# QUANTIFICATION GOES DIGITAL

# 10+ YEARS OF dPCR DEVELOPMENTS IN GMO DIAGNOSTICS

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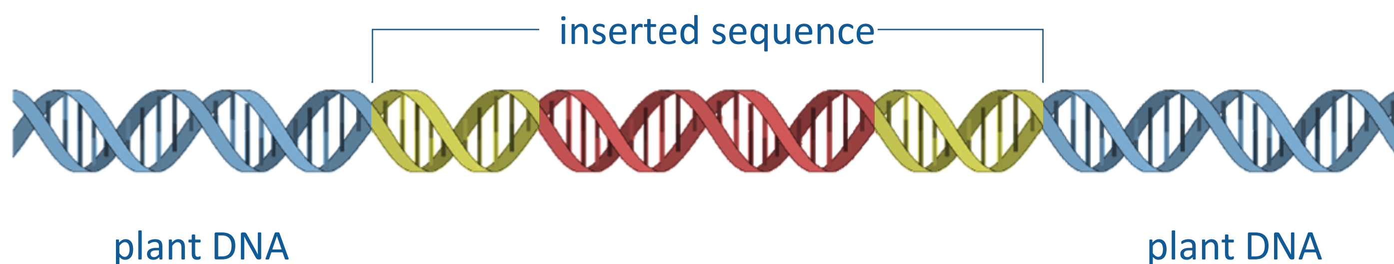
# Genetically Modified Organisms

…are by the EU legal definition[1]: organisms, with the exception of humans, in which the genetic material has been altered through the use of biotechnological methods, in a way that does not occur naturally by mating and/or natural recombination.

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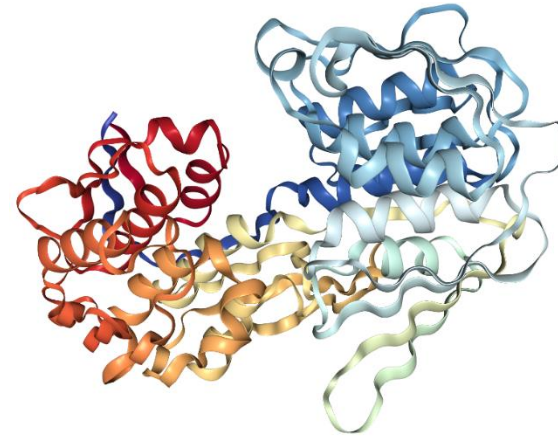
[1] Directive 2001/18/EC of the European Parliament and the Council of 12 March 2001 on the deliberate release in to the environment of genetically modified organisms and repealing Council Directive

# GMO Detection and Quantification

DNA-based

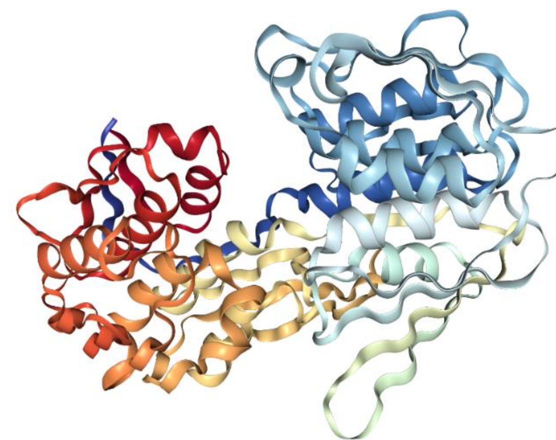


protein-based

# GMO Detection and Quantification

DNA-based

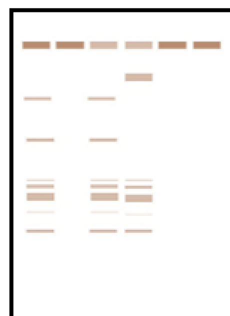
Enables identification of specific GM-lines, enables quantification, but is more time consuming.



