NATIONAL INSTITUTE OF BIOLOGY

Digital PCR for GMO quantification

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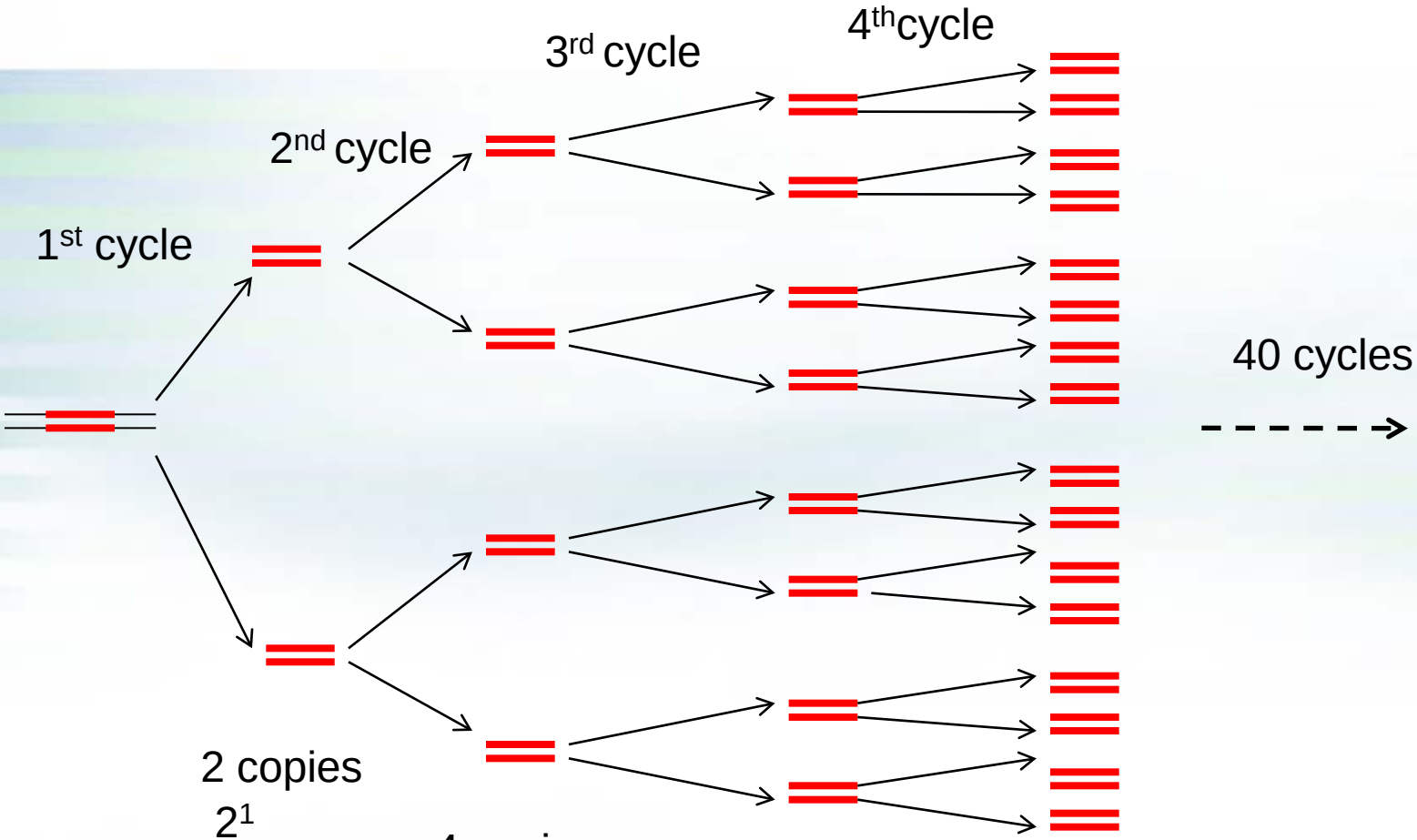
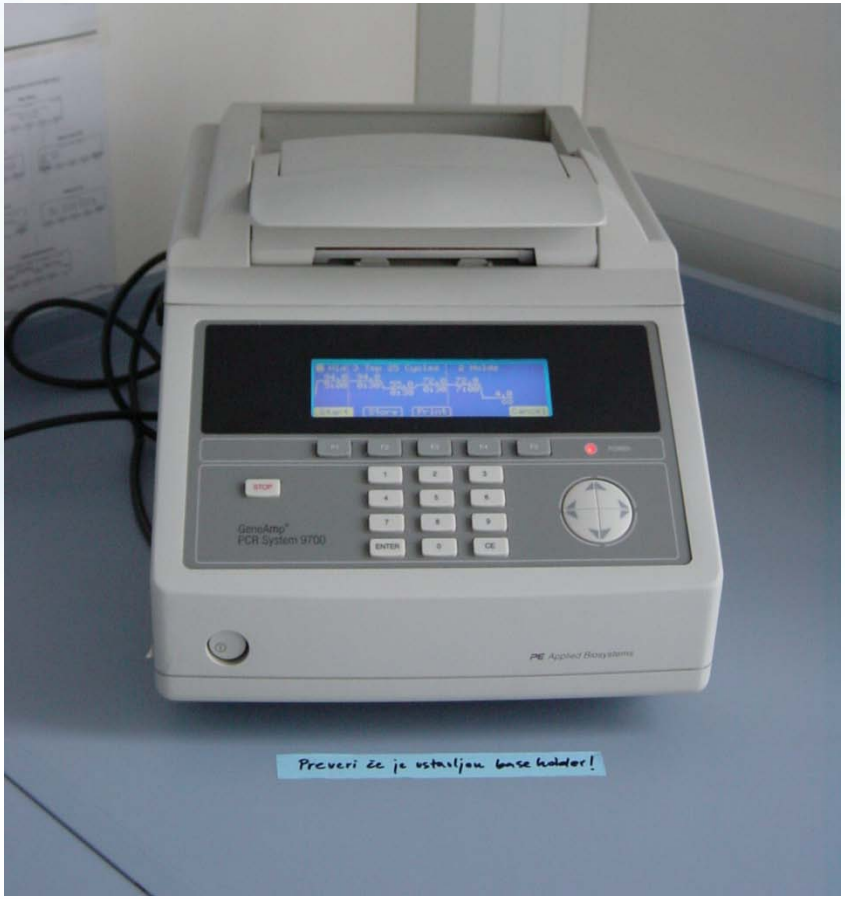
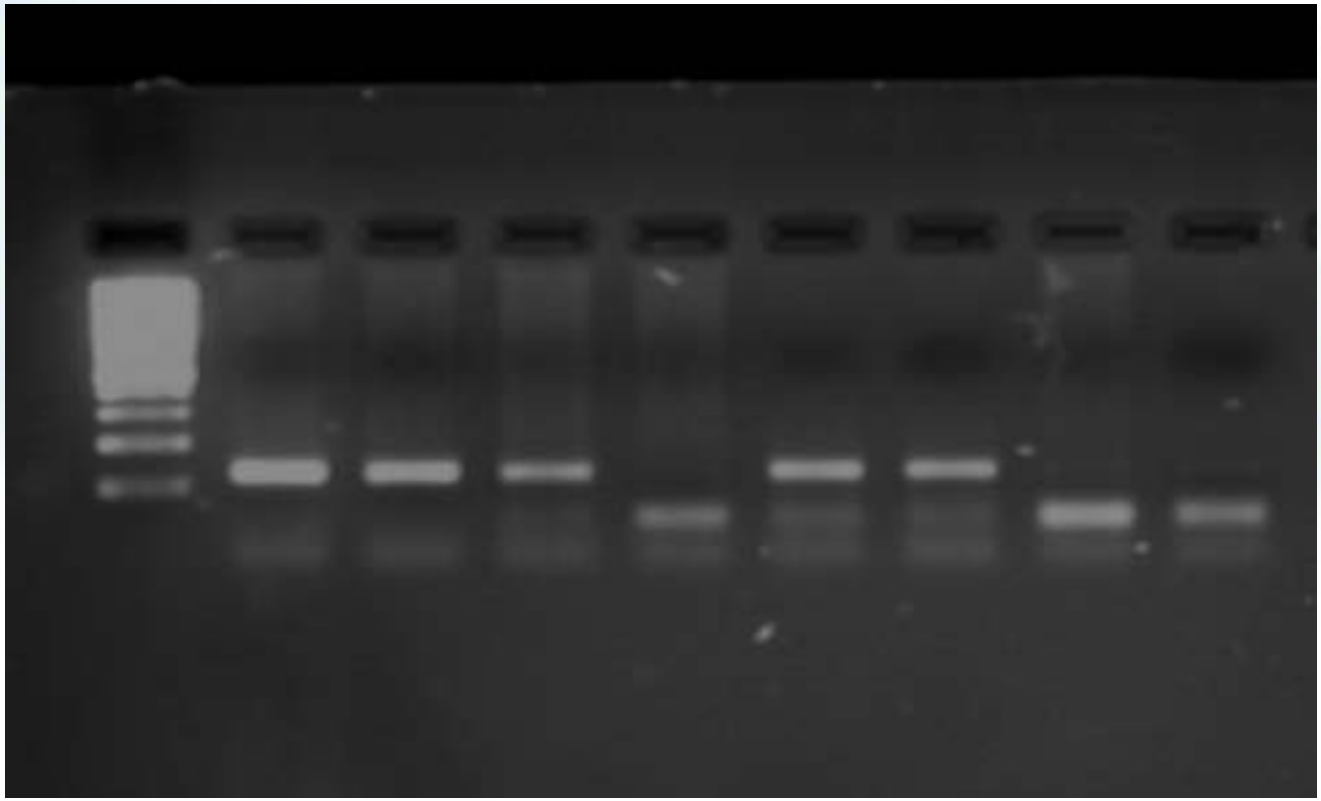
Polymerase chain reaction (PCR)



