Droplet Digital PCR

Publications for Food Applications

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GMO Testing

Iwobi et al. (2016)
Droplet digital PCR for routine analysis of genetically modified foods (GMO) – A comparison with real-time quantitative PCR. Food Control. Nov;69(1):205-213. doi: 10.1016/ j.foodcont.2016.04.048.

In this work, the authors applied ddPCR on selected GM-food and feed samples that were recently analyzed on the qPCR platform in inter-laboratory proficiency tests and showed good performance of the ddPCR method. Applying ddPCR, different concentrations of GM material, specifically transgene DNA at different levels (.1-1%) useful as reference DNA, were generated from 1% non GM material and 1% transgene plant material respectively, and key performance parameters of the ddPCR assay evaluated. ddPCR performed well, indicating its suitability for the production of reference GM materials.

Koppel et al. (2015)
Rapid establishment of droplet digital PCR for quantitative GMO analysis. European Food Research and Technology 241: 4,521-527 doi.org/10.1007/s00217-015-2475.

A detailed study of how different parameters of the ddPCR workflow affect the quantification of genetically modified plant traits. This group found little optimization is required to develop a robust ddPCR assay. Furthermore, they found ddPCR produced more accurate results than qPCR, presumably because ddPCR eliminates the need for reference materials, which contributes to variability in the results.

Morisset D et al. (2013)
Quantitative Analysis of Food and Feed Samples with Droplet Digital PCR. PLOS ONE. May;8(5):291-3. doi: 10.1371/journal.pone.0062583.

In this study, the applicability of ddPCR for routine analysis in food and feed samples was demonstrated with the quantification of genetically modified organisms (GMOs). The ddPCR system is shown to offer precise absolute and relative quantification of targets, without the need for calibration curves. 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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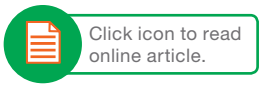

Niu et al. (2018)
Ultrasensitive Single Fluorescence-Labeled Probe-Mediated Single Universal Primer–Multiplex–Droplet Digital Polymerase Chain Reaction for High-Throughput Genetically Modified Organism Screening. Analytical Chemistry. Apr 13;90 (9):5586-5593. doi: 10.1021/acs.analchem.7b03974

This fiveplex screening method is a new development in GMO screening. Utilizing an optimal amplification assay, the specificity, limit of detection (LOD), and limit of quantitation (LOQ) were validated. The LOD and LOQ of this GMO screening method were 0.1% and 0.01%, respectively, with a relative standard deviation (RSD) < 25%. This method could serve as an important tool for the detection of GM maize from different processed, commercially available products. Further, this screening method could be applied to other fields that require reliable and sensitive detection of DNA targets.

Dobnik et al. (2014)
Multiplex Droplet Digital PCR Protocols for Quantification of GM Maize Events, in Karlin-Neumann, George; Bizouarn, Frank (eds.). Digital PCR: Methods and Protocols, Chapter 5. May:69-98. doi: 10.1007/978-1-4939-7778-9.

Two different protocols for multiplex quantification of GM maize events are describe in this chapter: (1) nondiscriminative multiplex quantification of GM maize targets as a group, i.e. per ingredient or plant species (multiplex quantification per ingredient, MQI) and (2) discriminative multiplex quantification of individual GM events (multiplex event quantification, MEQ).

Meat Speciation & Authenticity

Ren J et al. (2017)
A digital PCR method for identifying and quantifying adulteration of meat species in raw and processed food. PLOS ONE. Mar2; 12(3): e0173567. doi: 10.1371/journal.pone.0173567

In this paper, a new ddPCR method was developed for the quantitative determination of the presence of chicken in sheep and goat meat products. Meanwhile, a constant (multiplication factor) was introduced to transform the ratio of copy numbers to the proportion of meats.

Floren et al. (2015)
Species identification and quantification in meat and meat products using droplet digital PCR (ddPCR). Food Chemistry. Apr 15;173(1): 1054-1058. doi: 10.1016/j.foodchem.2014.10.138

The authors describe a reliable two-step ddPCR assay targeting the nuclear F2 gene for precise quantification of cattle, horse, and pig in processed meat products. The ddPCR assay is advantageous over qPCR showing a limit of quantification (LOQ) and detection (LOD) in different meat products of 0.01% and 0.001%, respectively. The specificity was verified in 14 different species. Hence, determining F2 in food by ddPCR can be recommended for quality assurance and control in production systems.

