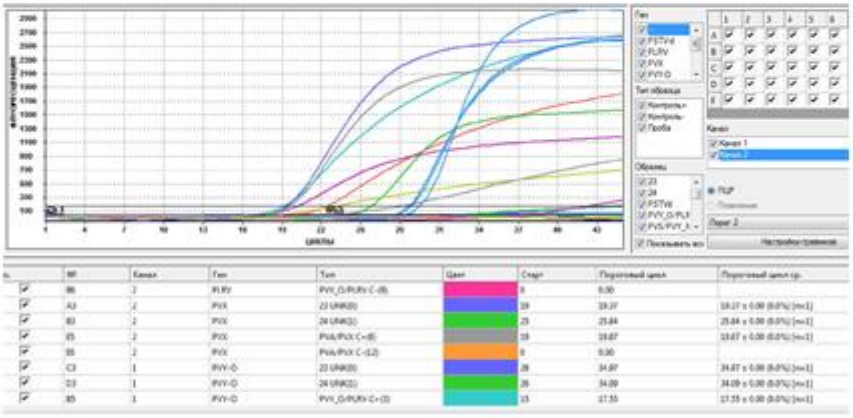


Guaranteed life span of preserved (lyophilized) PRC matrices:
6 months – DNA
3 months - RNA



Interpretation of results

Results are analyzed with the authentic "AriaDNA" software



Proposed *vs.* Conventional

Proposed	Conventional
• Target: crop • Multiple objects in a single test • Time required: ~2 hrs (including DNA/RNA isolation) • Could be used "anywhere" • No need in highly trained personnel • "Pre-serves" for a chosen range of pathogens that could be kept for ~6 months under room conditions	• Target: object/pathogen • Separate test for a single pathogen • Time required: > 1 day • Stationary conditions • Does require highly trained personnel • Reaction mixture for each object/pathogen

S.W.O.T.

S - Strength	W - Weakness
• User friendliness • Multiple objects in a single test • Speed • High sensitivity and accuracy • Mobility • End-users can easily develop their own applications	• High qualification required for development of the test systems • Not approved yet by international standards
O - Opportunities	**T - Threats**
• Use for screening and monitoring "anywhere" • Use for seed quality assessment and for IPR protection • End-users can easily develop their own applications	• Possible unpreparedness of the society to instantly accept new, differing significantly from traditional approaches


РЕГИСТРАЦИОННОЕ УДОСТОВЕРЕНИЕ
НА МЕДИЦИНСКОЕ ИЗДЕЛИЕ

+

ISO 9001:2008
№ Q-04.04.18f
from 12.02.2013

Potato - available matrices

DNA	RNA	Soil
• *Clavibacter michiganensis subsp. sepedonicus*	• PLRV	• *Globodera rostochiensis*
• *Pectobacterium atrosepticum*	• PVY-o+c	• *Globodera pallida*
• *Dickeya dianthicola*	• PVY-ntv	• Phytoplasm:
• *Erwinia carotovora subsp. atroseptica*	• PVX	▪ Aster yellows (16 Sr I)
• *Ralstonia solanacearum*	• PVA	▪ X-disease (16 Sr III)
• *Phytophthora infestans*	• PVM	▪ Clover proliferation (16 Sr VI)
	• PVS	
	• PMTV	
	• PSTVd	

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Potato – sampling

Viruses

Bacteria

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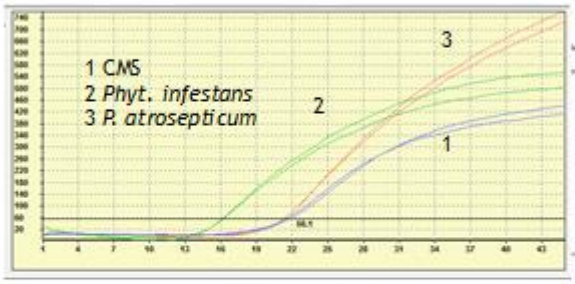
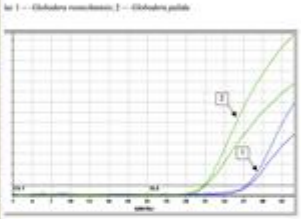
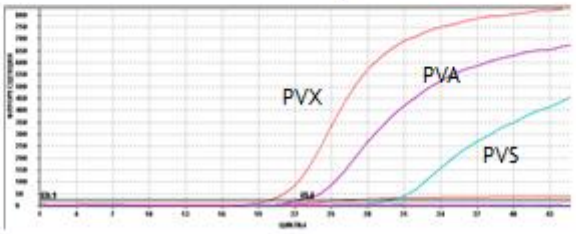
Potato – PCR-matrices