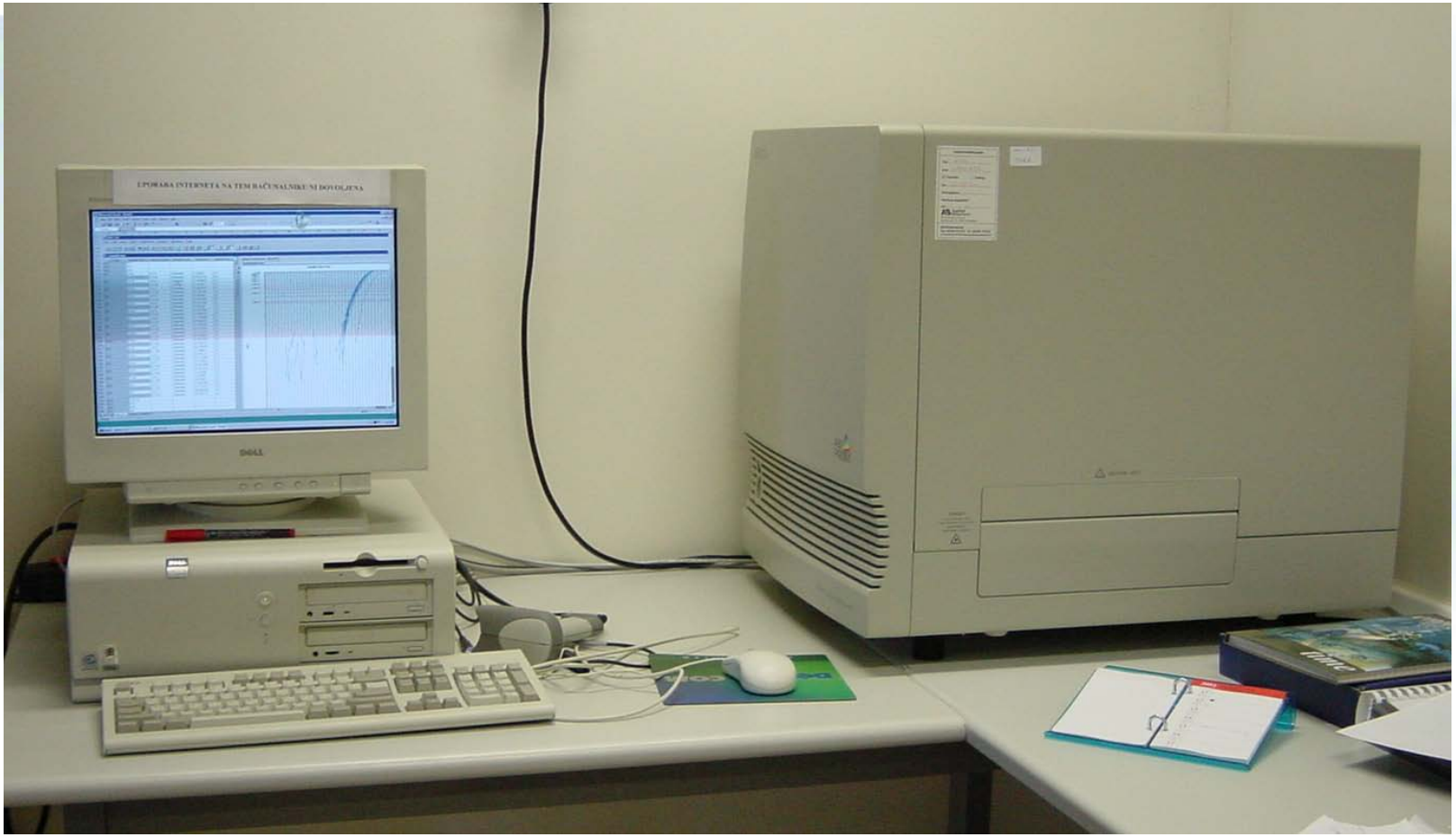
Polymerase chain reaction (PCR)



