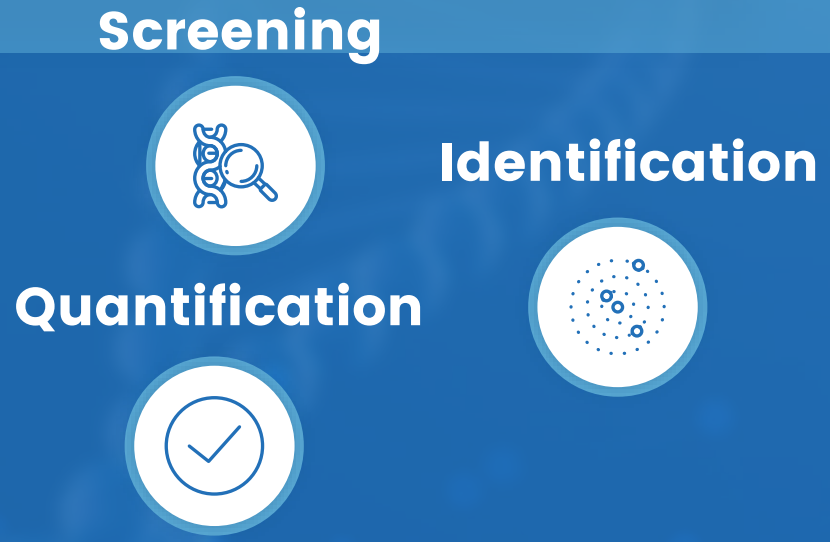
STILLA

Crystal Digital PCR™ : a fast and high-plex solution for GMO quantification

Romain Parillaud, PhD, Application Specialist

Overview of the presentation

- Principles of Digital PCR
- GMOs detection & quantification with 3-color
- Highly Multiplexed assay for GMO Detection
- Quantification in Complex Matrix with Crystal Digital PCR™
- Going further with our next innovation: 6-color



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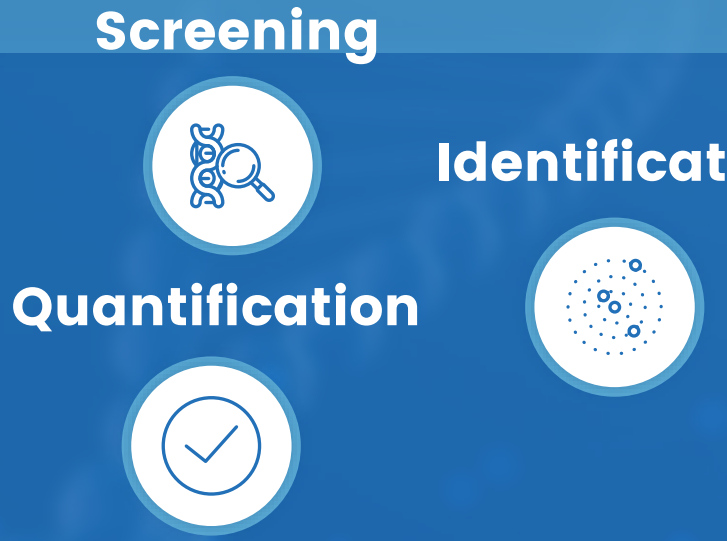
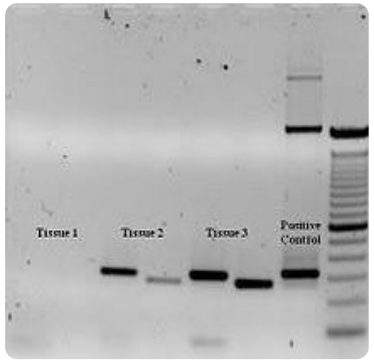


NEXT GENERATION OF PCR

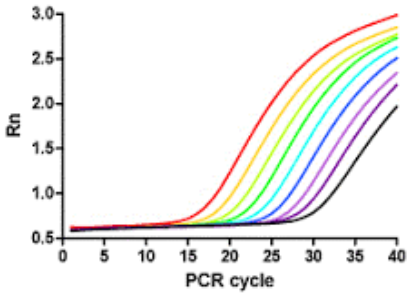
dPCR

PCR



Amplify Target DNA

Quantitative PCR

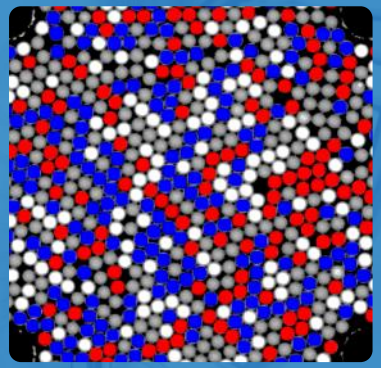


Relative quantification (std curves)

Real-time

Gold Standard

Digital PCR



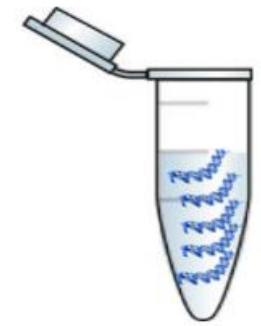
Absolute quantification

Endpoint PCR

Increased sensitivity

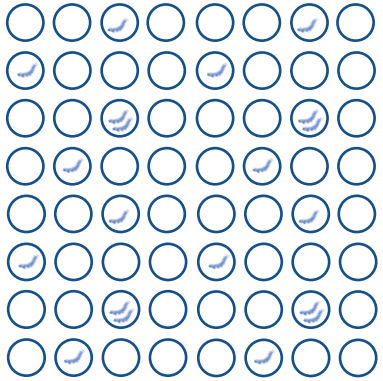
PRINCIPLE
OF DIGITAL PCR

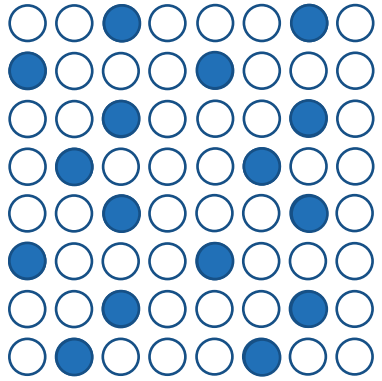
PARTITIONING



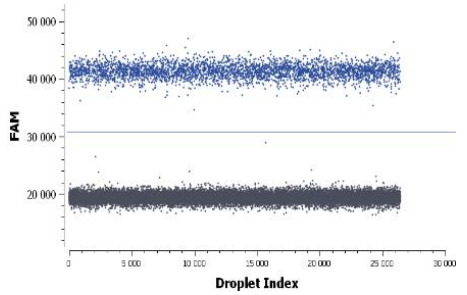
Wild-type DNA

PCR





READING & ANALYSIS



RESULTS
636 cp/µL with **2.2 %** uncertainty

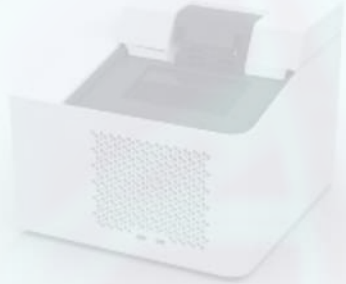
POISSON STATISTICS

$$\frac{N_{pos}}{N_{tot}}$$

Naica™ System Workflow

Crystal Digital PCR™

Sapphire Chip
(consumable)



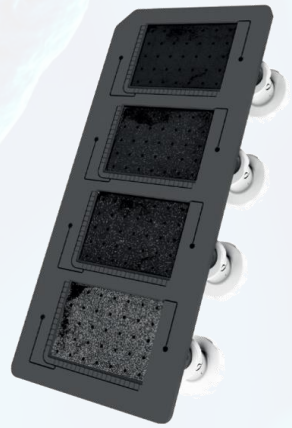
Sapphire Chip : pre-filled with oil

Input volume 25 µL

› **A unique and patented partitioning technology: droplet crystals.**

Droplets per sample ~30 000

Droplet volume 0.59 nL



Droplet crystal:
Self-assembled array of droplets

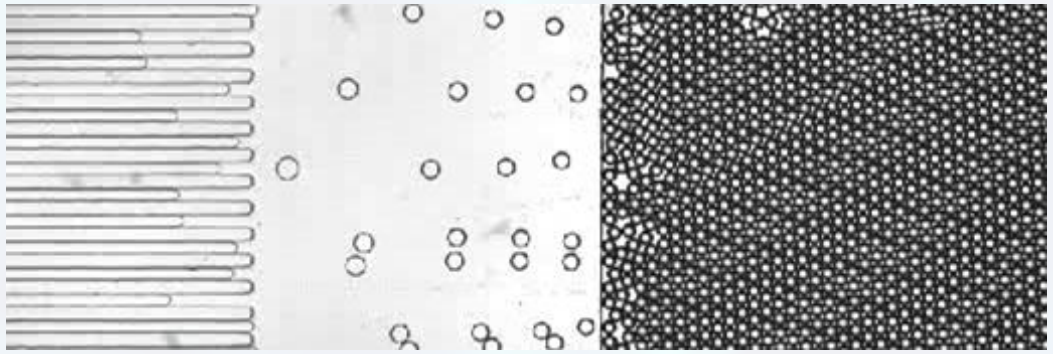
Naica™ System Workflow
Crystal Digital PCR™