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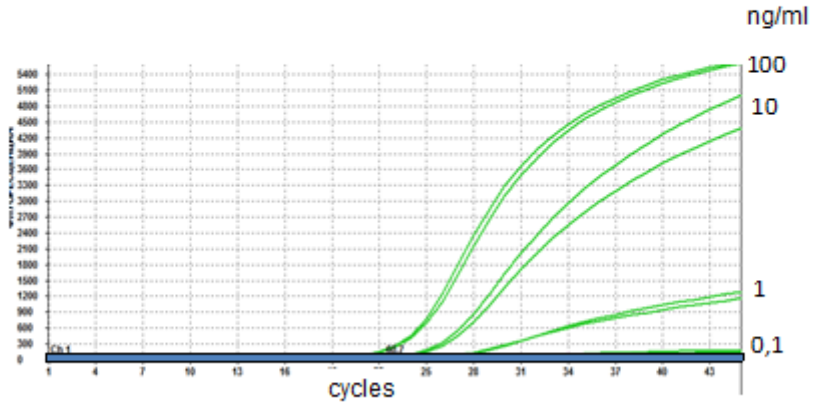
Potato - PCR-matrices

Analytical sensitivity



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Potato – PCR-matrices, field tests

Together with the Russian Agricultural Center, Leningrad region, Shushary, 2014

Samples: foliage and stems, 10 potato varieties

VARIETY	DESCRIPTION	ELISA	GenBit - (rt) qPCR
Nevskiy	Suspected: *Dickeya*	???	*Erwinia carotovora subsp. atroseptica*
Nevskiy	Suspected: Y-virus	-	PVY (o)
Avrora	Suspected: Y-virus	-	PVY (o+n)
Impala	Striated veins	PVM	PVM + PVY (o)
Asterisk	Spotted leaves	-	PVY (o+n)
Lausnak	Suspected: X-virus	PVX	PVX + PVY (o)

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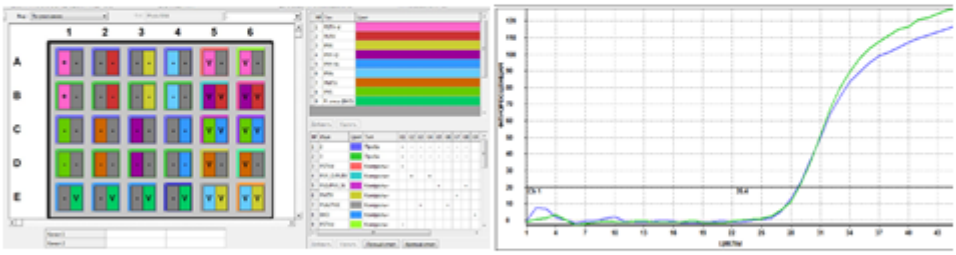
Potato – PCR-matrices, PSTV-d

Two samples by 150 mg each were taken from eyes (slices) of two mini-tuber suspected of being infected with the viroid



template

presence of PSTV-d in both samples (~0.5 ng/ml)

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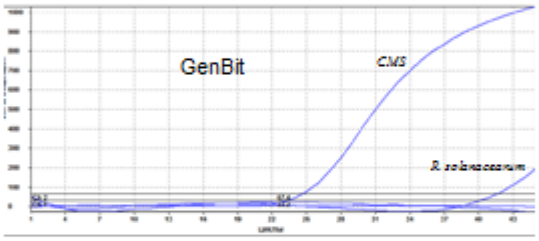
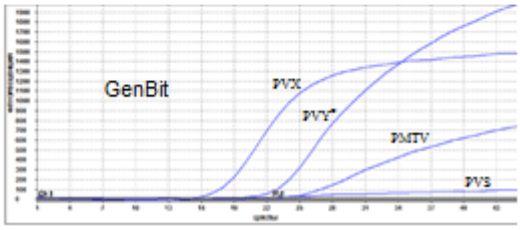
Potato – PCR-matrices, multiple pathogens