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Shehata et al. (2019)
Re-visiting the occurrence of undeclared species in sausage products sold in Canada. Food Research International. Jan 15. doi: 10.1016/j.foodres.2019.01.030.

In this study, the authors collaborated with the Canadian Food Inspection Agency. First, all samples were tested using DNA barcoding using universal primers, which revealed that 97% of the samples contained the declared species, presumably as the predominant species. Second, all samples were tested using ddPCR assays specifically targeting beef, pork, chicken, and turkey, which revealed that five beef samples, three chicken samples and two turkey samples contained undeclared species. Additionally, ddPCR revealed the presence of undeclared sheep in five samples. The results from this study can be used to support decision-making processes for future inspection and monitoring activities in order to control species substitution or adulteration to protect consumers.

Cai et al. (2014)
Quantitative Analysis of Pork and Chicken Products by Droplet Digital PCR. BioMed Research International. Aug;2014:6 pages. doi: 10.1155/2014/810209.

In this project, a highly precise quantitative method based on the ddPCR technique was developed to determine the weight of pork and chicken in meat products. The quantitative data indicated that ddPCR is highly precise in quantifying pork and chicken in meat products and therefore has the potential to be used in routine analysis by government regulators and quality control departments of commercial food and feed enterprises.

Köppel et al. (2019)
Duplex digital PCR for the determination of meat proportions of sausages containing meat from chicken, turkey, horse, cow, pig and sheep. European Food Research and Technology. April;245(4):853-862. doi: 10.1007/s00217-018-3220-3.

The authors present the measurement of proportions of beef, pork, chicken, turkey, sheep and horse meat in a cooked sausage matrix and a procedure to calculate the proportions (w/w) based on target DNA concentrations measured using ddPCR. Six laboratories applied these methods and determined the w/w proportions of 20 sausage samples. It was shown that these methods in conjunction with conversion factors can be used to estimate meat proportions in mixed meat products with superior accuracy and precision compared to results generated by real-time PCR.

Microbial Detection & Enumeration

Jang et al. (2017)
Droplet-based digital PCR system for detection of single-cell level of foodborne pathogens. BioChip Journal. Dec 12;11(4):329-337. doi: 10.1007/s13206-017-1410-x.

The authors show in the report a ddPCR system which allows identifying single-cell level of foodborne pathogens. E. coli O157:H7 and *Salmonella* cells were selected as model bacterial foodborne pathogens. The ddPCR system could be a useful platform for the quantitative detection of foodborne pathogens without any pretreatment process.

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Witte et al. (2016)
Evaluation of the performance of quantitative detection of the *Listeria monocytogenes prf*A locus with droplet digital PCR. Analytical and Bioanalytical Chemistry. Aug;408(27): 7583–7593. doi: 10.1007/s00216-016-9861-9.

Using the *Listeria monocytogenes* specific *prf*A assay, authors focused on the questions of whether the assay was directly transferable to ddPCR and whether ddPCR was suitable for samples derived from heterogeneous matrices, such as foodstuffs that often included inhibitors and a non-target bacterial background flora. Moreover, results demonstrated the outstanding detection limit of one copy. However, while poorer DNA quality, such as resulting from longer storage, can impair ddPCR, *internal amplification control* (IAC) of *prf*A by ddPCR, that is integrated in the genome of *L. monocytogenes Δprf*A, showed even slightly better quantification over a broader dynamic range.

Creminoesi et al. (2016)
Development of a Droplet Digital Polymerase Chain Reaction for Rapid and Simultaneous Identification of Common Foodborne Pathogens in Soft Cheese. Frontiers in Microbiology. 7:1725. doi: 10.3389/fmicb.2016.01725.

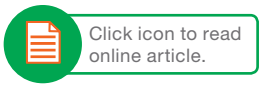
Researchers developed an accurate quantitative protocol based on ddPCR involving eight individual TaqMan reactions to detect simultaneously, without selective enrichment, *Listeria* spp., *L. monocytogenes, Salmonella* spp., verocytotoxin-producing *E. coli* and *Campylobacter* spp. in cheese.