Sapphire Chip
(consumable)

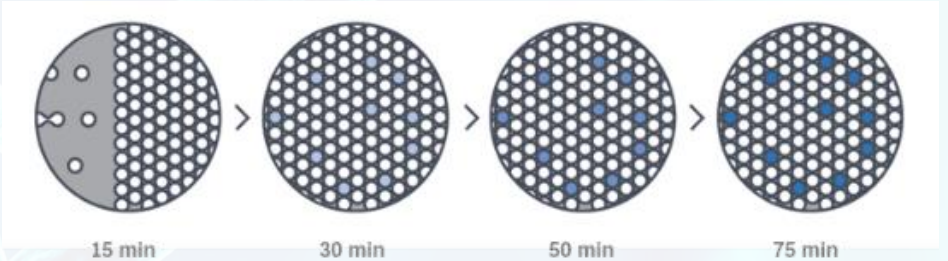
Naica™ Geode

Step 2.1 – Partition

- 1-3 chips and 1-12 samples / run
- Contactless fluid injection



Step 2.2 – Amplify

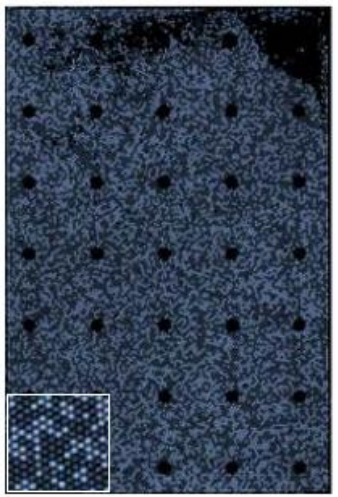


Naica™ System Workflow
Crystal Digital PCR™

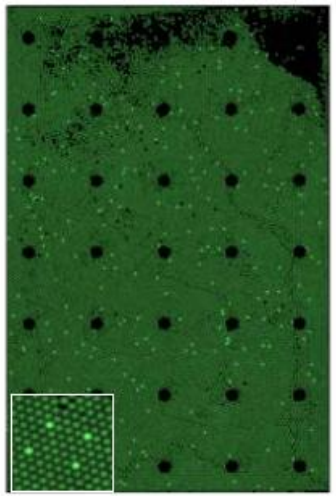
Sapphire Chip
(consumable)

Naica™ Geode

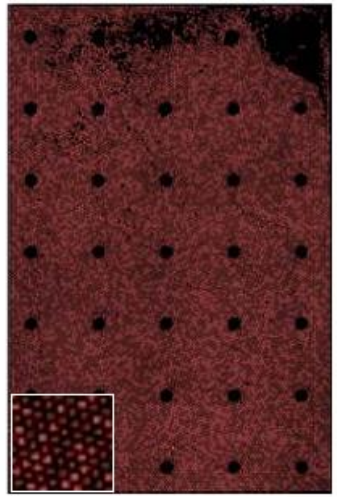
Naica™ Prism3



Blue
Ex: 415-480 nm
Em: 495-520 nm
FAM…



Green
Ex: 530-550 nm
Em: 560-610 nm
HEX…



Red
Ex: 615-645 nm
Em: 655-720 nm
Cy®5…

Naica™ System Workflow

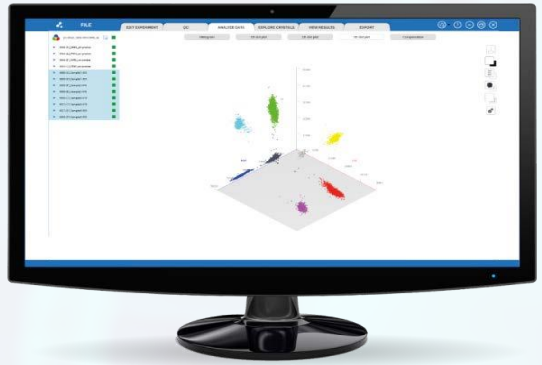
Crystal Digital PCR™

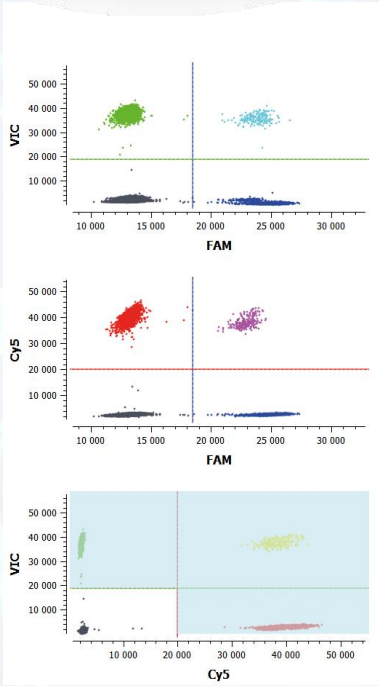
Sapphire Chip (consumable)

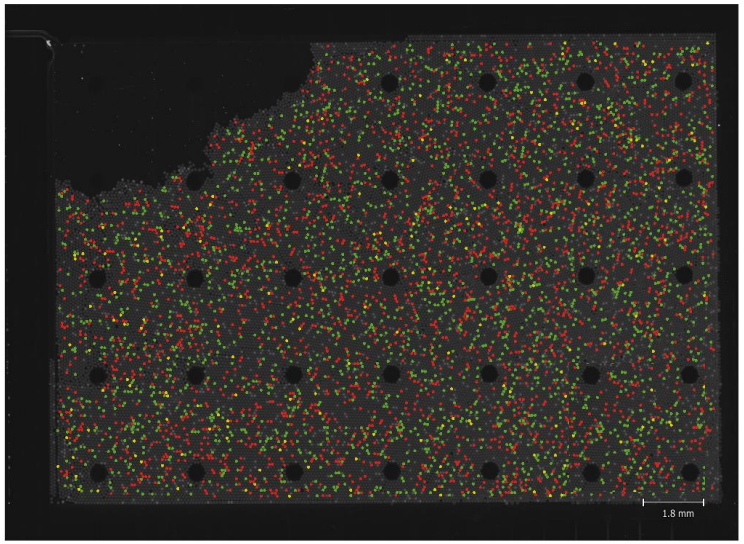
Naica™ Geode

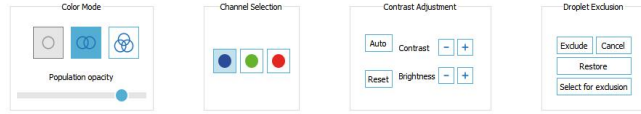
Naica™ Prism3

Crystal Miner™ (software)



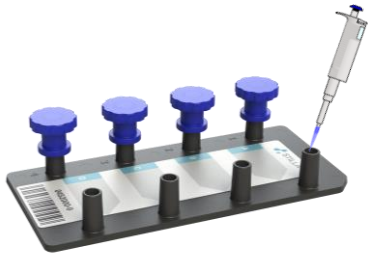


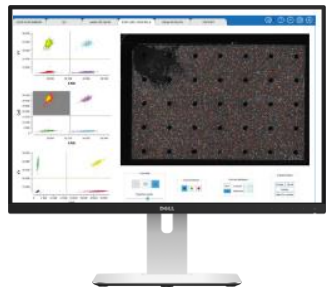




PERFORM CRYSTAL DIGITAL PCR™

IN 2H30 WITH MINIMUM HANDS-ON TIME





DESCRIPTION	Pipette 25 µL of PCR mix into the Sapphire Chips and seal with cap	Place Sapphire chip into the Geode and launch the combined partitioning and thermocycling program	Image Chips using three fluorescent detection channels	Analyze results using our intuitive Crystal Miner software
PROCESS TIME 2H30	**5 min**	**2h10 min**	**10 min**	**5 min**
HANDS-ON TIME 5 min	**5 min**	**15 s**	**15 s**	

Overview of the presentation

- Principles of Digital PCR
- **GMOs detection & quantification with 3-color**
- **Highly Multiplexed assay for GMO Detection**
- **Quantification in Complex Matrix with Crystal Digital PCR™**
- Going further with our next innovation: 6-color

BIOTECON Diagnostics

Highly Multiplexed assay for GMO Detection in Soybean by Crystal Digital PCR™

Motivation

EU Legislation :

⇒ Labelling: set at 0.9% per ingredient

⇒ Tolerate: technical zero limit of 0.1%

Does the sample contain GMO material?

Screening

If yes, which GMO and does it have an approval?