17.02.2015 Federal Enterprise "The All-Russian Center for Plant Quarantine" (VNIIKR), "blind" samples, mix of DNA/RNA of bacterial and viral plant pathogens

VNIIKR:
Clavibacter michiganesis subsp. *Sepedonicus*
+
Ralstonia solanacearum





VNIIKR:
PMTV+PVS+PVY+PVX

GMO

EU Database of Reference Methods for GMO Analysis

JRC EU: Compendium of reference methods for GMO analysis

The CropLife International Detection Methods Database

GM-soybean, element specific detection

GM-soybean lines approved for FFP in the Russian Federation

LINE	CaMV P-35S	P-FMV	T-*nos*	*npt* II	*bar*
A 2704-12	+	-	-	-	-
A 5547-127	+	-	-	-	-
BPS-CV127-9	-	-	-	-	-
GTS-40-3-2	+	-	+	-	-
MON87701	-	-	-	-	-
MON89788	-	+	-	-	-
SYHTOH2	-	-	-	-	-
FG72	-	-	-	-	-

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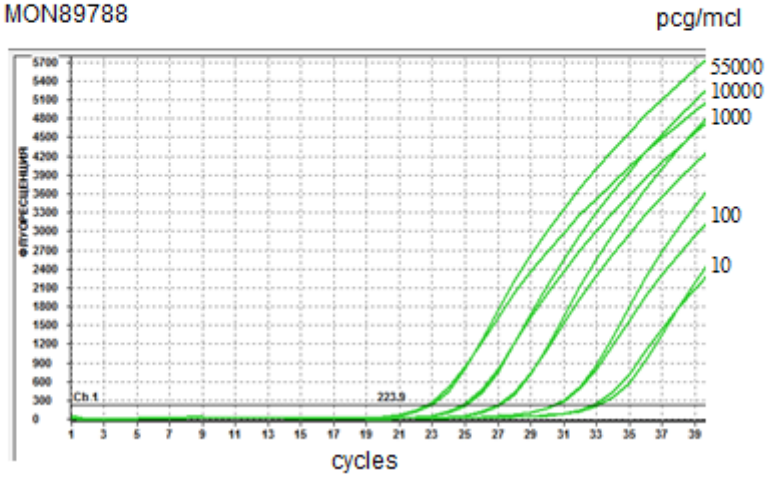
GM-soybean, PCR-matrices, event specific

Analytical sensitivity

MON89788



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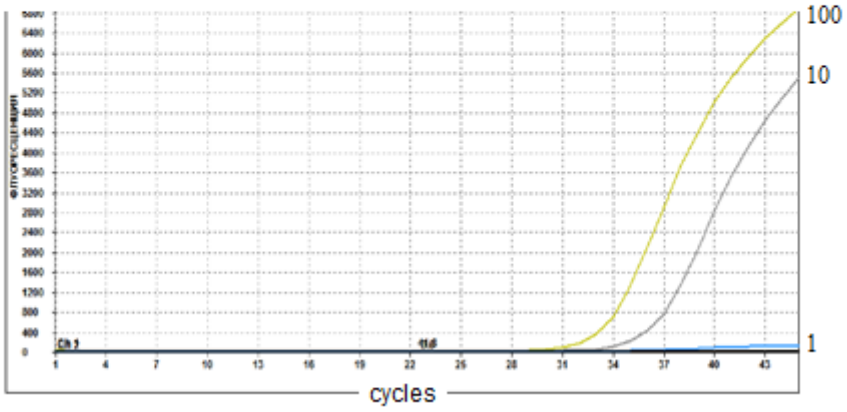
GM-soybean, PCR-matrices, event specific

