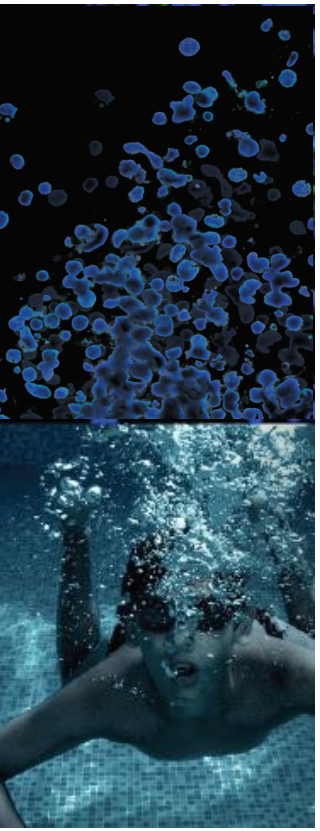
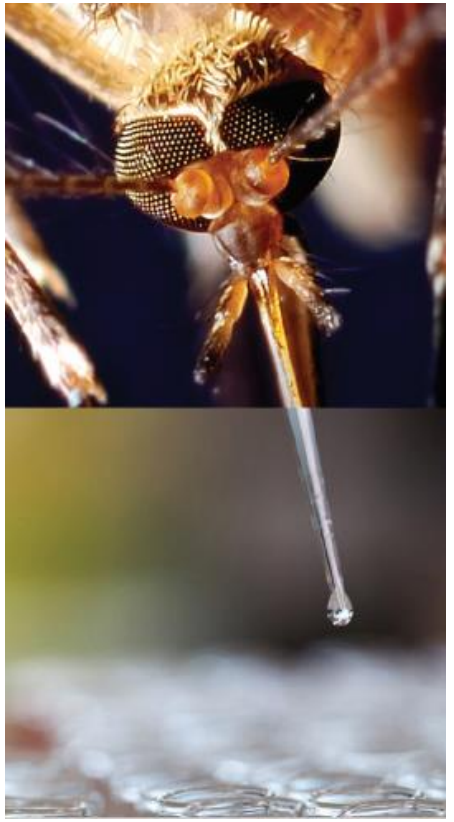
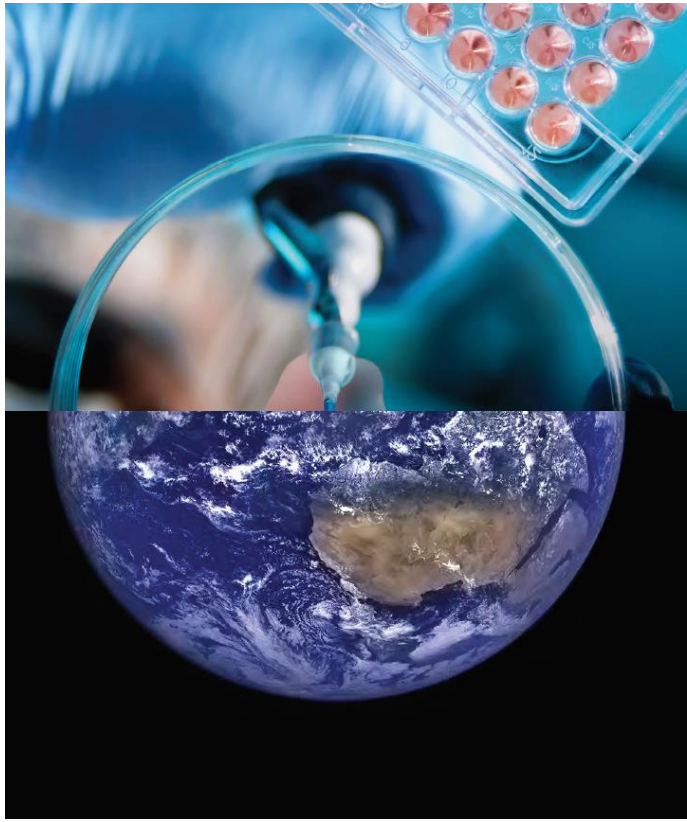
Absolute Quantification of Organisms by Droplet Digital PCR (ddPCR) – An Overview of the Technology and Method