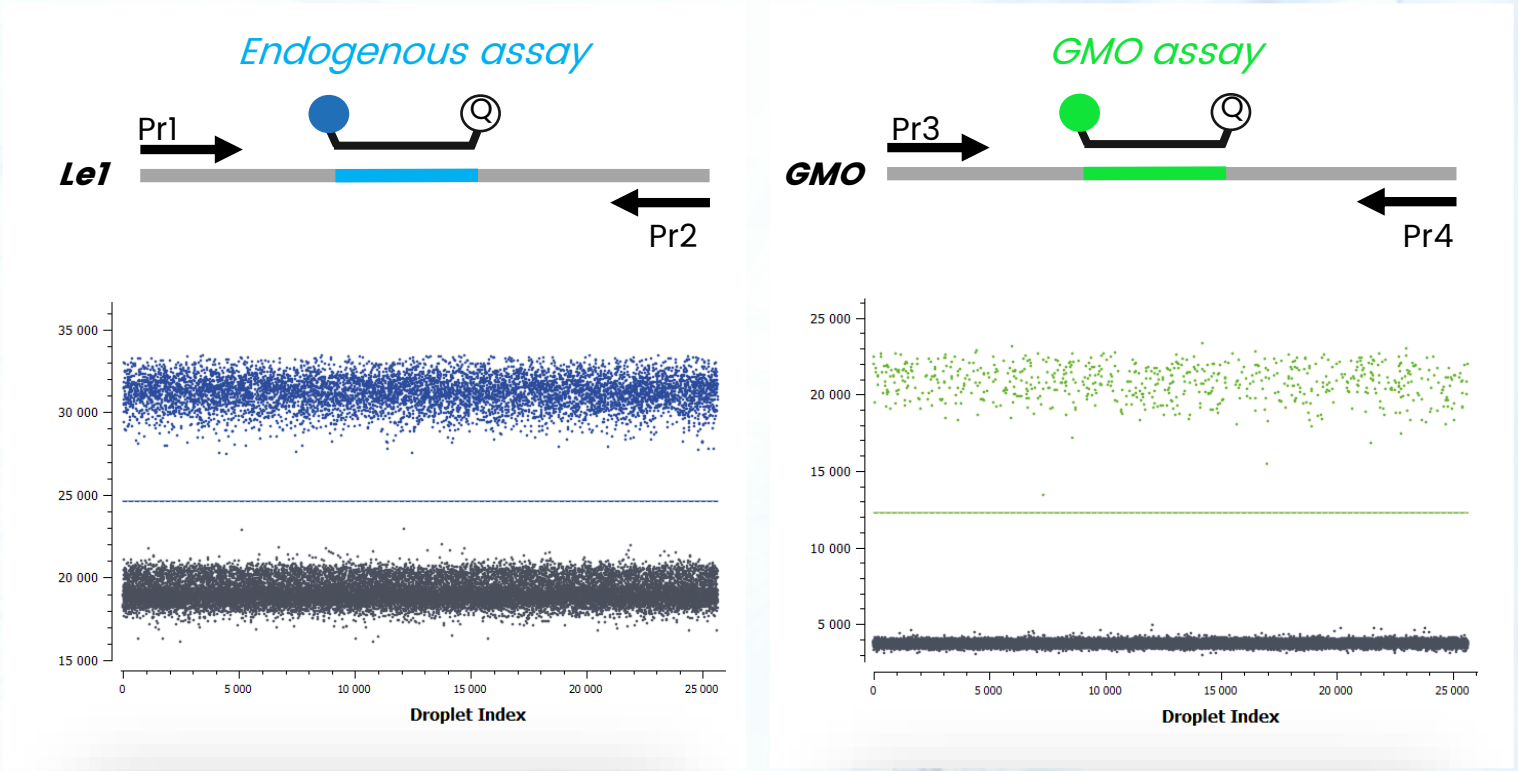
Identification

If it is approved, how high is the relative content?

Quantification

Highly Multiplexed assay for GMO Detection in Soybean by Crystal Digital PCR™

- GMO detection with TaqMan Detection assay



Total content concentration

GMO concentration

Total GMO Content [%]

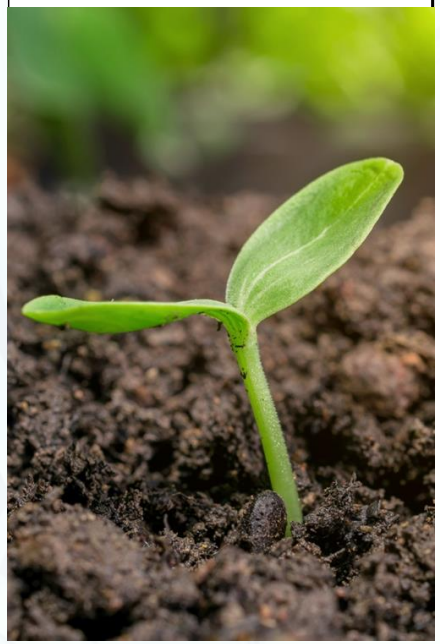
Highly Multiplexed assay for GMO Detection in Soybean by Crystal Digital PCR™

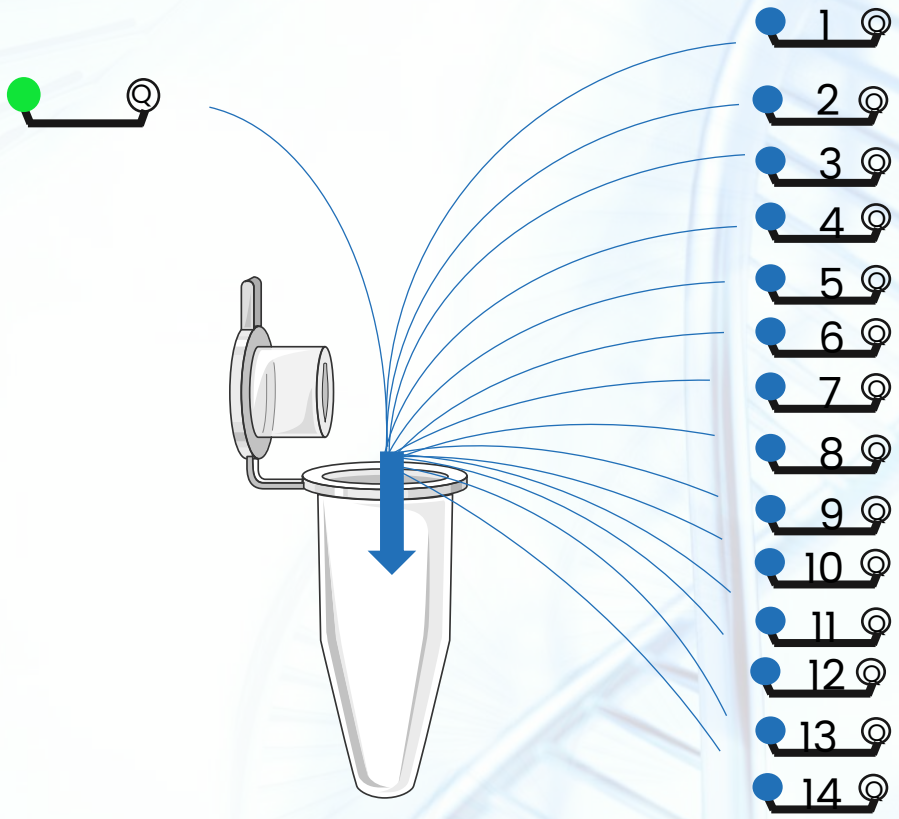
Detection and quantification of highly multiplex kit