Analytical sensitivity

SYHT0H2

pcg/mcl



GM-soybean, PCR-matrices, event specific

GM-soybean, PCR-matrices, event specific

17.02.2015 Federal Enterprise "Centre of Molecular Diagnostics (CMD) - The All-Russian State Center for Quality and Standardization of Veterinary Drugs and Feed (VGNKI)", three "blind" samples, mix of DNA of 7 GM-soybean lines each, one matrix

0.1%

LINE	GenBit
A 2704-12	+
A 5547-127	+
BPS-CV127-9	+
GTS-40-3-2	+
MON87701	+
MON89788	+
SYHTOH2	+

0.5%

LINE	GenBit
A 2704-12	+
A 5547-127	+
BPS-CV127-9	+
GTS-40-3-2	+
MON87701	+
MON89788	+
SYHTOH2	+

1.0%

LINE	GenBit
A 2704-12	+
A 5547-127	+
BPS-CV127-9	+
GTS-40-3-2	+
MON87701	+
MON89788	+
SYHTOH2	+

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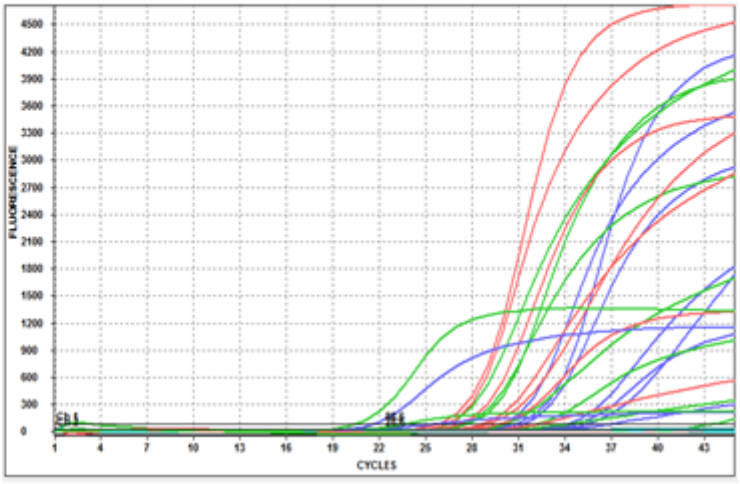
GM-soybean, PCR-matrices, event specific

17.02.2015 CMD-VGNKI: A2704-12+A5547-127+BPS-CV127-9+GTS-40-3-2+MON87701+MON89788+SYHTOH2

0,1%

0,5%

1,0%



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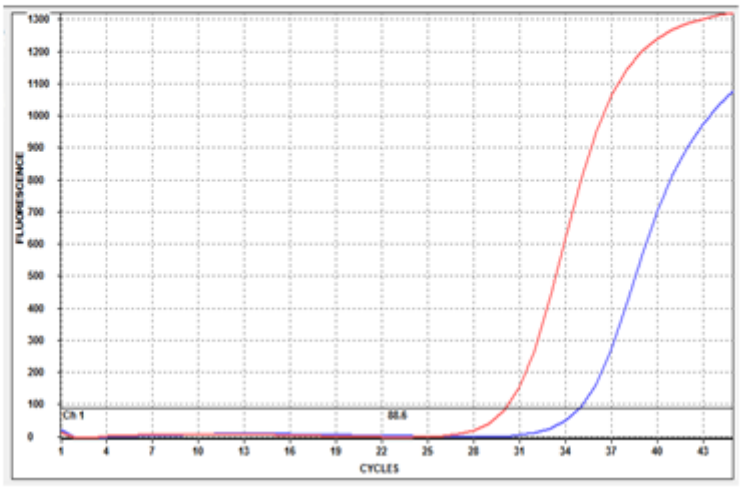
GM-soybean, PCR-matrices, event specific

