Absolute Quantification of Organisms by Droplet Digital PCR (ddPCR) – An Overview of the Technology and Method

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Overview

The technology behind ddPCR
Method of ddPCR
Advantages and Limitations of ddPCR
Case Study using ddPCR Technology
Applications of ddPCR for Probiotics
ATCC – Life Science Innovations That Touch People

Company highlights...

- Trusted partner to the global scientific community since 1925
- One of world’s largest, most diverse biological materials and information resource standards - the “gold standard”
- Leading global supplier of authenticated cell line, viral and microorganism standards
- An innovative R&D company
  - Gene editing, microbiome, NGS, primary cells and advanced cell models
- cGMP biorepository
- Partner with government, industry and academia
- Customer focused
  - Sales & Marketing, Customer Care Center and Tech Support, global cold chain supply
The technology behind ddPCR
What is Droplet Digital PCR (ddPCR)?

- More recent technology
- Commercial availability in 2011
- Uses water-oil emulsion to create thousands of nanoliter-sized droplets
- Key feature: Massive sample partitioning
Sample partitioning

Droplet dispensing

Sample partitioning

Random distribution of DNA or RNA molecules
Droplet Reading

1. Place on droplet reader
2. Sample Injection
3. Droplet detection
Example scatter plot

Positive droplets

Threshold

Negative droplets
Method of ddPCR
ddPCR Assay Development

Amplicon Design

Select a gene target

Common design guidelines for qPCR apply to ddPCR amplicon design

Design forward and reverse primers along with a probe
ddPCR Assay Optimization

- Perform a gradient ddPCR run
- Select the best annealing temperature
- Look for greatest separation between positive and negative events

Gradient ddPCR with temperatures decreasing left to right from 64.7°C to 55.0°C
Manual Droplet Generation

1. Prepare ddPCR reaction mix
2. Add samples and oil to cartridge
3. Droplet generation
4. Droplet dispensing
Manual Droplet Generation (continued)

Droplet aspiration → Droplet dispensing → Plate sealing → Thermal cycling
Automated Droplet Generation

Prepare ddPCR reaction mix

Add samples to sample plate

Sample plate sealing
Automated Droplet Generation (continued)
A well-designed assay will have good separation between positive and negative events.

A threshold is chosen by the biologist to separate the positive events from the negative events.
Data Analysis (continued)

- Copy number values for each sample replicate are displayed on the Setup page
- Instead of using a standard curve like qPCR, ddPCR uses Poisson statistics to determine the absolute copy number of the sample
- Copy number provided by software is multiplied by ddPCR dilution factor and serial dilution factor to obtain copy number of starting sample

Example: \[1236 \times 10,000 \times 4 = 4.94 \times 10^7 \text{ copies/µL}\]
Advantages and Limitations of ddPCR
Advantages of ddPCR

- Absolute quantification of a sample
- Massive partitioning of sample template
- Greatly enhanced sensitivity
- Two optical channels allow for multiplexing
- Isn’t dependent on amplification efficiency
- Capable of high-throughput sample analysis
Limitations of ddPCR

- Need a single copy gene or known number of gene copies in genome
- ddPCR of organisms with large genomes, multiple chromosomes or polyploid cells
- Range of detection for the droplet reader
- Extraction method used could be a limitation
- Droplets are unstable and can easily rupture prior to PCR amplification
Case study using ddPCR technology

ddPCR is used for production of the virome product, MSA-2008

Used during production to determine genome copy number of extracted DNA and RNA

Used for QC of final product

This same method could be applied to the Probiotics industry to make standard controls for production processes
Applications of ddPCR for Probiotics

Strain-specific quantification of organisms used in the production of probiotics

- For the production of poultry feed, it is critical to measure the amount of the active strain after addition of the probiotic product to the feed.
- Previous research has found that the use of ddPCR might be a better method compared to the qPCR method currently being used (Raurich et al., 2019)\(^1\).

Quantification of probiotic products throughout the production process

- A study compared ddPCR to commonly used quantification methods of plate counting and flow cytometry for the quantification of viable probiotics (Hansen et al., 2020)\(^2\).
- All three methods were comparable in quantifying viable concentrations.

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Key Points of today’s presentation

- Greatly enhanced sensitivity
- Strain-specific absolute quantification
- Massive partitioning
- High-throughput capabilities
Thank You

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