Michael Geimer
October 6, 2022







Credible Leads to Incredible™

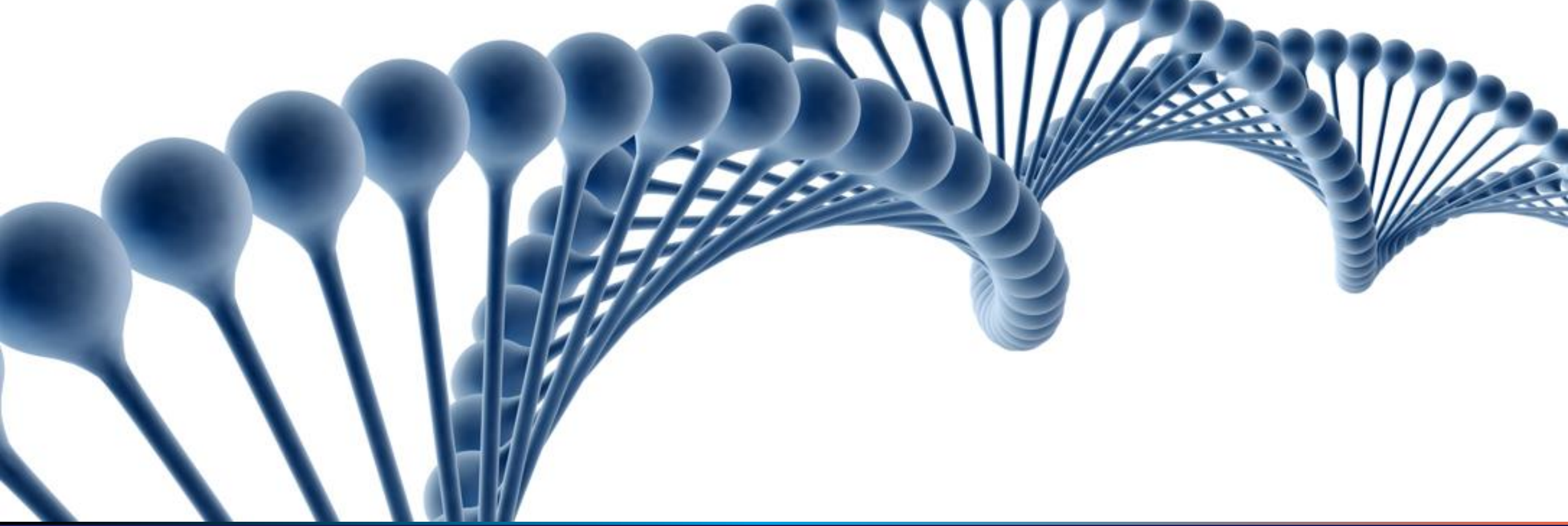
Overview

- 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*

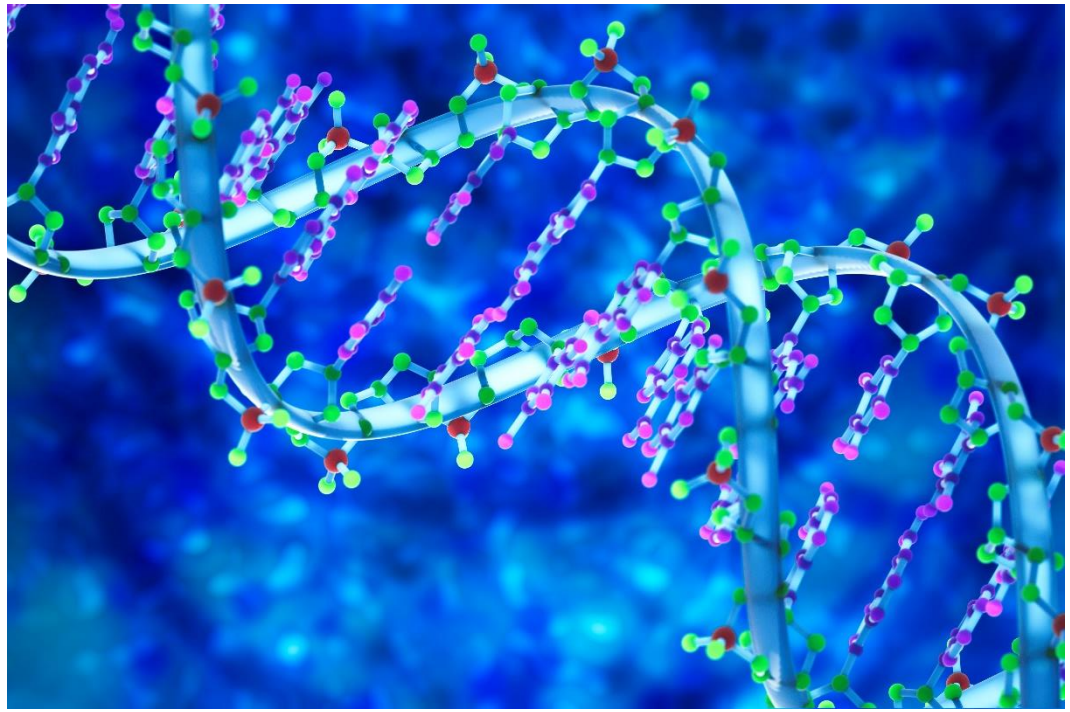
Company highlights…

- 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)?