17.02.2015 CMD-VGNKI: A2704-12 - Ct 30,12 (1%) Ct 34,99 (0,1%)

0,1%

1,0%

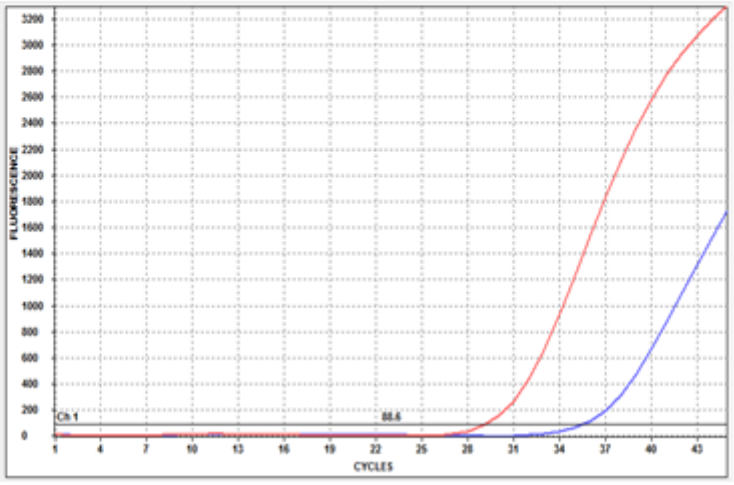


GM-soybean, PCR-matrices, event specific

17.02.2015 CMD-VGNKI: A5547-127 - Ct 29,24 (1%) Ct 30,57 (0,1%)

0,1%

1,0%

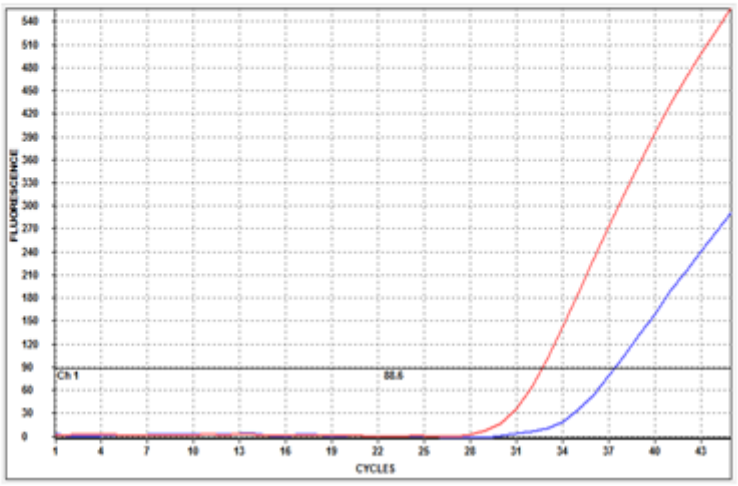


GM-soybean, PCR-matrices, event specific

17.02.2015 CMD-VGNKI: BPS-CV-127 Ct 32,71 (1%) Ct 37,43 (0,1%)

0,1%

1,0%

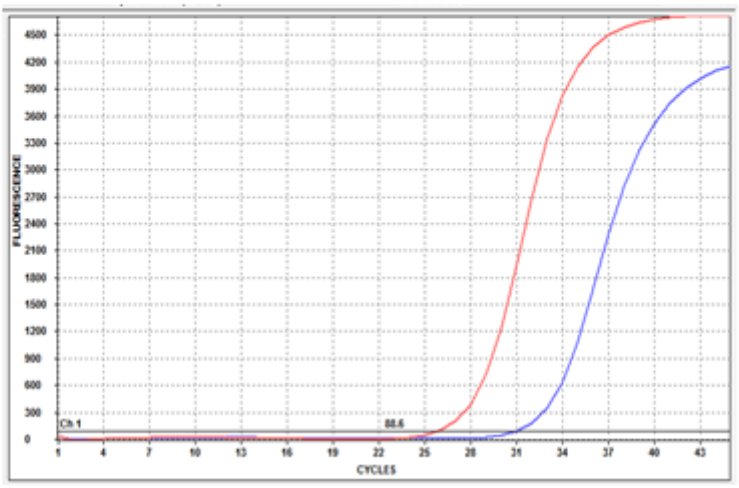


GM-soybean, PCR-matrices, event specific

17.02.2015 CMD-VGNKI: GTS 40-3-2 Ct 26,01 (1%) Ct 31,08 (0,1%)

0,1%

1,0%

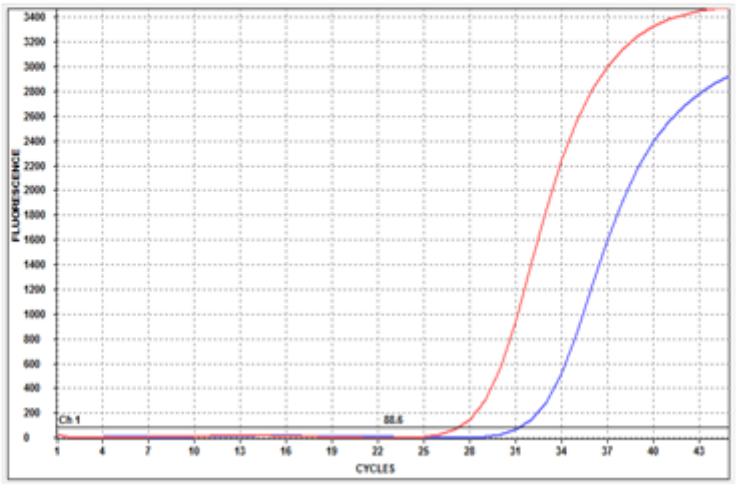