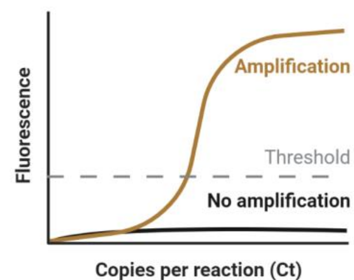
protein-based

Faster but cannot differentiate between specific GM-lines, not applicable for quantification

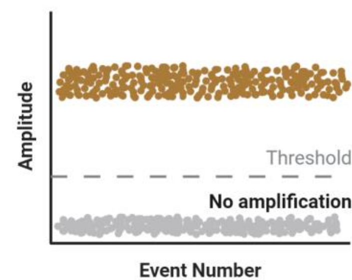
# Evolution of PCR-based methods



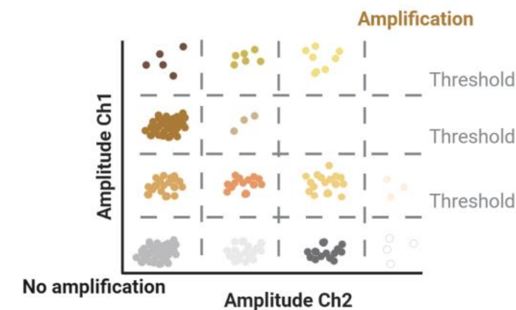
PCR → 




qPCR →

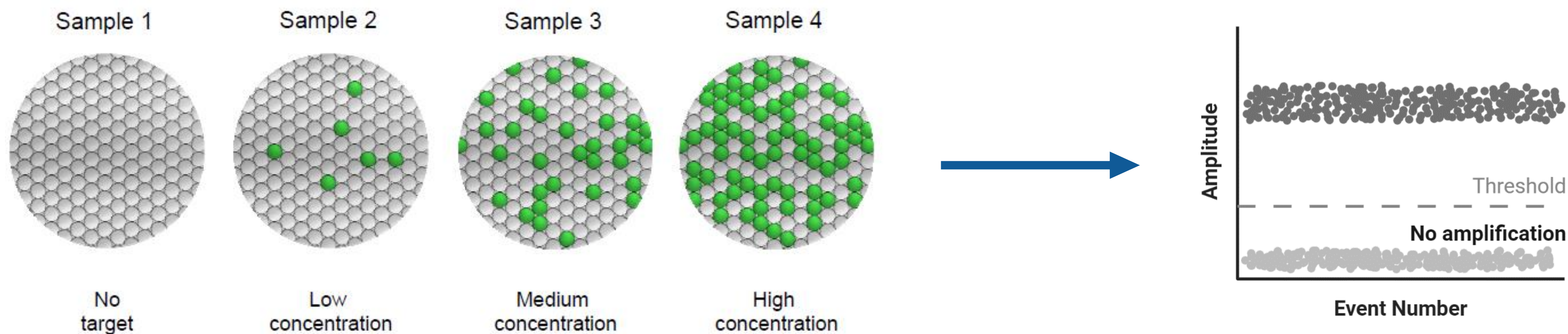



dPCR →




multiplex dPCR

# Why digital PCR?




The partitioning renders dPCR more **resistant to inhibitors**, which enables GMO quantification in complex matrices.

Enables multi target – **multiplex quantification** → time- and cost-effective

# Digital PCR gets it’s name

*Proc. Natl. Acad. Sci. USA*
Vol. 96, pp. 9236–9241, August 1999
Genetics

## Digital PCR

BERT VOGELSTEIN* AND KENNETH W. KINZLER

The Howard Hughes Medical Institute and the Johns Hopkins Oncology Center, Baltimore, MD 21231

*Contributed by Bert Vogelstein, June 9, 1999*

ABSTRACT The identification of predefined mutations expected to be present in a minor fraction of a cell population is important for a variety of basic research and clinical applications. Here, we describe an approach for transforming the exponential, analog nature of the PCR into a linear, digital signal suitable for this purpose. Single molecules are isolated by dilution and individually amplified by PCR; each product is then analyzed separately for the presence of mutations by using fluorescent probes. The feasibility of the approach is demonstrated through the detection of a mutant *ras* oncogene in the stool of patients with colorectal cancer. The process provides a reliable and quantitative measure of the proportion of variant sequences within a DNA sample.