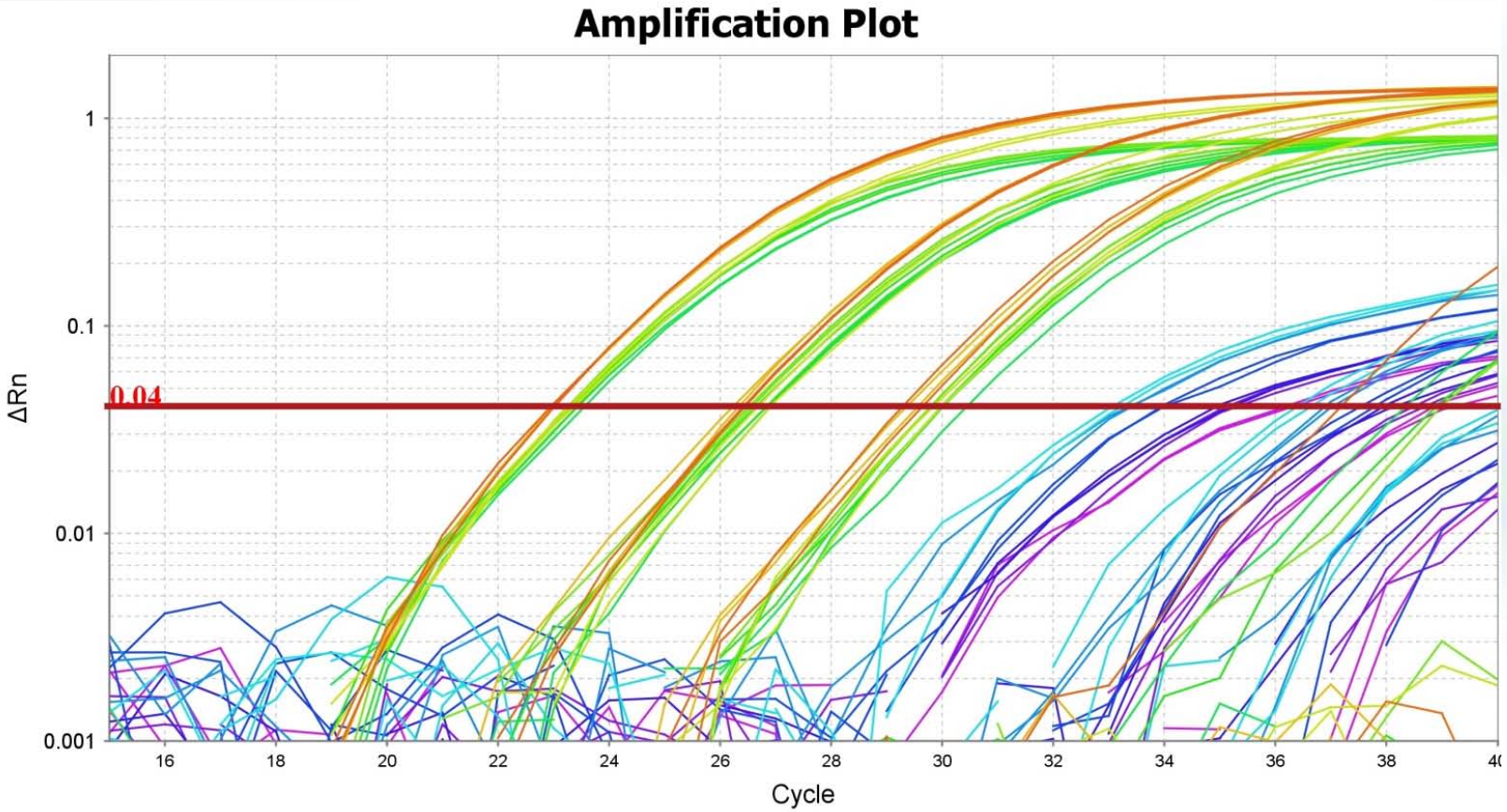
PCR results



Real time PCR

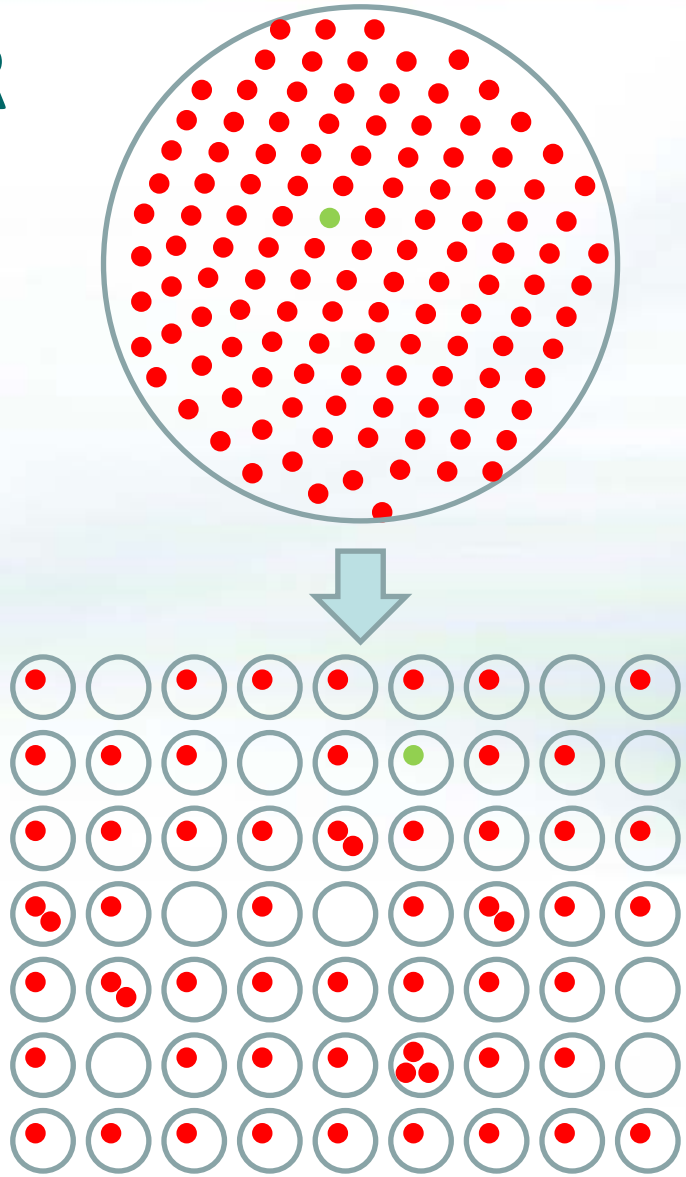


Real time PCR results



Digital PCR

- Concept of Limiting Dilutions
- To enrich minority targets by partitioning
- Divide initial DNA sample into multiple partitions
  - 0, 1 or few copies per reaction
- Diluted background
- All partitions subjected to standard PCR