GM-soybean, PCR-matrices, event specific

17.02.2015 CMD-VGNKI: MON87701 Ct 27,36 (1%) Ct 31,41 (0,1%)

0,1%

1,0%



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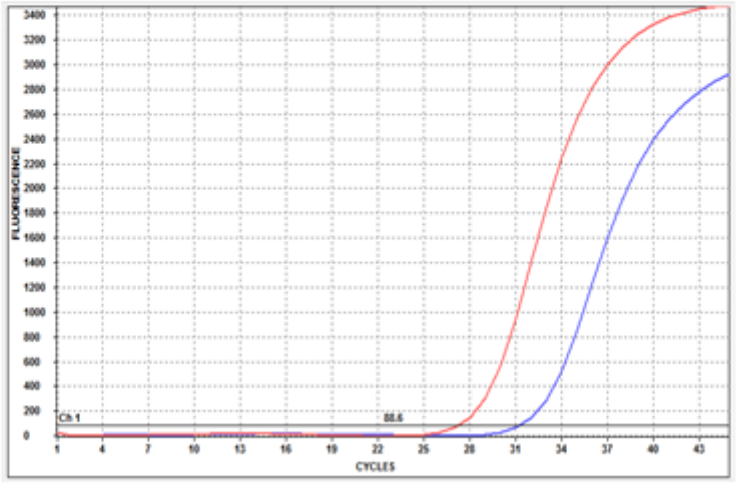
GM-soybean, PCR-matrices, event specific

17.02.2015 CMD-VGNKI: MON89788 Ct 28,95 (1%) Ct 34,39 (0,1%)

0,1%

1,0%



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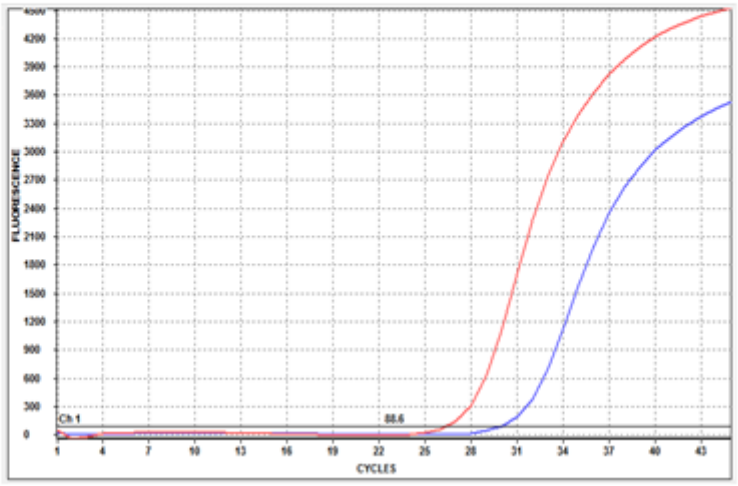
GM-soybean, PCR-matrices, event specific

17.02.2015 CMD-VGNKI: SYHTOH2 Ct 26,50 (1%) Ct 30,10 (0,1%)

0,1%

1,0%



GM-soybean, PCR-matrices, event specific (pattern)