# Comercialisation and application in GMO field

2006

Anal Bioanal Chem (2009) 394:457–467
DOI 10.1007/s00216-009-2729-5

ORIGINAL PAPER

**Single molecule detection in nanofluidic digital array enables accurate measurement of DNA copy number**

**Somanath Bhat · Jan Herrmann · Paul Armishaw · Philippe Corbisier · Kerry R. Emslie**

2009

NIB
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# Development of new platforms

# Development of new platforms

2012

NIB first beta tester in Europe

2021

2021

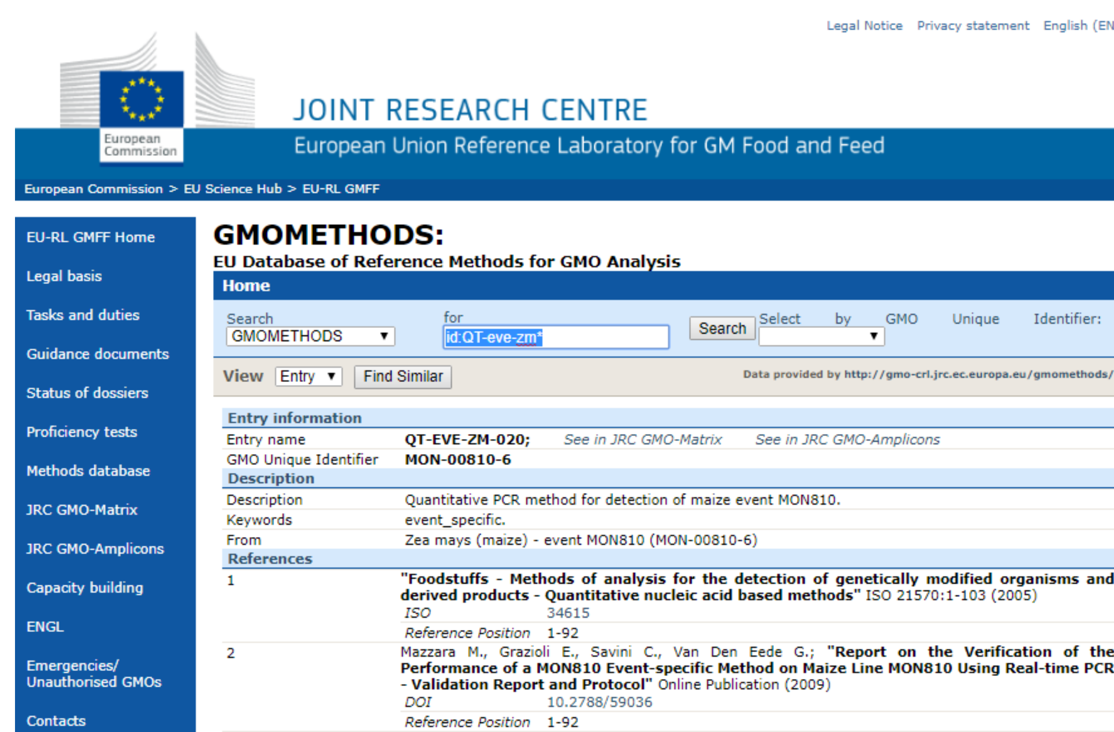
2020

2022

2022

# From qPCR to dPCR – testing transferability of assays

Direct transferal of *hmgA* and MON810 assays from qPCR to dPCR platform.



**Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing**

**European Network of GMO Laboratories (ENGL)**

13 October 2008

Date of application: 13 April 2009

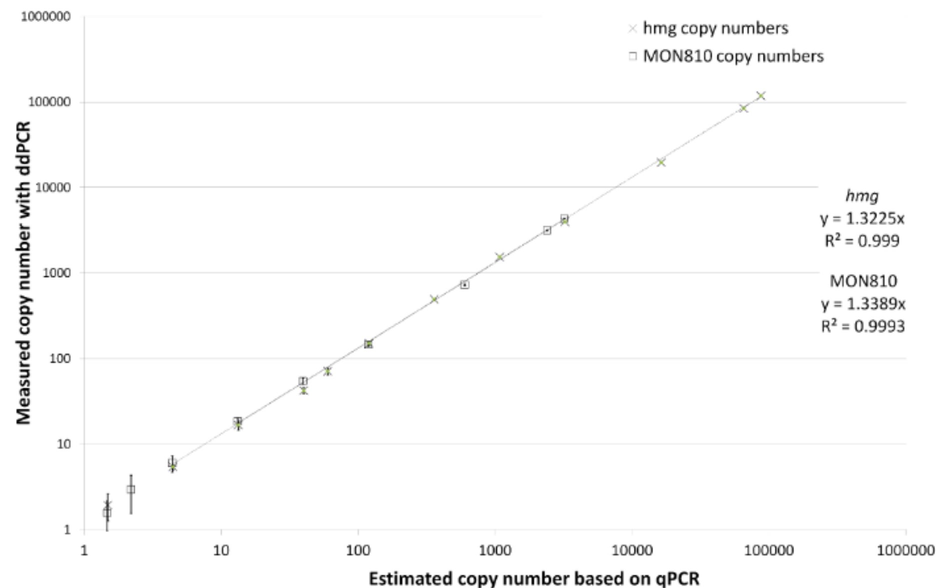
CAC/GL 74-2010 Page 1 of 22

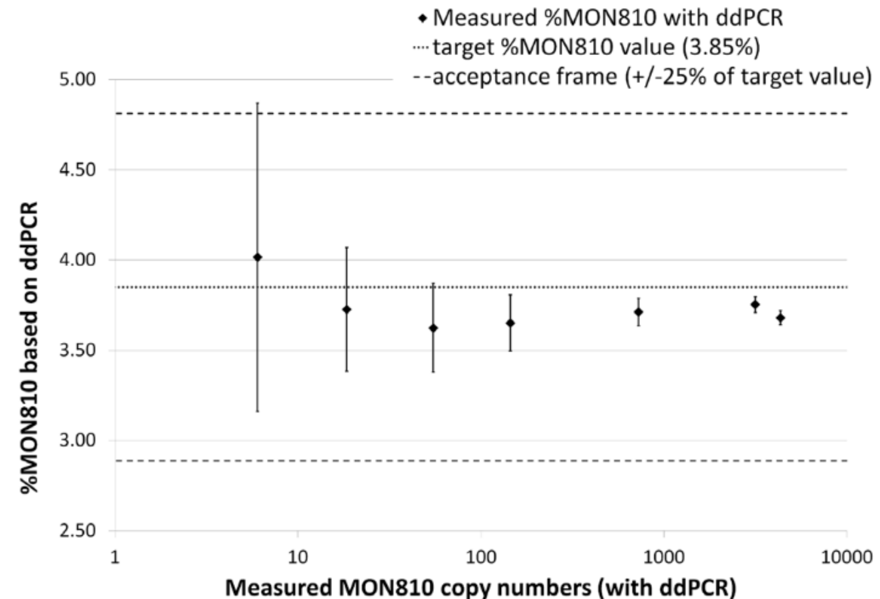
**GUIDELINES ON PERFORMANCE CRITERIA AND VALIDATION OF METHODS FOR DETECTION, IDENTIFICATION AND QUANTIFICATION OF SPECIFIC DNA SEQUENCES AND SPECIFIC PROTEINS IN FOODS***

**CAC/GL 74-2010**

# From qPCR to dPCR – testing transferability of assays

Trueness within acceptance criteria (±25% of the target value) down to 6 copies of event specific target (MON810)




Good linearity beyond 10 copies with $R^2 > 0.999$ for both targets

# Combining multiple assays – multiplex dPCR

**General idea:** GMOs labelled with one fluorophore, reference gene/endogen labelled with the other

Legal grounds → regulation No. 1829/2003

Multiplex quantification per ingredient or MQI

12 maize lines

Maize MQI
- 10-plex
- 4-plex

11 + 4 soybean lines

Soybean MQI
- 6-plex
- 7-plex → 11-plex

# MQI Practicability

## Comparison to possible qPCR scenarios

| Ingredient | Method | No. of tested sample | Relative final price per sample | Hands on time in hours (including analysis) | Number of 96-well plates required |
|---|---|---|---|---|---|
| maize | dPCR | 1 | 100 | 3 | 1 |
| | | 5 | 100 | 4 | 1 |
| | | 11 | 100 | 5 | 1 |
| | qPCR | 1 | 272 | 8 | 7 |
| | | 5 | 292 | 16 | 18 |
| | | 11 | 300 | 25 | 37 |
| soybean | dPCR | 1 | 100 | 4 | 1 |
| | | 5 | 100 | 7 | 1 |
| | | 10 | 100 | 8 | 1 |
| | qPCR | 1 | 368 | 14 | 7 |
| | | 5 | 445 | 31 | 18 |
| | | 10 | 483 | 63 | 36 |