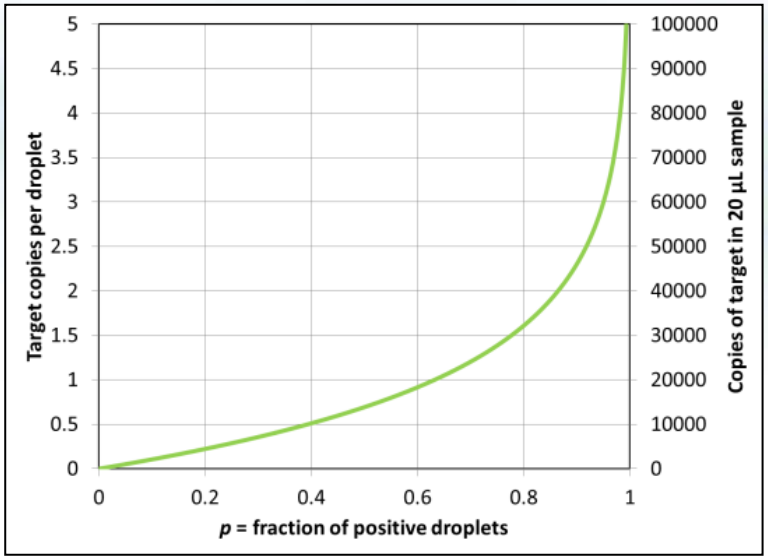


Digital PCR

Poisson law of small numbers

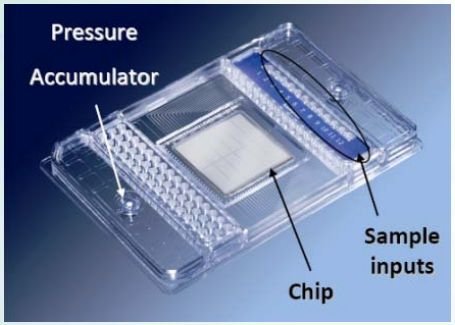
Siméon Denis Poisson (1781-1840)

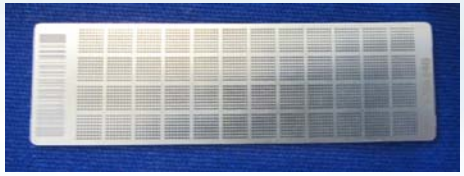
- Number of positive partitions is directly related to concentration
- Fraction of negatives is fit to a Poisson algorithm to determine absolute copy number, results in copies per input µl of sample



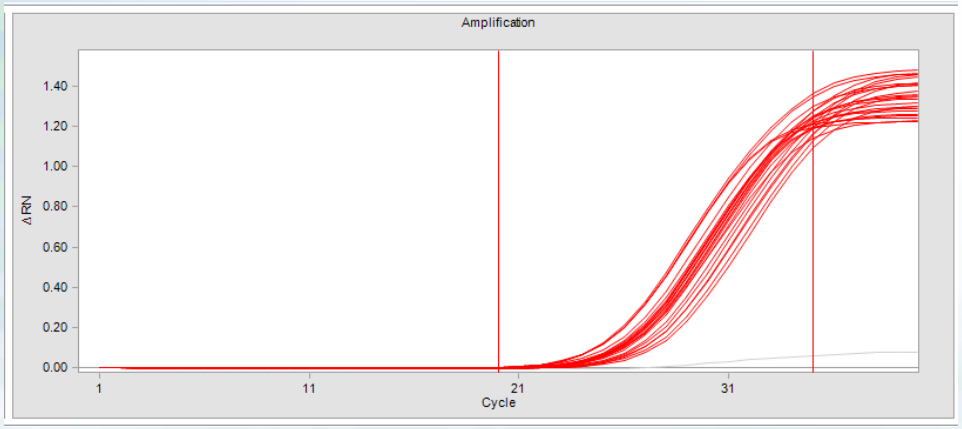
Modeling as Poisson copies per droplet = - ln (1 – p) where p = fraction of positive droplets