17.02.2015 CMD-VGNKI: A2704-12+A5547-127+BPS-CV127-9+GTS-40-3-2+MON87701+MON89788+SYHTOH2

GM-soybean, PCR-matrices, event specific

19.02.2015 Federal Enterprise "Institute of Nutrition", three "blind" samples, DNA mix, one matrix:
MON89788+SYHT0H2+A2704 (№2)
BPS CV-127+SYHT0H2 (№4)
BPS CV-127+SYHT0H2+GTS 40-3-2 (№6)

№2

LINE	GenBit
A 2704-12	+
A 5547-127	-
BPS-CV127-9	-
GTS-40-3-2	-
MON87701	-
MON89788	+
SYHTOH2	+

№4

LINE	GenBit
A 2704-12	-
A 5547-127	-
BPS-CV127-9	+
GTS-40-3-2	-
MON87701	-
MON89788	-
SYHTOH2	+

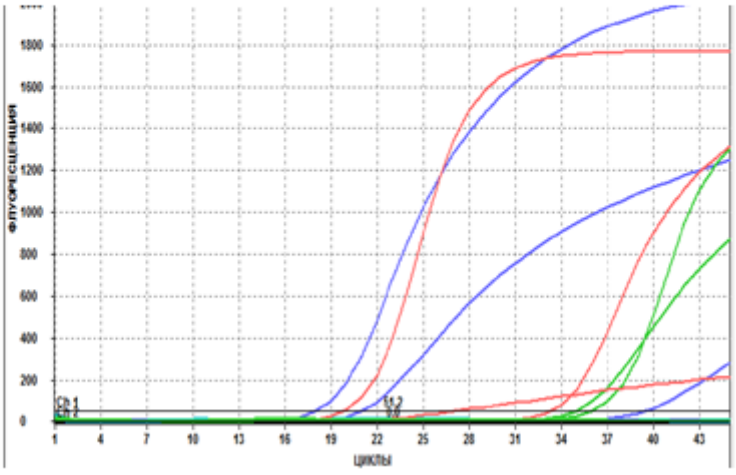
№6

LINE	GenBit
A 2704-12	-
A 5547-127	-
BPS-CV127-9	+
GTS-40-3-2	+
MON87701	-
MON89788	-
SYHTOH2	+

GM-soybean, PCR-matrices, event specific

19.02.2015 Federal Enterprise "Institute of Nutrition", three "blind" samples, DNA mix, one matrix:

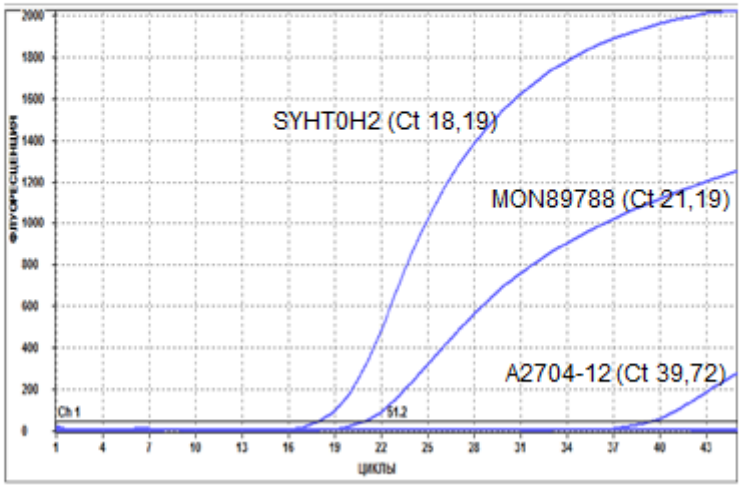
№2
№4
№6



GM-soybean, PCR-matrices, event specific

19.02.2015 Federal Enterprise "Institute of Nutrition", three "blind" samples, DNA mix, one matrix:

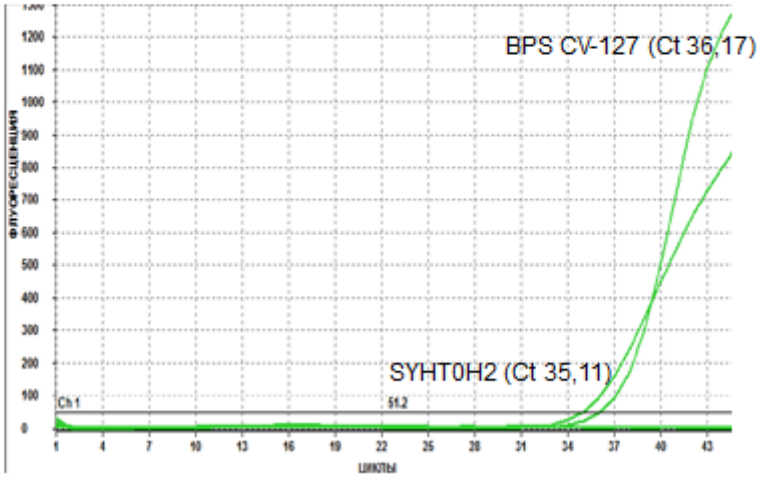
№2



GM-soybean, microchips, event specific

19.02.2015 Federal Enterprise "Institute of Nutrition", three "blind" samples, DNA mix, one matrix:

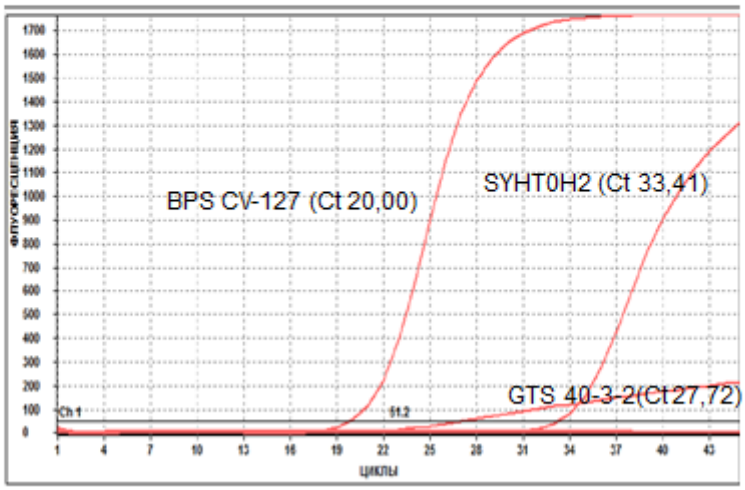
№4



GM-soybean, microchips, event specific

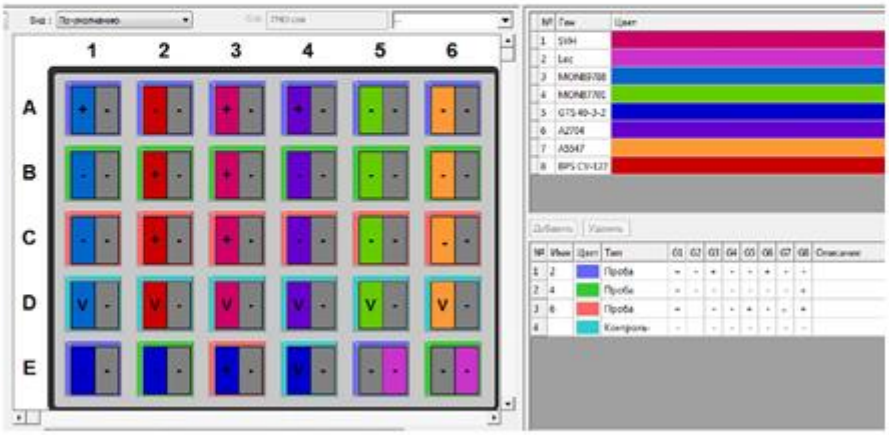
19.02.2015 Federal Enterprise "Institute of Nutrition", three "blind" samples, DNA mix, one matrix:

№6



GM-soybean, microchips, event specific (pattern)

19.02.2015 Federal Enterprise "Institute of Nutrition", three "blind" samples, DNA mix, one matrix:



PCR-matrices, practice

We do exist

[End of Annex and of document]