- Detection of 14 GM soybean events and the soybean lectin gene

BIOTECON Diagnostics

Endogene Soybean
soybean lectin gene



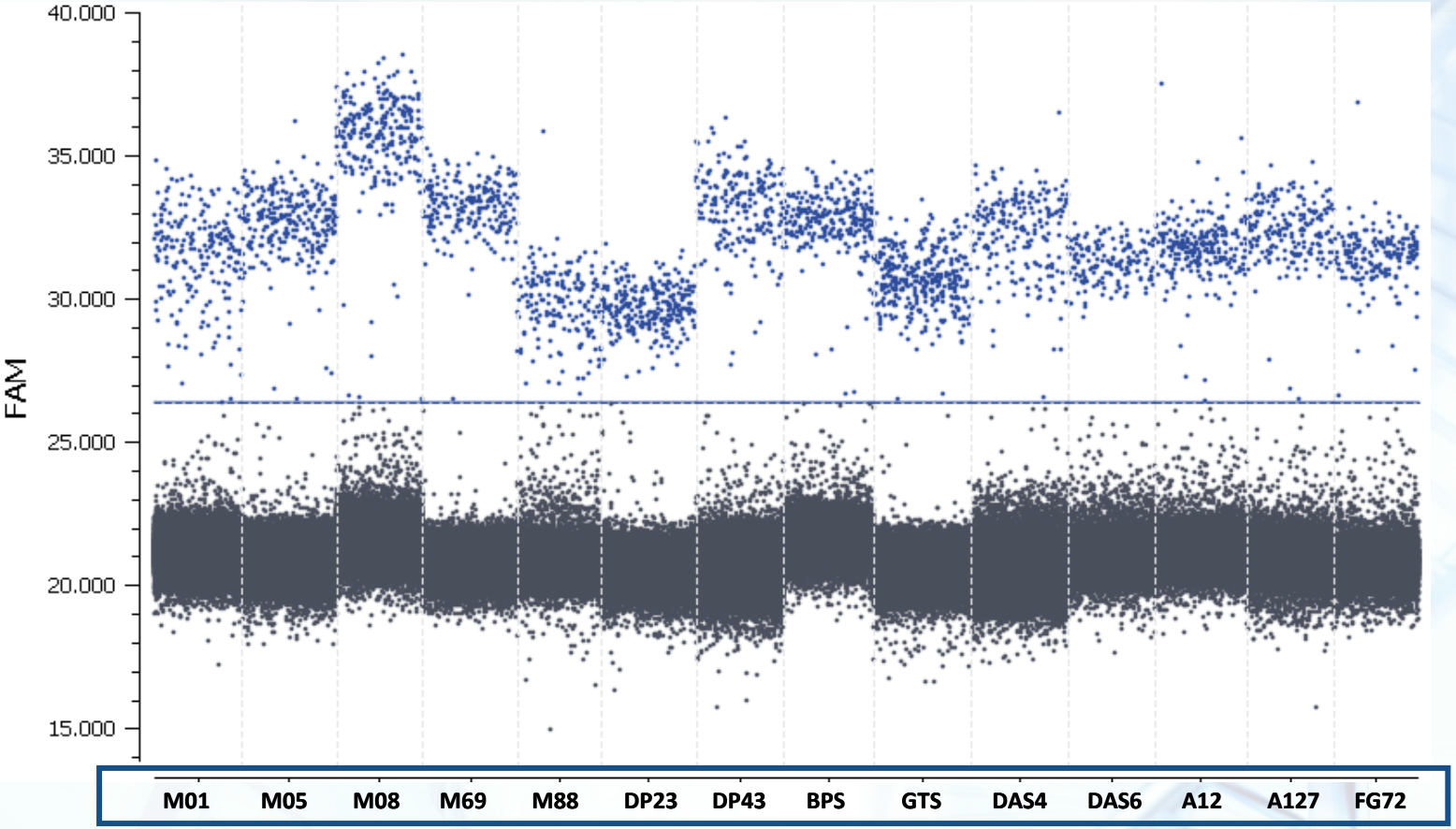


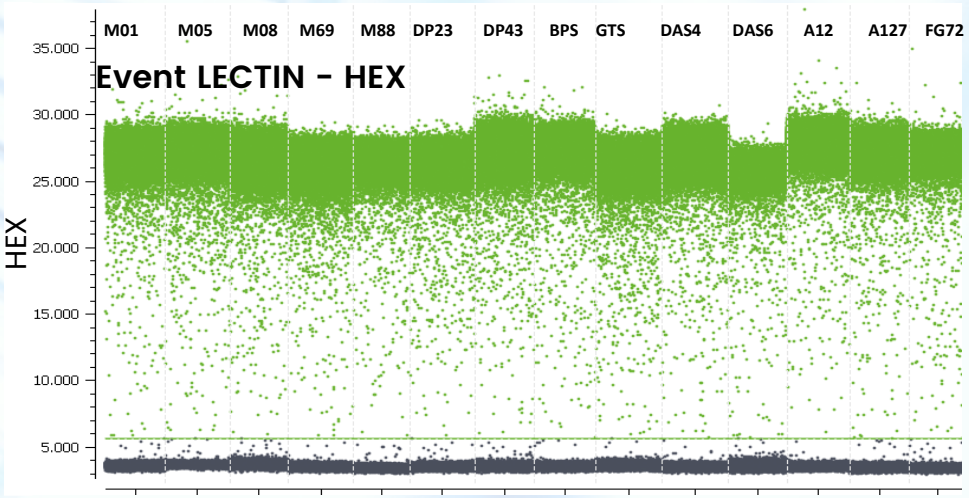
GM Soybean Line	Abbreviation
A5547-127	A127
A2704-12	A12
BPS-CV127-9	BPS
DP-305423	D23
DP-356043	D43
DAS-68416-4	DAS4
DAS-44406-6	DAS6
FG72	FG72
GTS 40-3-2	GTS
MON87701	M01
MON89788	M88
MON87705	M05
MON87769	M69
MON87708	M08

Highly Multiplexed assay for GMO Detection in Soybean by Crystal Digital PCR™

Assay Development

- DNA of reference material from all 14 events was diluted (GMO content 1%) with DNA extracted from GMO-free soy flour





1% each

BIOTECON Diagnostics

Highly Multiplexed assay for GMO Detection in Soybean by Crystal Digital PCR™

Event	GMO Content %	Total GMO Content %
MON89788	40.5	87.6 =
MON87701	47.1	
Multiplex		88.8

Sample	Event	GMO Content [%]	Total GMO Content [%]
Soy flour 1	GTS 40-3-2	0.4	1.3 =
	MON89788	0.0	
	DP305423	0.4	
	A2704-12	0.5	
	Multiplex		1.2
Soy flour 2	GTS 40-3-2	0.8	2.3 =
	MON89788	0.7	
	DP305423	0.7	
	A2704-12	0.0	
	Multiplex		2.1

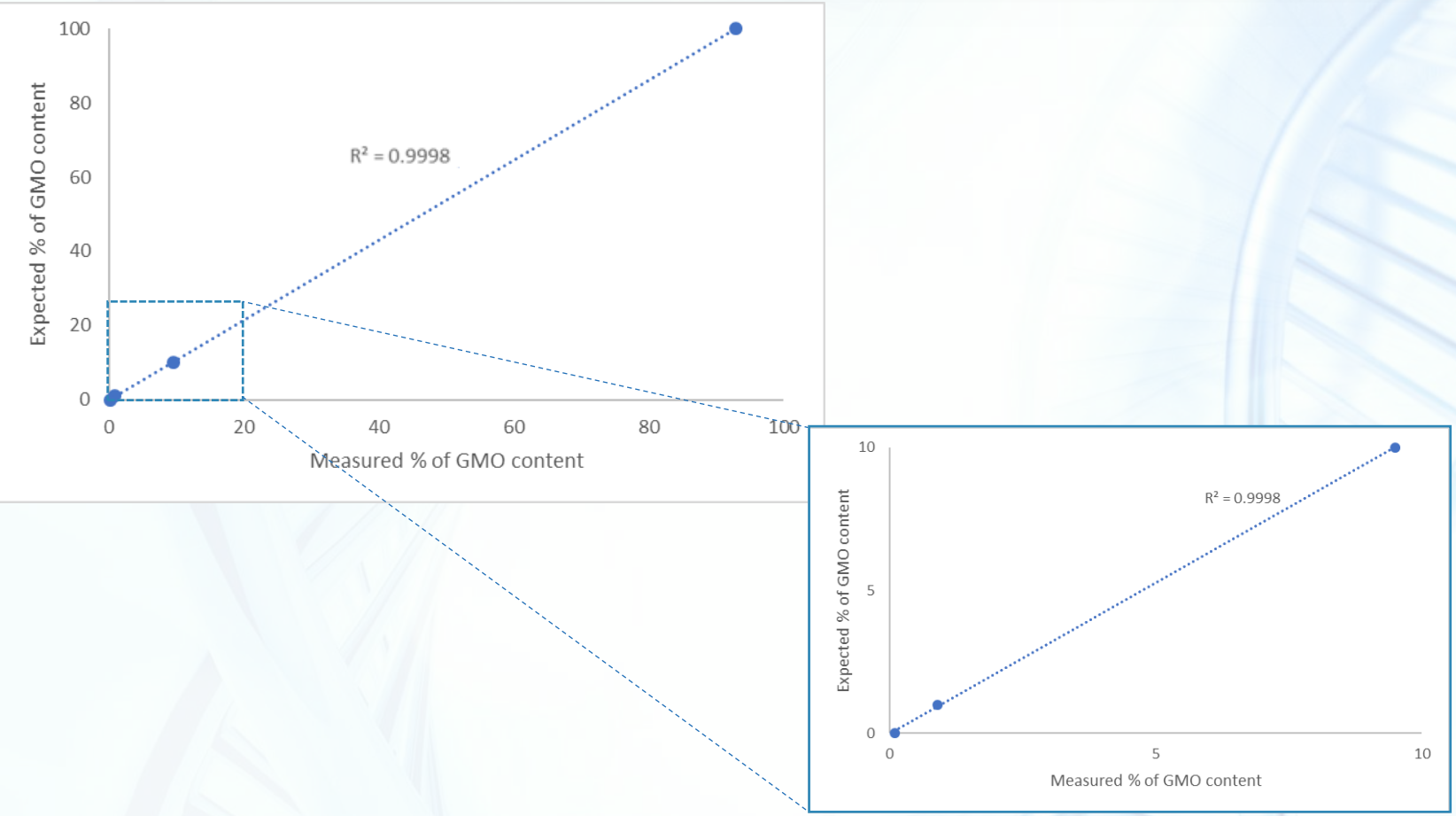
→ **Testing the full GMO content in one assay**