Wang et al. (2018)
Comparison between digital PCR and real-time PCR in detection of *Salmonella* Typhimurium in milk. International Journal of Food Microbiology. Feb 2; 266:251-256. doi: 10.1016/j.ijfoodmicro.2017.12.011.

To compare the effect between qPCR and ddPCR in detecting *Salmonella* Typhimurium, a series of nucleic acid, pure strain culture and spiking milk samples were applied and the resistance to inhibitors referred in this article as well. Compared with qPCR, ddPCR exhibited more sensitive (10–4ng/μl or 102cfu/ml) and less pre-culturing time (saving 2 hr). Moreover, ddPCR had stronger resistance to inhibitors than qPCR, yet absolute quantification hardly performed when target's concentration over 1ng/μl or 106cfu/ml. This study provides an alternative strategy in detecting foodborne *Salmonella* Typhimurium.

Porcelato et al. (2016)
Detection and quantification of Bacillus cereus group in milk by droplet digital PCR. Journal of Microbiological Methods. Aug; 127:1-6. doi: 10.1016/j.mimet.2016.05.012.

The researchers optimized and used a new ddPCR assay for the detection and quantification of the *Bacillus cereus* group in milk. They also compared ddPCR to a standard qPCR assay. The new ddPCR assay showed a similar coefficient of determination and a better limit of detection compared to the qPCR assay during quantification of the target molecules in the samples. This new assay was then tested for the quantification of the *B. cereus* group in 90 milk samples obtained over three months from two different dairies and the milk was stored at different temperatures before sampling. The ddPCR assay showed good agreement with the qPCR assay for the quantification of the *B. cereus* group in milk, and due to its lower detection limit more samples were detected as positive.



Kim et al. (2014)
Comparison of droplet digital PCR and quantitative real-time PCR for examining population dynamics of bacteria in soil. Appl Microbiol Biotechnol. Jul;98(13):6105-13. doi: 10.1007/s00253-014-5794-4.

This proof of principle paper compares qPCR and ddPCR in the ability to quantitate changes in bacterial populations in soil over time. ddPCR proved to be more sensitive and reproducible compared to qPCR. Because absolute quantification does not require reference materials and a standard curve, ddPCR is a better tool for direct comparison of results between laboratories or experiments in ecological studies.

Other Food Applications

Mayer et al. (2019)
Quantification of the allergen soy (*Glycine max*) in food using digital droplet PCR (ddPCR). European Food Research and Technology. Feb;245(2):499-509. doi: 10.1007/s00217-018-3182-5.

The Austrian Agency for Health and Food Safety developed an in-house PCR system for the quantitation of allergens using ddPCR. ddPCR was selected for quantification for its particular advantages and the method has been validated in-house. It was found to be applicable to various matrices including meat products, flour, milk, and fatty creams, with recovery rates between 60 and 100%. The limit of detection and the limit of quantification (LOQ) are 0.16 mg/kg and 0.60 mg/kg, respectively. Repeated analysis of analyte-free food matrices spiked with reference material provided acceptable values for precision: The relative standard deviation (RSDoverall) of the whole method (including DNA extraction) is below 25%. The recovery of pure soy material (pulverized beans) was between 112.5 and 135.0%. The presented method is shown to be reliable and accurate, provided that samples and reference material are extracted and amplified in the same way.

Pierboni et al. (2018)
Digital PCR for analysis of peanut and soybean allergens in foods. Food Control. Apr;92: 128-136. Doi: 10.1016/j.foodcont.2018.04.039.

Researchers developed a digital PCR assay to detect hidden allergens in foods. They found that digital PCR is a suitable and reliable technology for peanut and soybean allergen analysis.

Bartsch et al. (2018)
Analysis of frozen strawberries involved in a large norovirus gastroenteritis outbreak using next generation sequencing and digital PCR. Food Microbiology. Dec;76:390-395. doi: 10.1016/j.fm.2018.06.019.

To provide new tools for virus detection and characterization in berries, next generation sequencing (NGS) and reverse transcription-digital PCR (RT-dPCR) techniques were tested with strawberries previously involved in a large-scale norovirus (NoV) gastroenteritis outbreak in Germany. The study shows that identification of human-pathogenic viruses in naturally contaminated frozen berries is possible using NGS technologies.

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