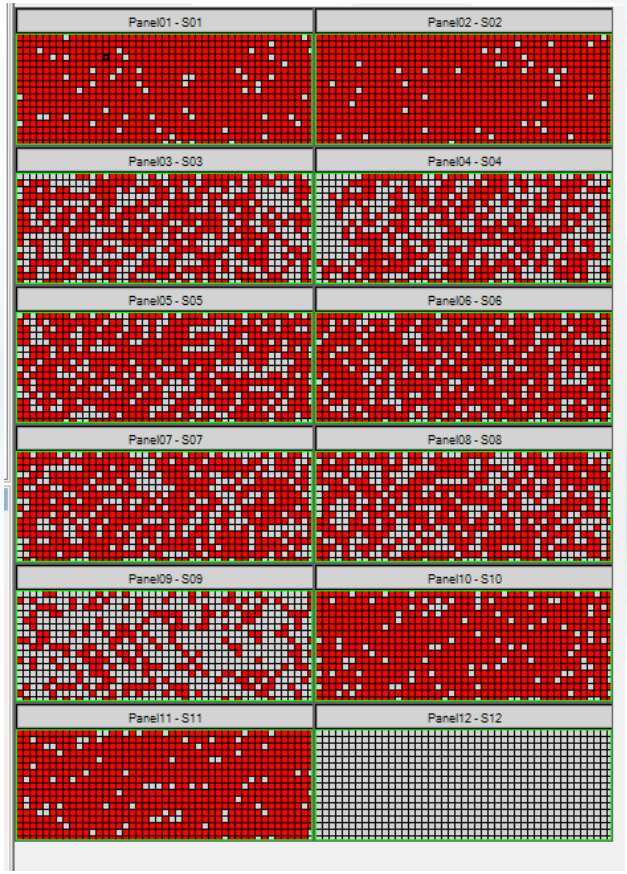
Digital PCR - arrays





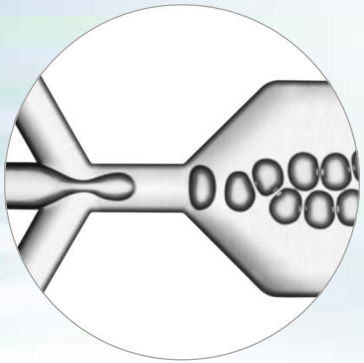
Digital PCR – arrays results



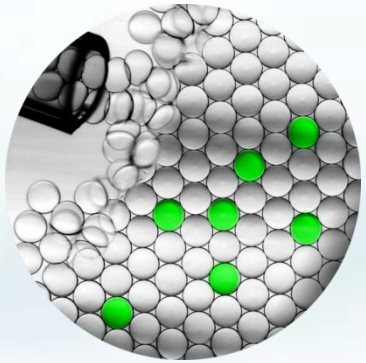


Digital PCR - droplets

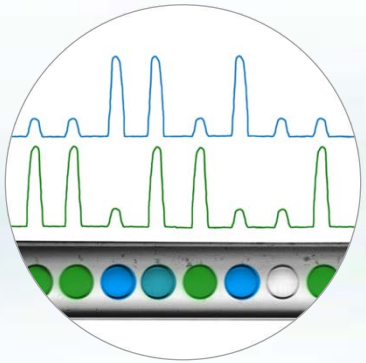
Digital PCR - droplets



Make Droplets



PCR Droplets

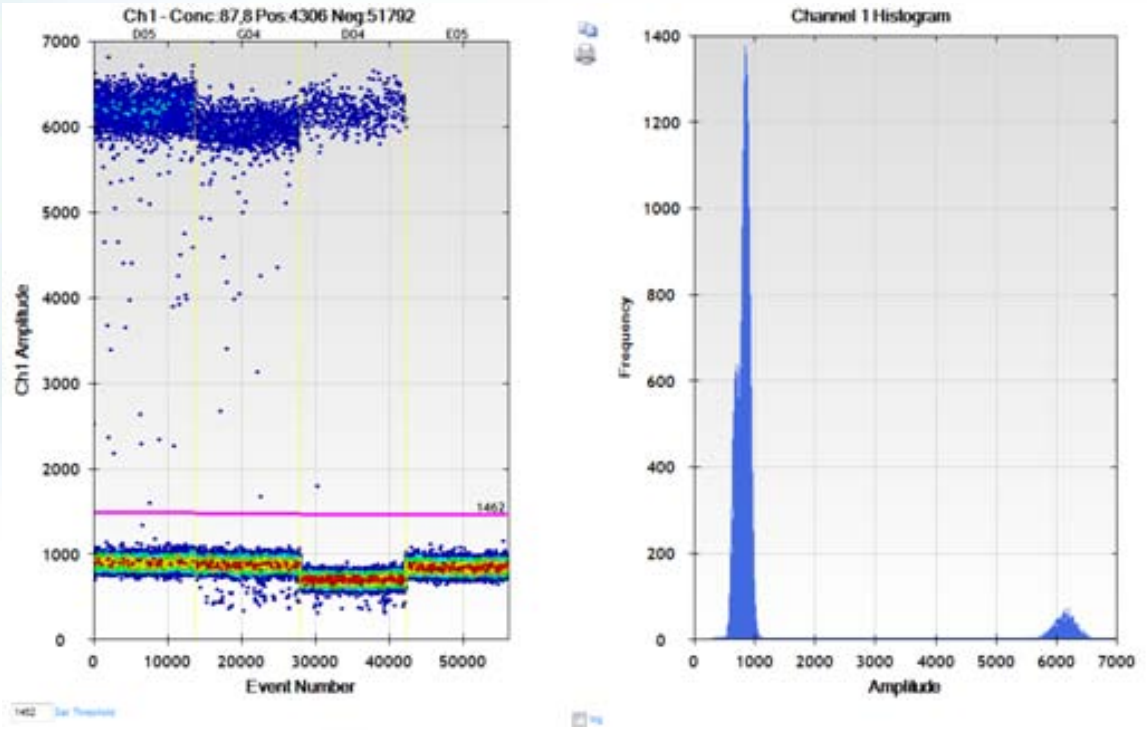


Read Droplets

“X” target copies

Results

Digital PCR – droplets results



Why going digital?

- Direct measurement: no need for standard or calibration curve (stil needed for development/ validation/verification of the method)
- No more matrix effect between calibrant and sample
- End-point: less impact of amplification efficiency
- Quantification from absolute transgene and endogene copies:
  - no conversion factor
  - in accordance with EU recommendations
- Quantification at low target levels (e.g. LLP)

Digital PCR - arrays

- GMO quantification already evaluated in chamber digital PCR:
  - Duplex possible: direct transgene/endogene ratio determination, lower uncertainty
  - Good sensitivity: <10 copies
  - Acceptable limit of quantification: 15-65 copies
  - 765 partitions (microfluidics).
    - Limited dynamic range: 2-3 logs.
    - Need to pre-determine concentration
    - Less room for duplex
    - ↑ replicates for ↓ uncertainty
  - High cost

Bhat *et al.*, Anal. Bioanal. Chem. 2009, 394
Corbisier *et al.*, Anal. Bioanal. Chem. 2010, 396
Burns *et al.*, Eur. Food and Res. Technology 2010, 231

Digital PCR - droplets

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PLOS ONE

Quantitative Analysis of Food and Feed Samples with Droplet Digital PCR

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Abstract