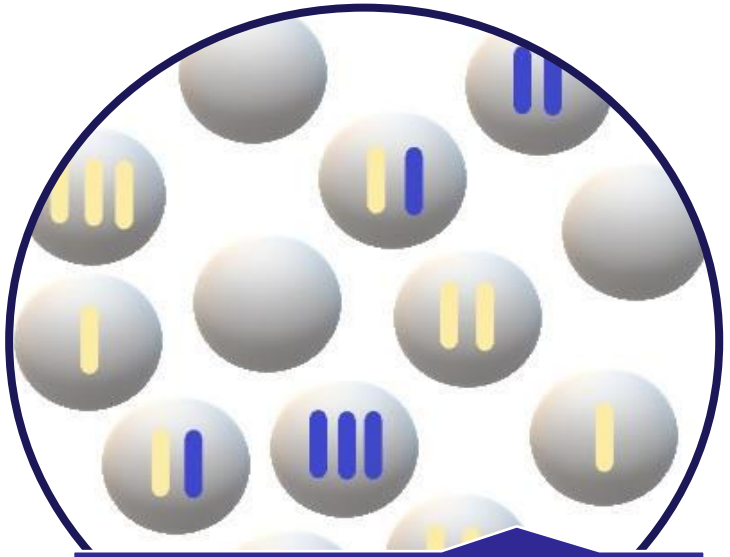


- More recent technology
- Commercial availability in 2011
- Uses water-oil emulsion to create thousands of nanoliter-sized droplets
- Key feature: Massive sample partitioning

Sample partitioning

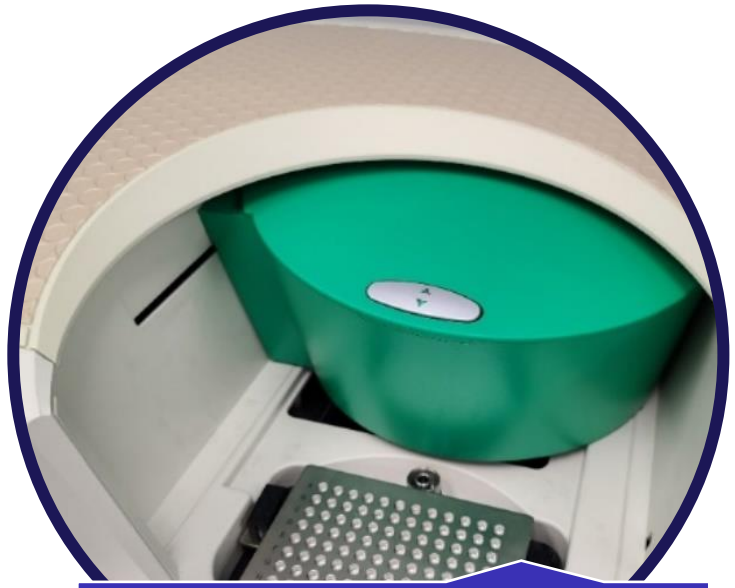
Droplet dispensing

Sample partitioning



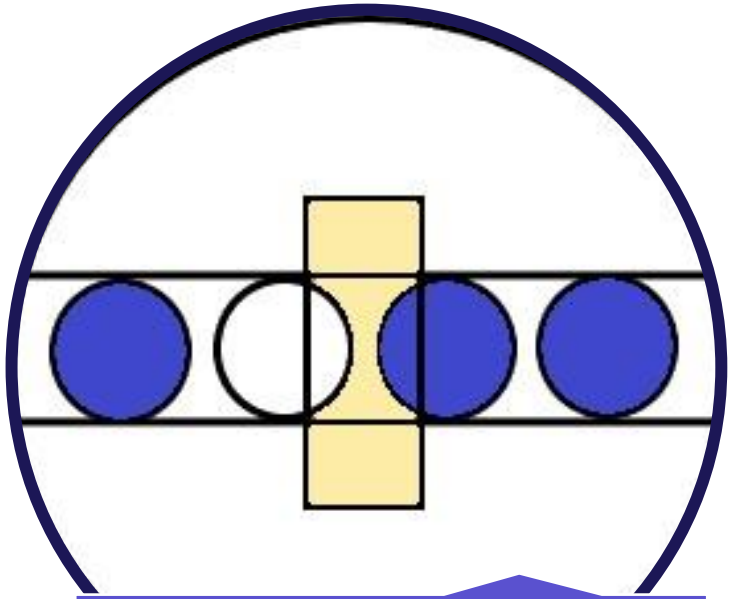
Random distribution of DNA or RNA molecules