*for qPCR calculations are done based on the use of 96-well plates

# Combining multiple assays – multiplex dPCR

**General idea:** quantification of specific GMOs in the same channel, by variation of probe concentrations

Multiple event quantification - MEQ

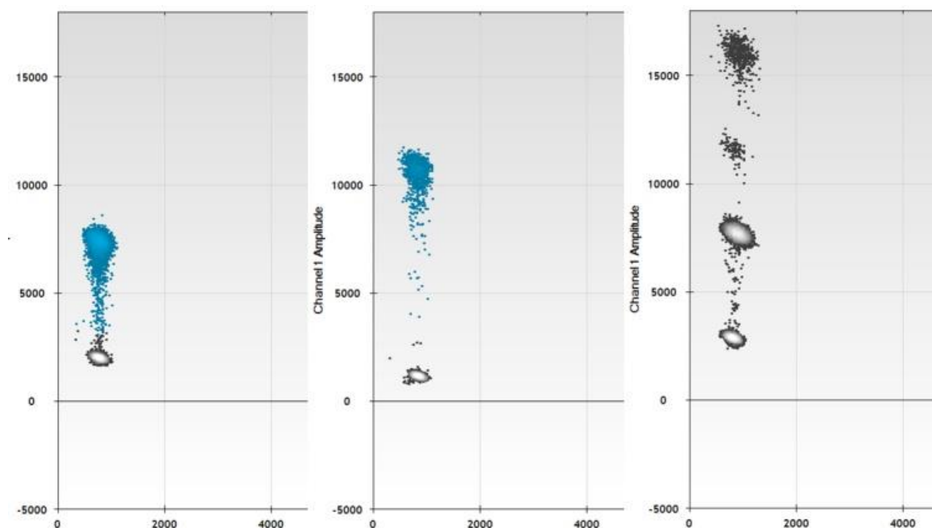
7 most common maize lines + maize reference gene (*hmgA*)