BIOTECON Diagnostics

Highly Multiplexed assay for GMO Detection in Soybean by Crystal Digital PCR™

Robust Peformance

- Dilution series of DNA reference material (MON87705) with DNA from GMO-free soybean flour



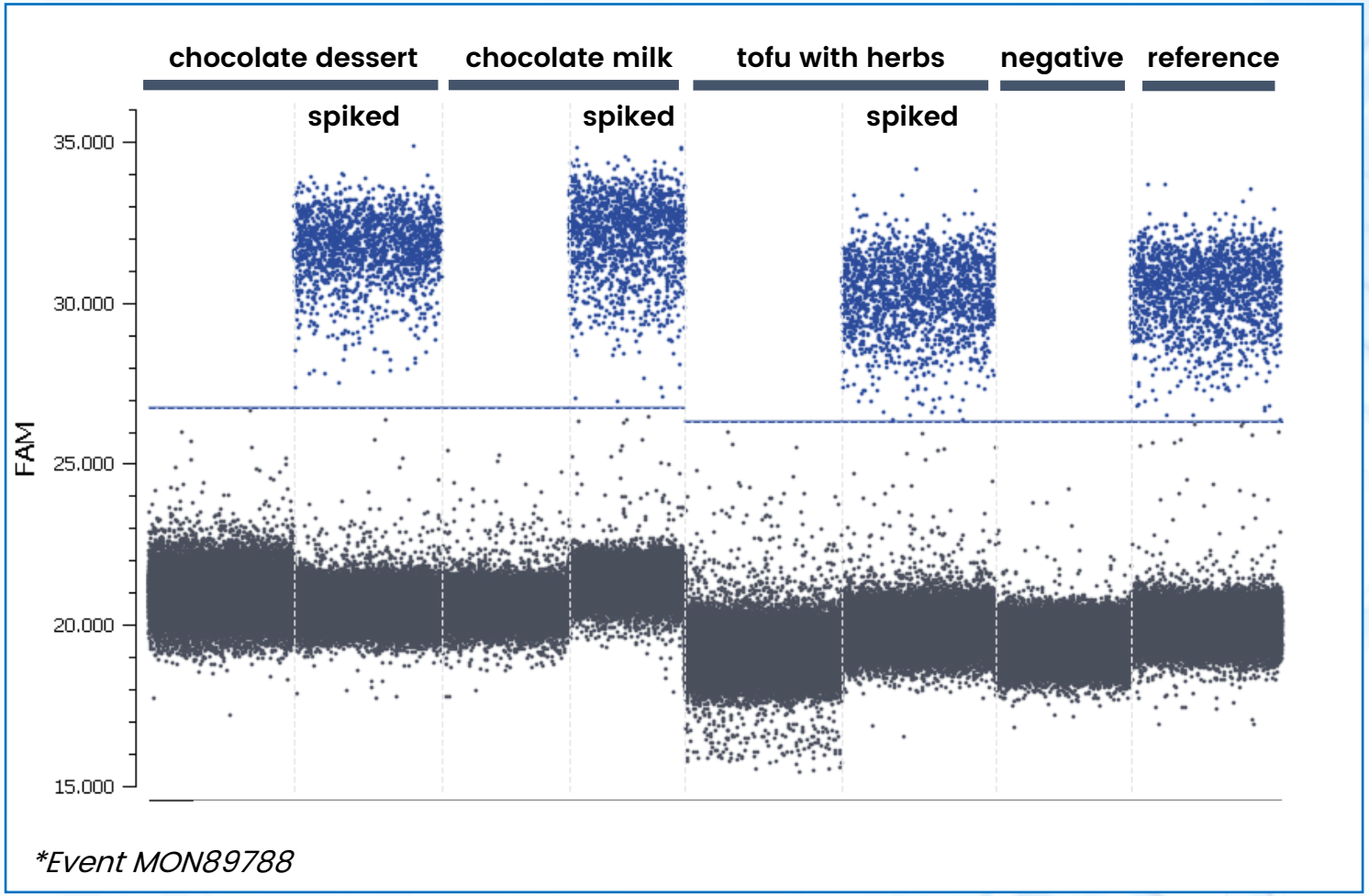
Sample* (GTS 40-3-2)	GMO Content %	GMO Content Standard Deviation [%]
GMO: 0.1%	0.10	9.1
GMO: 0.1%	0.12	
GMO: 0.1%	0.11	
GMO: 0.1%	0.11	

** DNA of reference material was diluted with DNA from GMO-free soybean flour*

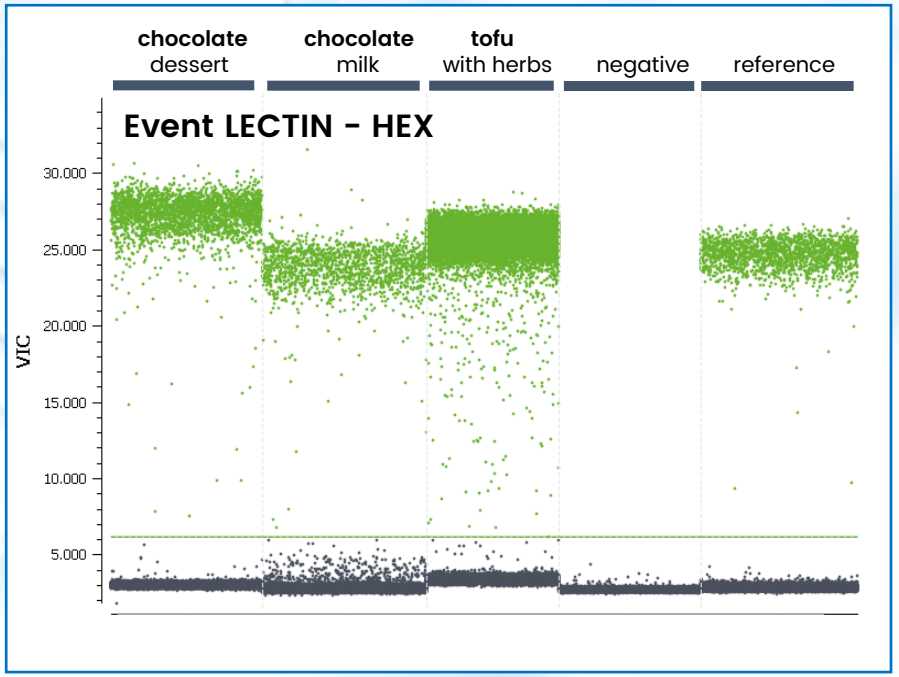
BIOTECON Diagnostics

Detection of GMOs in challenging food material by Crystal Digital PCR™

- DNA was extracted from 200 mg material
- 5 µL undiluted DNA, GMO* → *Spiked or not spiked*