Droplet Reading



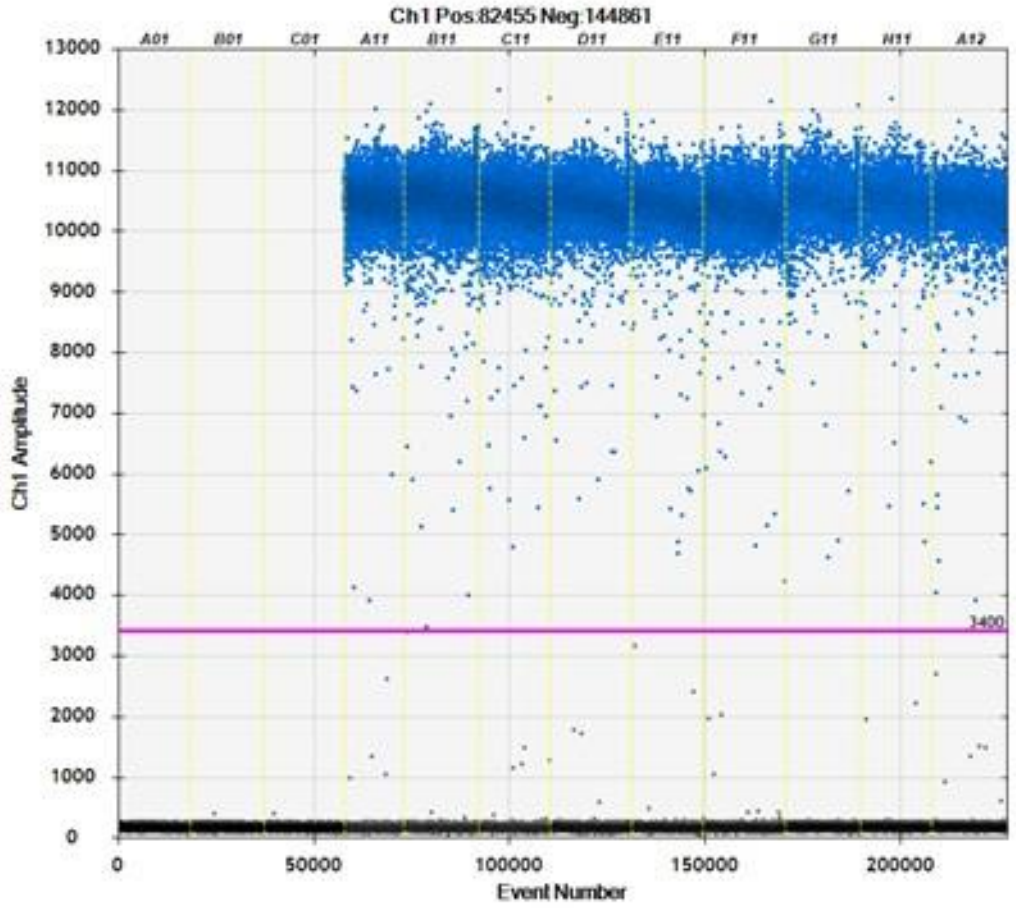
Place on droplet reader

Sample Injection



Droplet detection

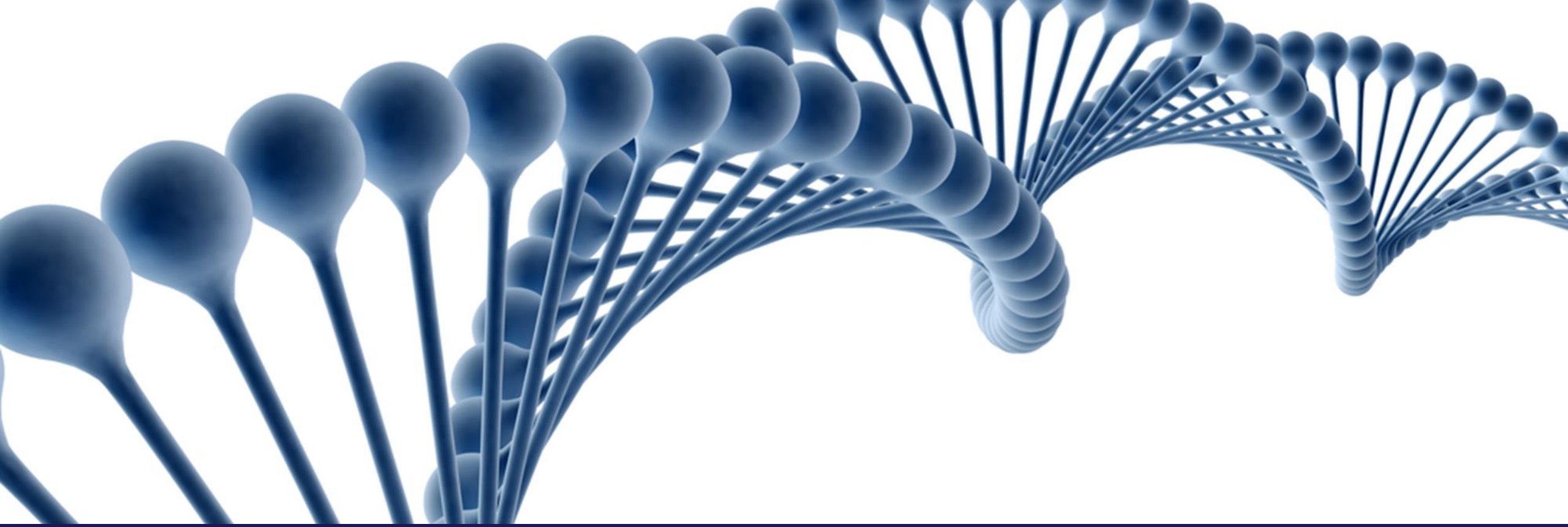
Example scatter plot



Positive droplets

Threshold

Negative droplets



Method of ddPCR

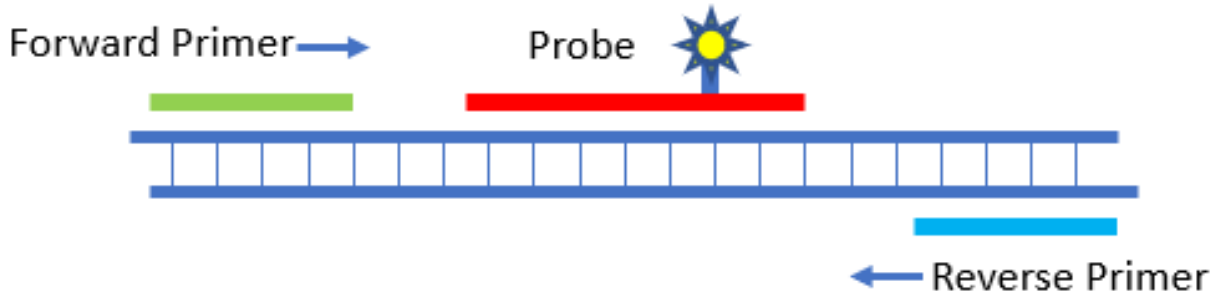
ddPCR Assay Development

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

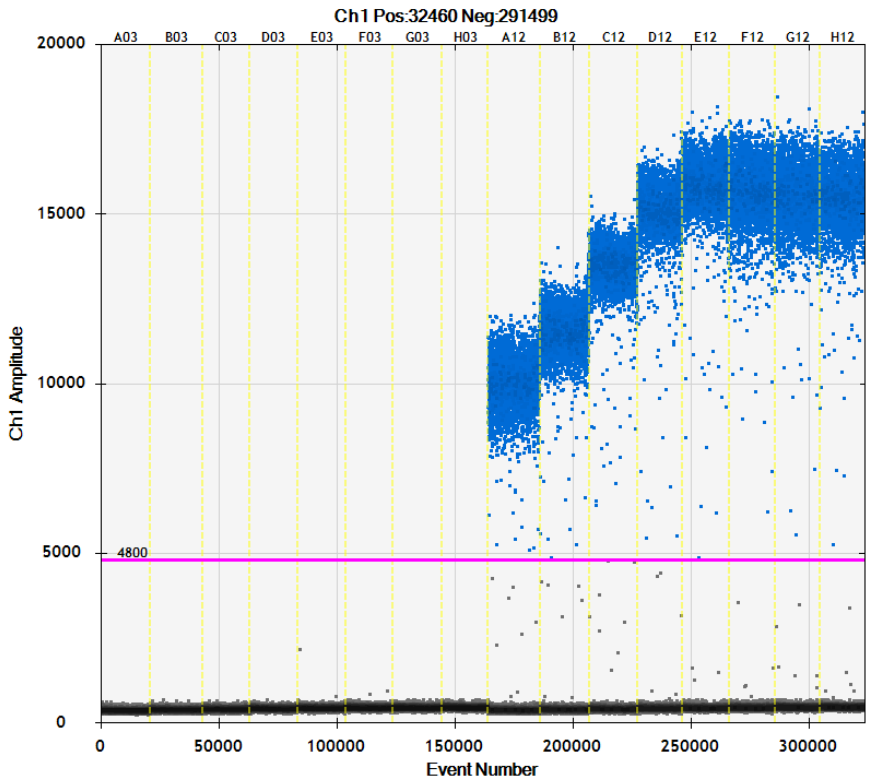


ddPCR Assay Optimization

Perform a gradient ddPCR run

Select the best annealing temperature