4-plex I

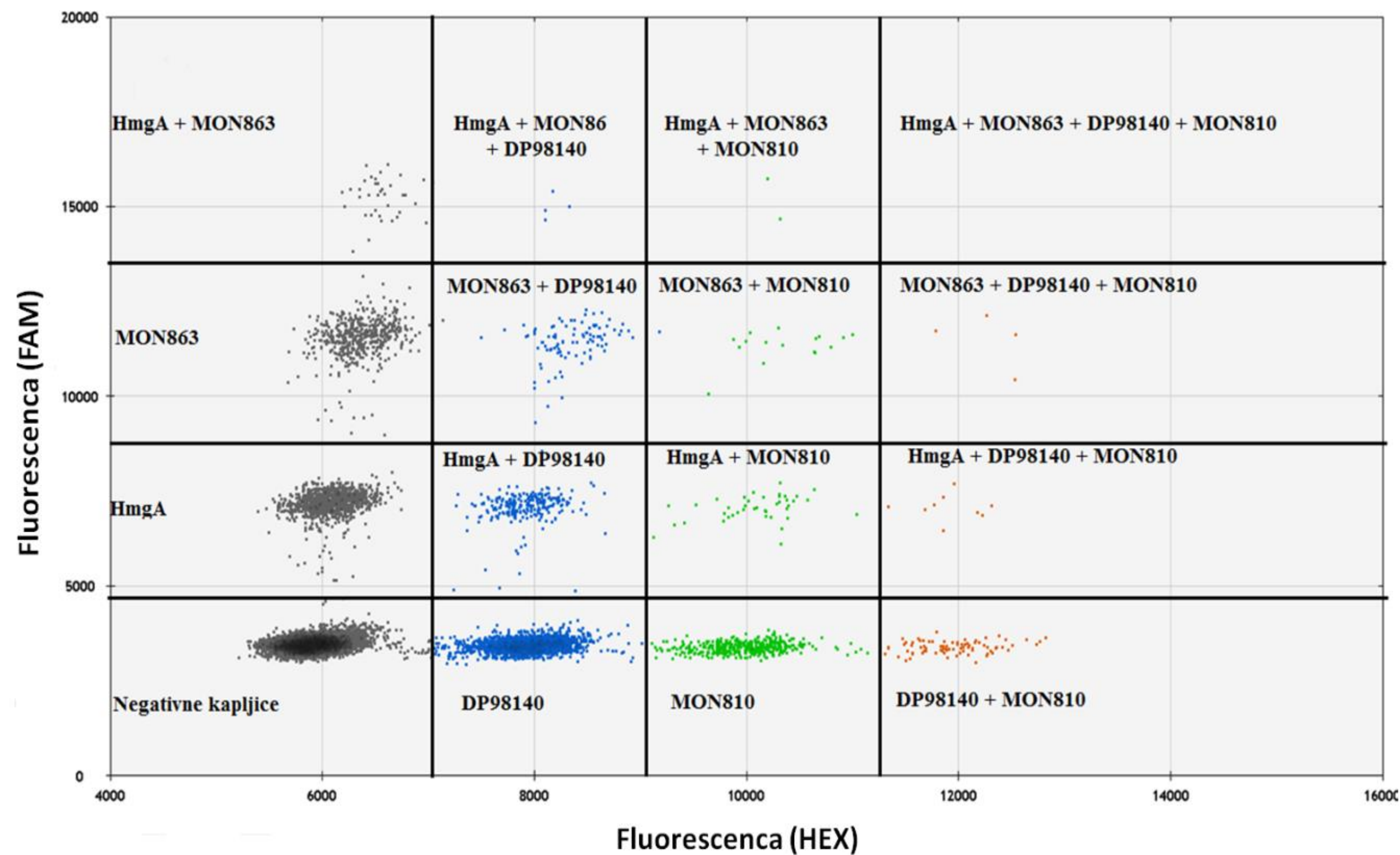
3 maize lines + reference gene

4-plex II

4 maize lines



# Multiple Event Quantification - MEQ



# Supporting diagnostics

vs.

© Bio-Rad

vs.

Use of dPCR for quantification of GM soybean line in complex samples, containing PCR inhibitors.

# Supporting diagnostics

Feed sample containing GM soybean – Roundup Ready

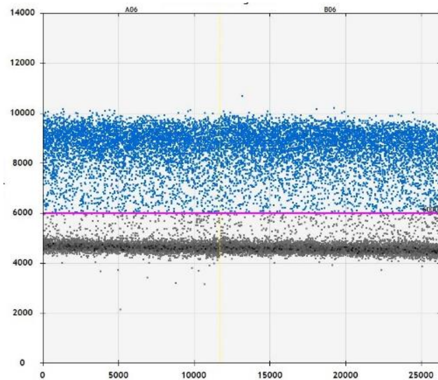
qPCR – sample exhibits great inhibition for both transgene and endogene up to 100 × dilution, sample cannot be quantified → pLOQ = 49 %

Comparison of GM% measured for real-life samples either with qPCR or ddPCR.

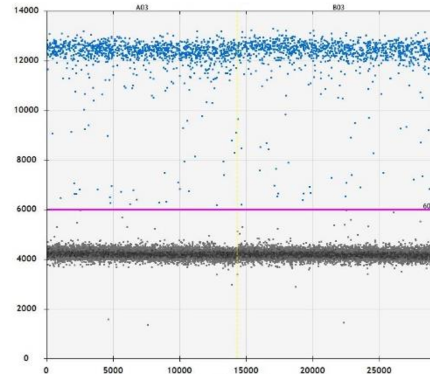
| Sample | Mean GM% | | | | | |
|---|---|---|---|---|---|---|
| | CTAB method | | | NucleoSpin Food kits | | |
| | qPCR | ddPCR [a] | Bias% | qPCR | ddPCR [a] | Bias% |
| A | < pLOQ [b] | 33.54 | *Na* [e] | *Na* [c] | 38.21 | *Na* [e] |
| B | 41 | 35.87 | 14.30 | *Na* [c] | 35.57 | *Na* [e] |
| C | *Na* [c] | 45.12 | *Na* [e] | *Na* [c] | < LOQ | *Na* [e] |
| D | 0.65 | 0.62 | 4.84 | < pLOQ[d] | < LOQ | *Na* [e] |