Event MON89788



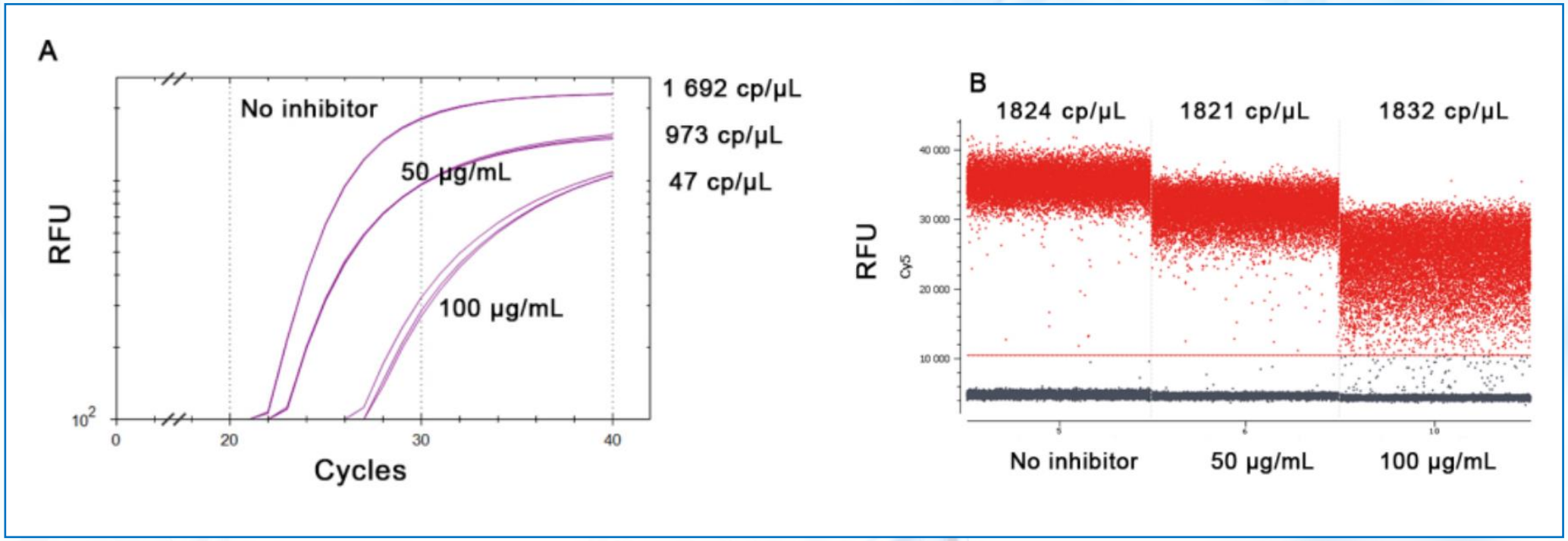
BIOTECON Diagnostics

CRYSTAL DIGITAL PCR™

INHERENT TOLERANCE TO INIHBITOR

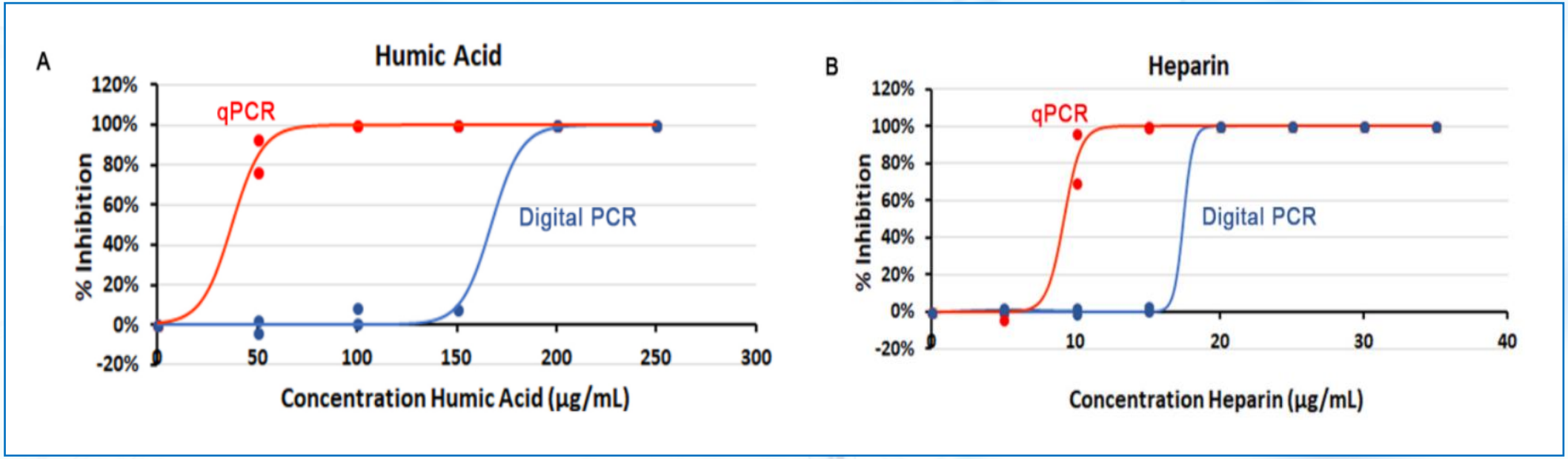
EXPERIMENT

- SAME DNA QUANTITY, VARIABLE CONCENTRATION OF INIHIBITOR (HUMIC ACID)



CRYSTAL DIGITAL PCR™

INHERENT TOLERANCE TO INIHBITOR



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- **Going further with our next innovation: 6-color**

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NATIONAL INSTITUTE OF **BIOLOGY**

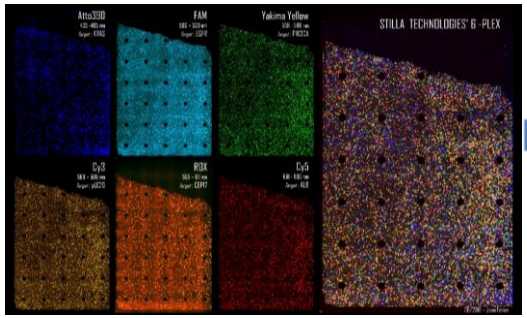
6-color Crystal Digital PCR™ for Quantification of Genetically Modified Soybean

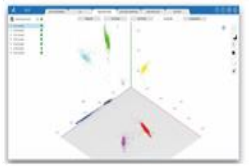
Workflow

Sapphire Chips

Naica™ Geode

6-Color Reader





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NATIONAL INSTITUTE OF **BIOLOGY**

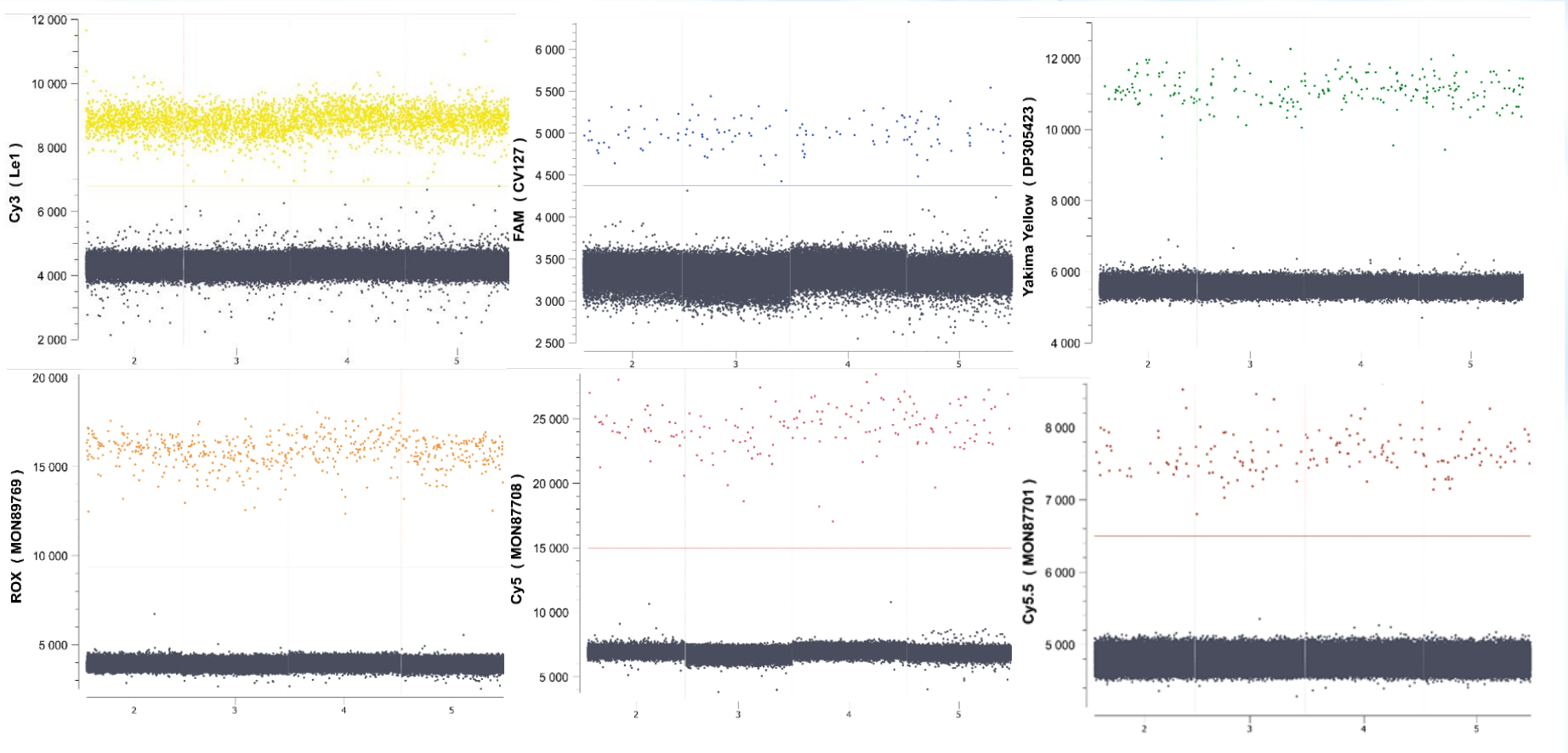
- 6 assays, 5 targeting GM soybean event and 1 soybean endogene

GM line	Fluorescent dye
DP305423	Yakima yellow
MON87708	Cy5
MON87701	Cy5.5
CV127	FAM
MON89769	ROX
Le1	Cy3