Look for greatest separation between positive and negative events



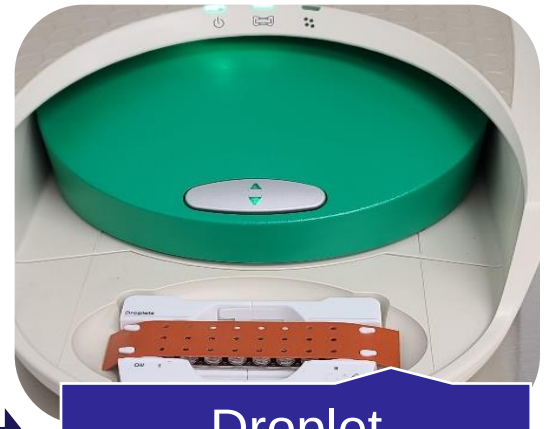
Well A12: 64.7°C
Well B12: 64.1°C
Well C12: 62.8°C
Well D12: 61.0°C
Well E12: 58.9°C
Well F12: 57.0°C
Well G12: 55.7°C
Well H12: 55.0°C

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

Manual Droplet Generation

Prepare ddPCR reaction mix

Add samples and oil to cartridge



Droplet generation

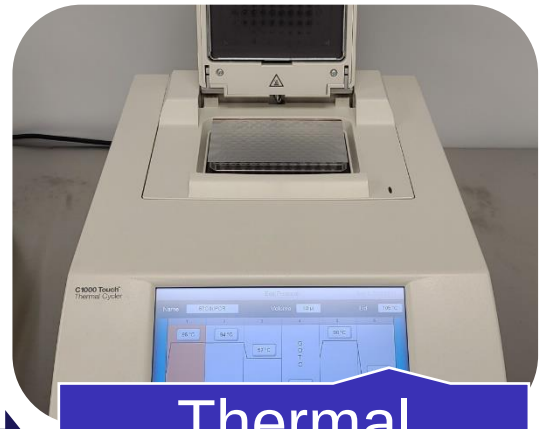
Droplet dispensing

Manual Droplet Generation (continued)