# dPCR is not completely „immune“ to inhibitors

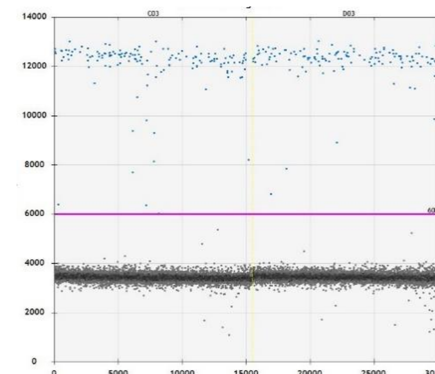
1× dilution – 14000 cp/µL



10× dilution – 2300 cp/µL

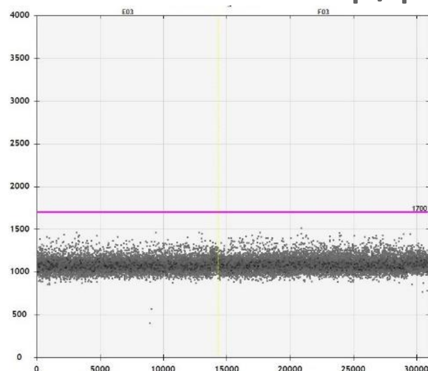


100× dilution – 260 cp/µL



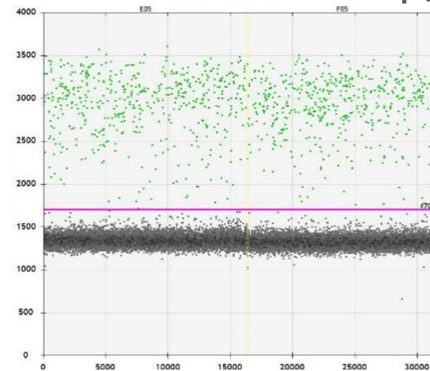
Reference gene

1× dilution – 0 cp/µL



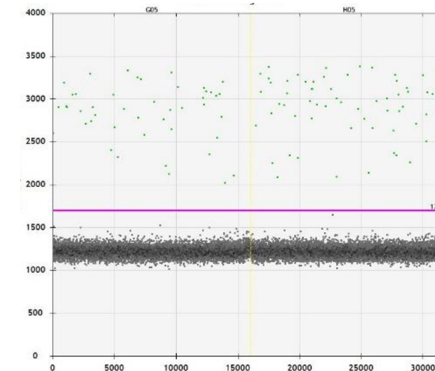
**0 %**

10× dilution – 810 cp/µL



**35.2 %**

100× dilution – 90 cp/µL



**34.6 %**

GMO

# Further work

Implementation of new technology which enables:

- Easier multiplexing – more than 2 channels
- Lower reaction volume
- Easier handling
- Faster turnaround time
- etc

# Acknowledgement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# Thank you for your attention!

analytical chemistry

Article

pubs.acs.org/ac

**Multiplex Quantification of 12 European Union Authorized Genetically Modified Maize Lines with Droplet Digital Polymerase Chain Reaction**

David Dobnik,[*,†,∥] Bjørn Spilsberg,[‡,∥] Alexandra Bogožalec Košir,[†,§] Arne Holst-Jensen,[‡] and Jana Žel[†]

OPEN ACCESS Freely available online

PLOS | ONE

**Quantitative Analysis of Food and Feed Samples with Droplet Digital PCR**

Dany Morisset*, Dejan Štebih, Mojca Milavec, Kristina Gruden, Jana Žel

Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia

**Chapter 5**

**Multiplex Droplet Digital PCR Protocols for Quantification of GM Maize Events**

David Dobnik, Bjørn Spilsberg, Alexandra Bogožalec Košir, Dejan Štebih, Dany Morisset, Arne Holst-Jensen, and Jana Žel

SCIENTIFIC REPORTS

OPEN

**Multiplex quantification of four DNA targets in one reaction with Bio-Rad droplet digital PCR syste[m] for GMO detection**

Received: 19 May 2016
Accepted: 30 September 2016
Published: 14 October 2016

David Dobnik, Dejan Štebih, Andrej Blejec, Dany Morisset & Jana Žel

SCIENTIFIC REPORTS

OPEN

**Development and inter-laboratory assessment of droplet digital PCR assays for multiplex quantification of 15 genetically modified soybean lines**

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**Digital PCR as an effective tool for GMO quantification in complex matrices**

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