6-color Soybean GMO Crystal Digital PCR™ Assay

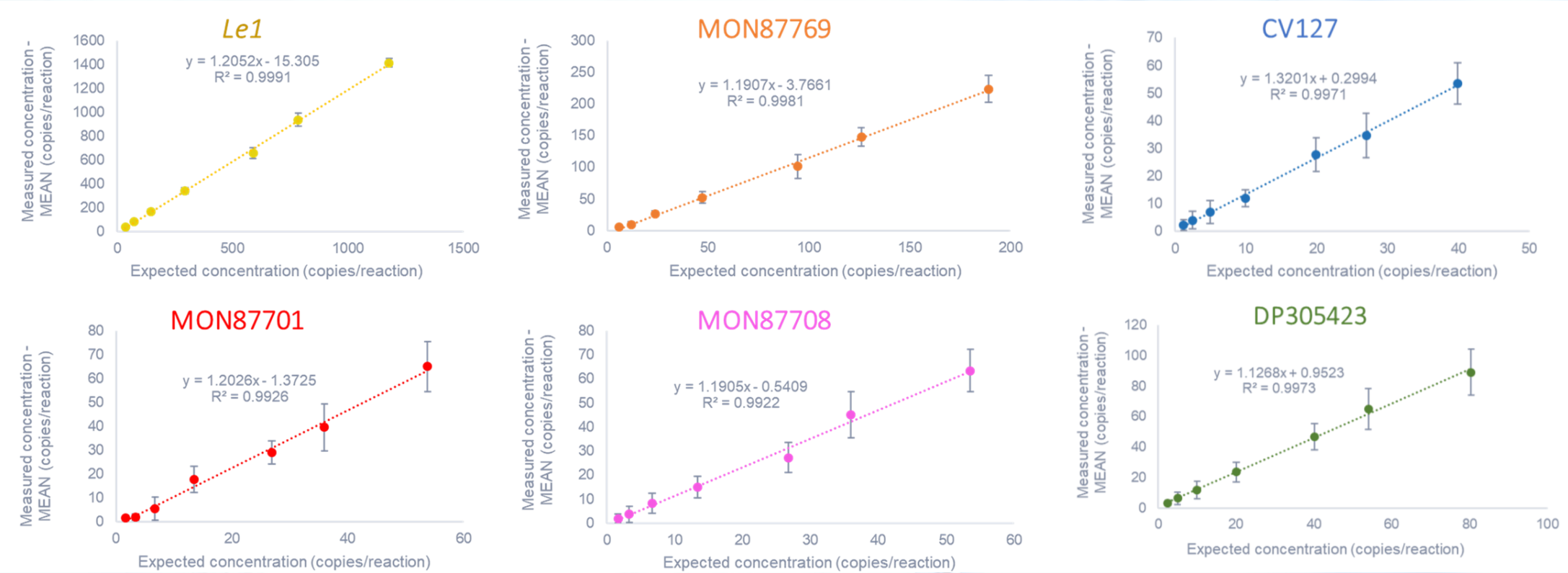
Crystal Miner 1D plots display robust separability between positive and negative clusters

GM line	Fluorescent dye
DP305423	Yakima yellow
MON87708	Cy5
MON87701	Cy5.5
CV127	FAM
MON89769	ROX
Le1	Cy3



Robust Performance

- Target sequences were detected with a 95% confidence level in serial dilutions (8 replicates per dilution)



Although assays were tested at the lower end of the dynamic range, the linearity is still very high (R^2>0.99)

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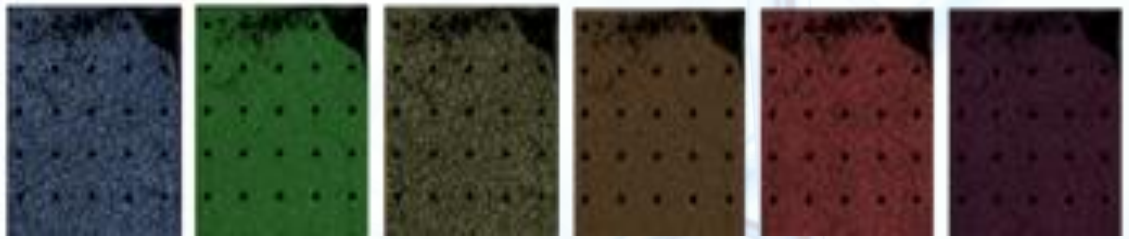
6-color Soybean GMO Crystal Digital PCR™ Assay

Crystal Digital PCR™ is a fast and cost-effective strategy for reliable simultaneous quantification of multiple GM soybean lines

GM Soybean line	Rep	Expected GM%	Mean % measured
CV127	1	3.38	3.89
	2		
DP305423	1	5.73	6.89
	2		
MON89769	1	13.47	16.06
	2		
MON87708	1	3.8	4.7
	2		
MON87701	1	3.82	4.83
	2		

CONCLUSION

- **NAICA™ system is a time and cost-effective solution to screen, quantify and identify GMO**
- **Crystal digital PCR™ is ideal for PCR inhibitory matrix**
- **6-color capability will allow high multiplexing for quantification and identification**



STILLA

THANK YOU FOR YOUR ATTENTION!

ANY QUESTIONS?
Visit Booth 23

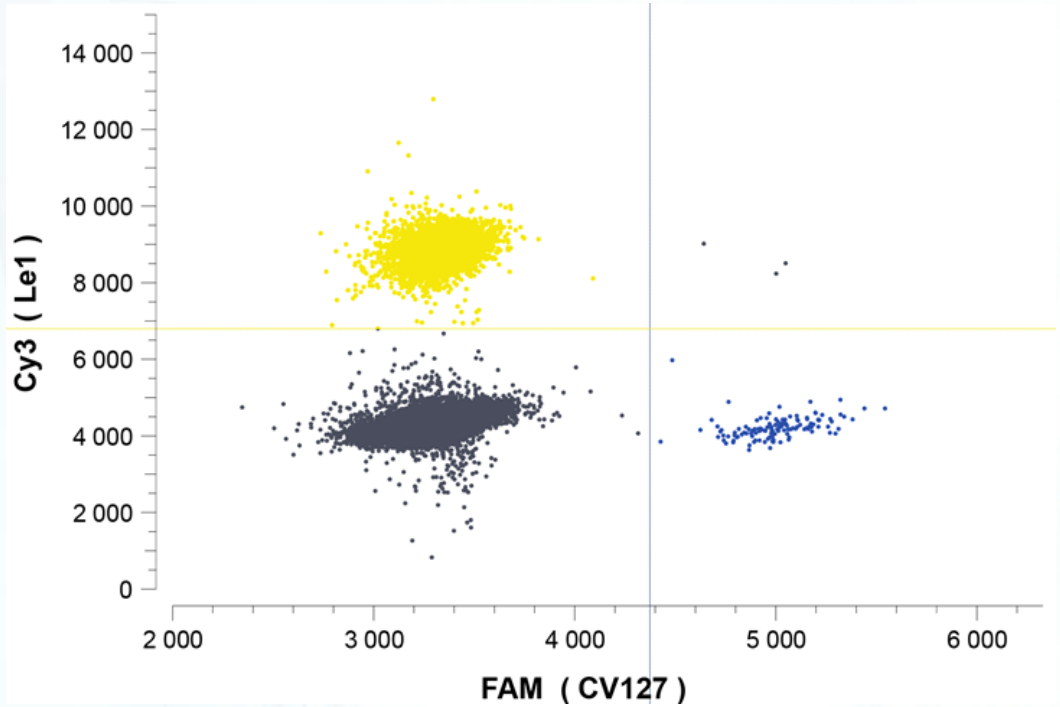
For more information on product and workflow, visit our website at