Droplet aspiration

Droplet dispensing

Plate sealing

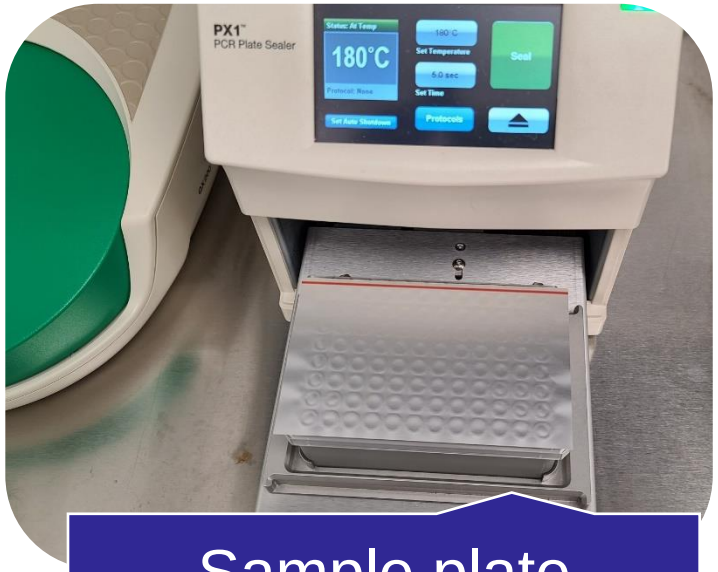


Thermal cycling

Automated Droplet Generation

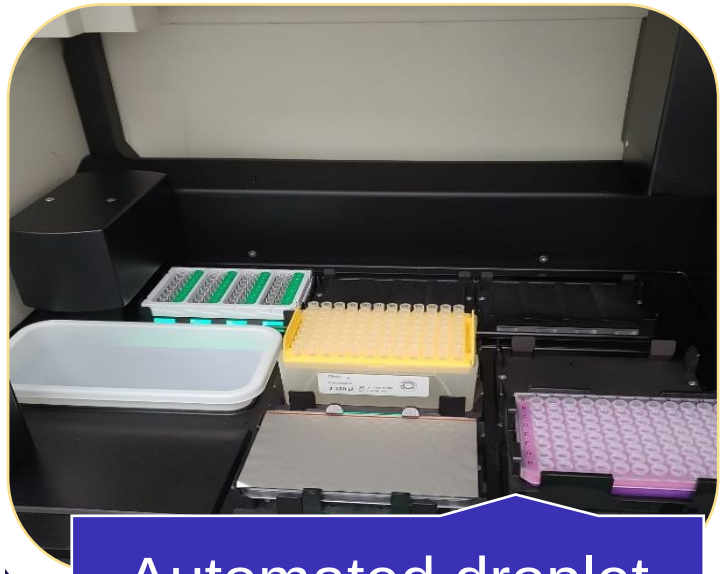
Prepare ddPCR reaction mix

Add samples to sample plate



Sample plate sealing

Automated Droplet Generation (continued)