In this study, the applicability of droplet digital PCR (ddPCR) for routine analysis in food and feed samples was demonstrated with the quantification of genetically modified organisms (GMOs). Real-time quantitative polymerase chain reaction (qPCR) is currently used for quantitative molecular analysis of the presence of GMOs in products. However, its use is limited for detecting and quantifying very small numbers of DNA targets, as in some complex food and feed matrices. Using ddPCR duplex assay, we have measured the absolute numbers of MON810 transgene and *hmg* maize reference gene copies in DNA samples. Key performance parameters of the assay were determined. The ddPCR system is shown to offer precise absolute and relative quantification of targets, without the need for calibration curves. The sensitivity (five target DNA copies) of the ddPCR assay compares well with those of individual qPCR assays and of the chamber digital PCR (cdPCR) approach. It offers a dynamic range over four orders of magnitude, greater than that of cdPCR. Moreover, when compared to qPCR, the ddPCR assay showed better repeatability at low target concentrations and a greater tolerance to inhibitors. Finally, ddPCR throughput and cost are advantageous relative to those of qPCR for routine GMO quantification. It is thus concluded that ddPCR technology can be applied for routine quantification of GMOs, or any other domain where quantitative analysis of food and feed samples is needed.

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Droplet digital PCR for routine GMO quantification

- ddPCR satisfies all parameters listed by current (and future) EURL-GMFF guidelines: Precision, accuracy, LOD, LOQ, dynamic range.
- It is applicable for routine quantification and practical (throughput, price, complexity)
- No standard curve
- Easier/faster to calculate %GMO
- Better harmonization
- Combined with real time PCR (screening/identification)