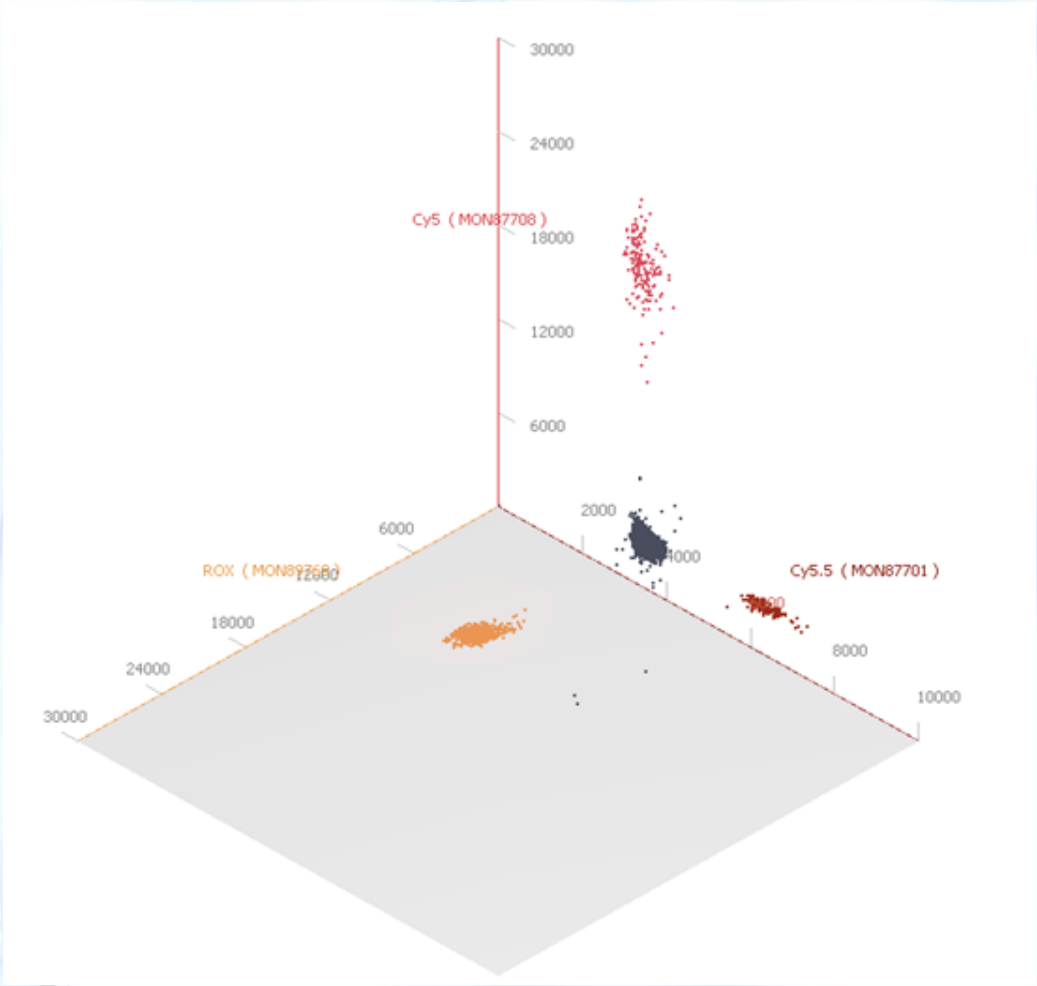
www.stillatechnologies.com

To learn more about Digital PCR, visit
www.Gene-Pi.com

6-color Soybean GMO Crystal Digital PCR™ Assay

2D and 3D plots to visualize robust separability of clusters

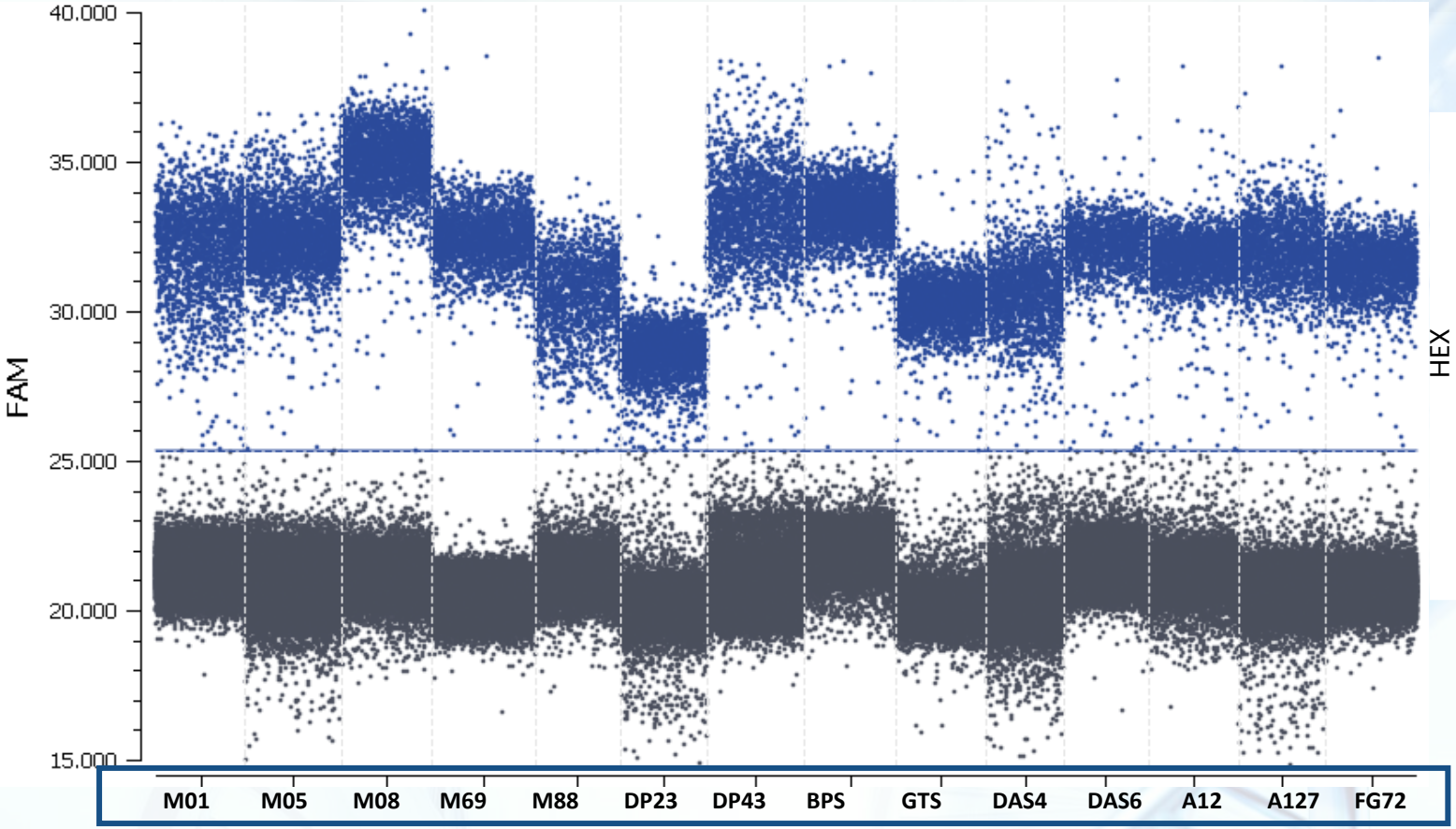


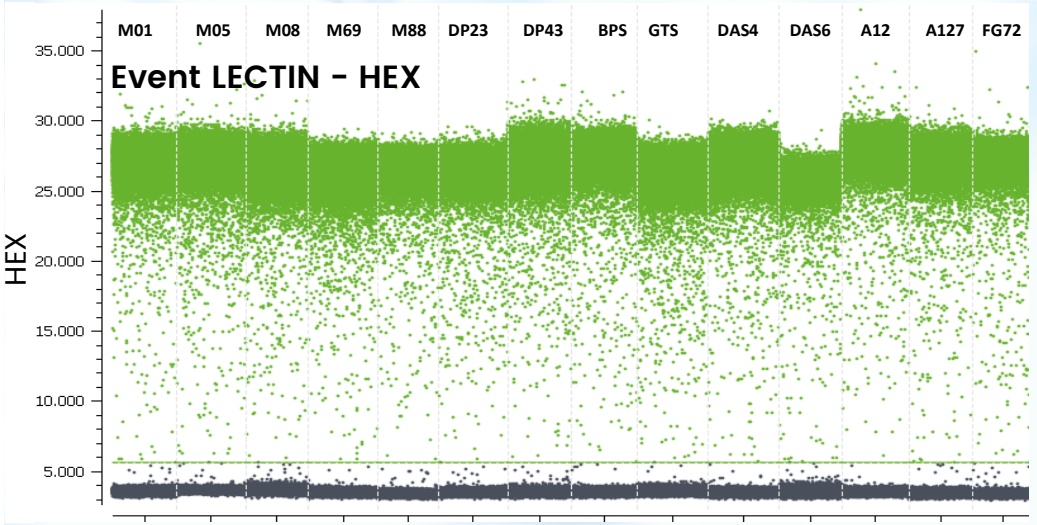


Highly Multiplexed assay for GMO Detection in Soybean by Crystal Digital PCR™

Assay Development

- DNA of reference material from all 14 events was diluted (GMO content 10%) with DNA extracted from GMO-free soy flour

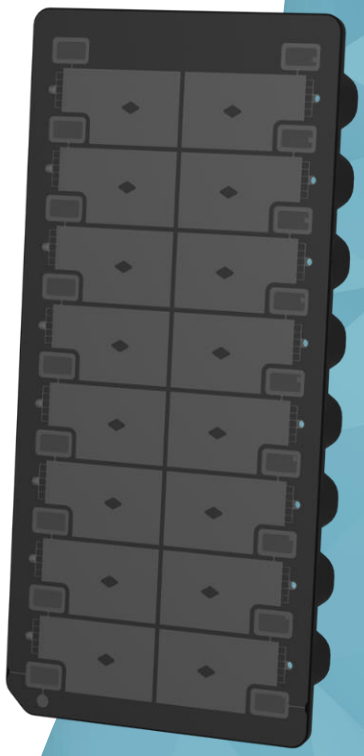




10% each

BIOTECON Diagnostics

INTRODUCING THE OPAL CHIP



Opal Chip

- Up to **16 samples** per chip
- Input volume **7 µL**
- Up to **3 chips** per run
- Droplet volume **0.22nL**
- **~ 20,000 droplets** per well
- Dynamic range **5 logs**

Introducing

6-color Crystal Digital PCR™

Compatible CHIPS:

- **Sapphire chips (72 targets in 2h30)**
- **Opal chips (288 targets in <3h)**

- **3 chips per run**
- **Reading time: ≤ 20 min, 6 channels, 3 chips**

6-Color Reader

6-color Crystal digital PCR

Example of compatible fluorophores:

Channel	Fluorophores
1	FAM
2	YY®
3	Atto 550
4	ROX
5	Cy®5
6	Atto 700