Automated droplet generation

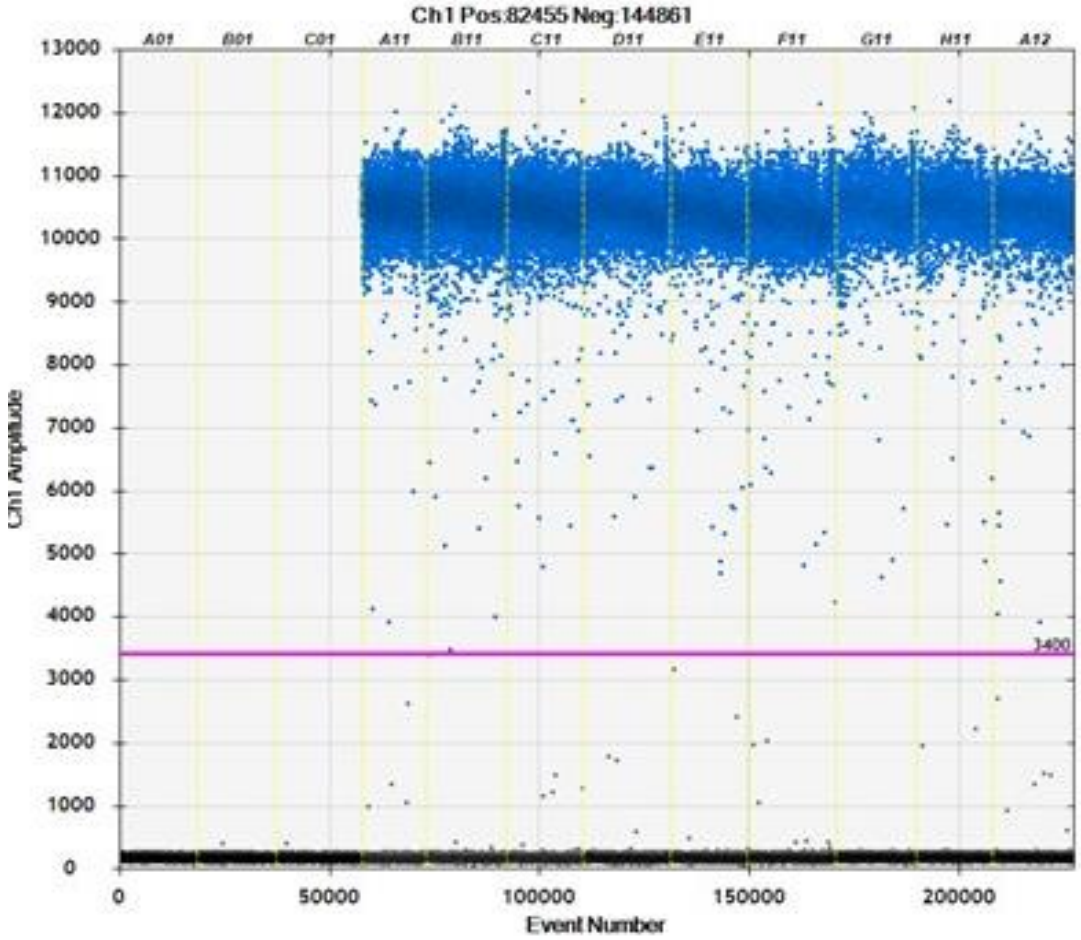
Plate sealing

Thermal cycling

Data Analysis

A well-designed assay will have good separation between positive and negative events

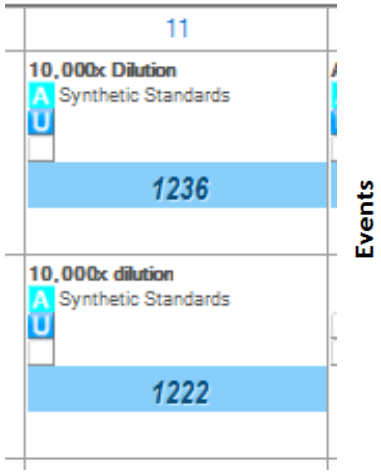
A threshold is chosen by the biologist to separate the positive events from the negative events

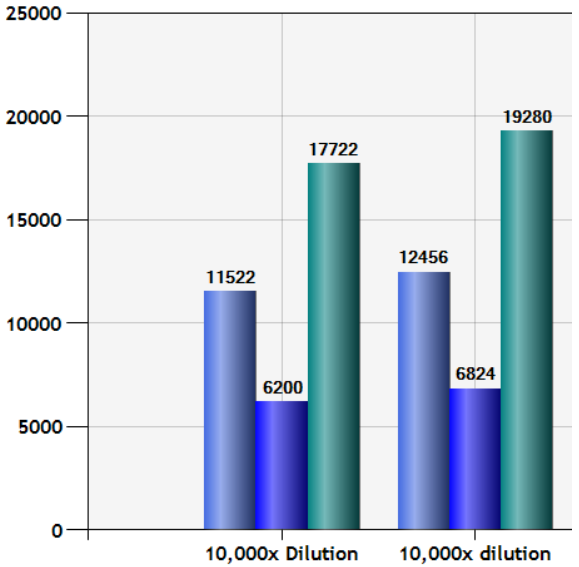


Data Analysis (continued)

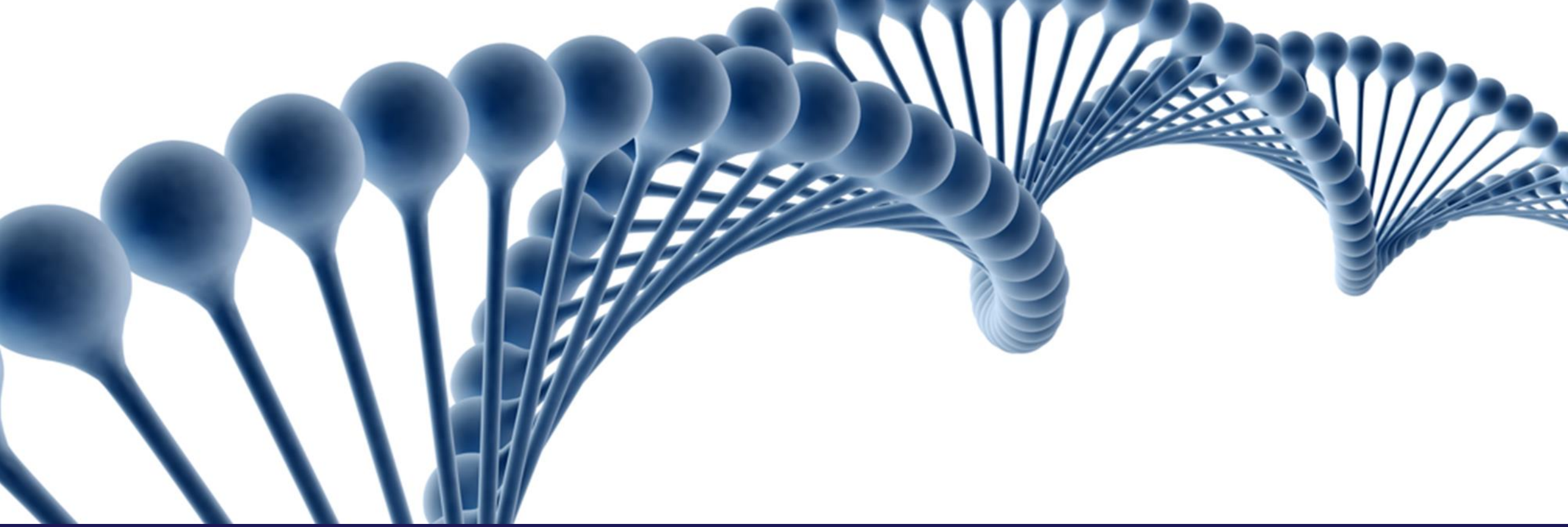
- Copy number values for each sample replicate are displayed on the Setup page
- Instead of using a standard curve like qPCR, ddPCR uses Poisson statistics to determine the absolute copy number of the sample
- Copy number provided by software is multiplied by ddPCR dilution factor and serial dilution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

Example: 1236 x 10,000 x 4 = 4.94E+07 copies/µL





Number of positive, negative, and total droplets for two sample replicates



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].

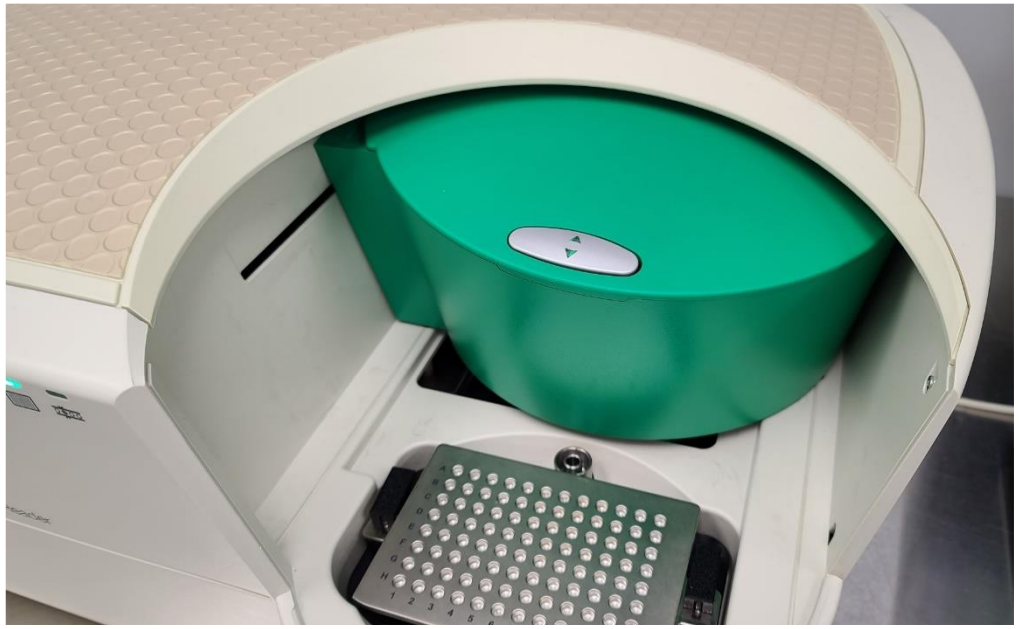
Quantification of probiotic products throughout the production process

- 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.

- [1]Raurich S, Weber B, Klose V, Mohnl M, Petri D, Fibi-Smetana S. Optimisation of a droplet digital PCR for strain specific quantification of a probiotic Bifidobacterium animalis strain in poultry feed. J Microbiol Methods. 2019 Aug;163:105646. doi: 10.1016/j.mimet.2019.105646. Epub 2019 May 30. PMID: 31152751.
- [2]Hansen SJZ, Tang P, Kiefer A, Galles K, Wong C, Morovic W. Droplet Digital PCR Is an Improved Alternative Method for High-Quality Enumeration of Viable Probiotic Strains. Front Microbiol. 2020 Jan 22;10:3025. doi: 10.3389/fmicb.2019.03025. PMID: 32038522; PMCID: PMC6987037.

ATCC

Key Points of today's presentation



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

Thank You

Acknowledgements

- Dr. Katalin Kiss, Senior Manager, Manufacturing Science & Technology (MSAT)
- Dr. Sung-Oui Suh, Lead Scientist, MSAT
- ATCC MSAT Molecular Team
- ATCC Sales & Marketing Team