



Digital PCR -
sensitive and specific method for quantification of CTC

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BIO-RAD



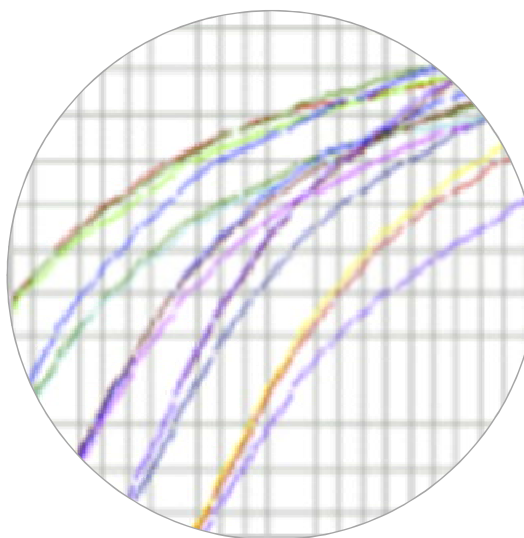
Droplet Digital PCR – The 3rd Generation of PCR

1st



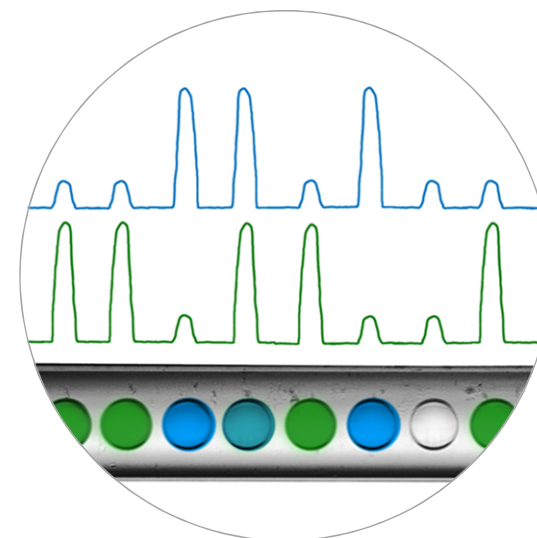
PCR
Qualitative

2nd



Real-time PCR
Relative Quantitation

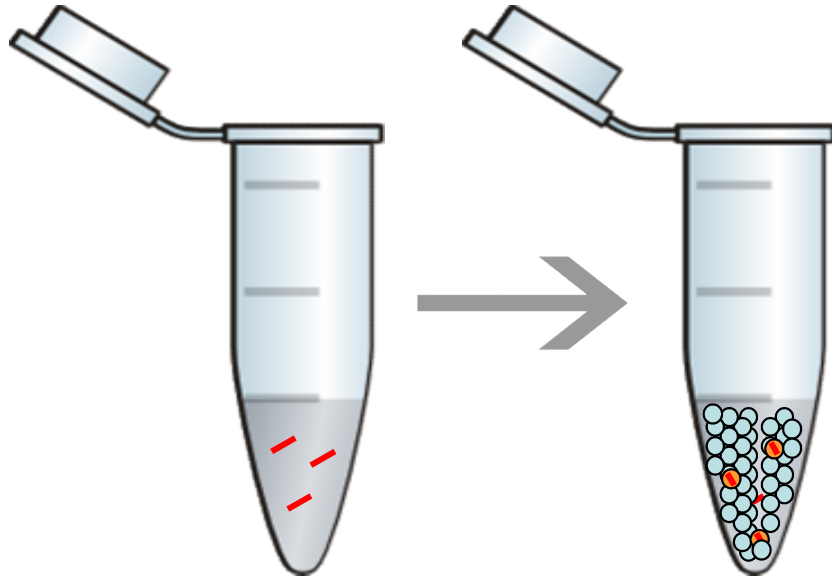
3rd



Droplet Digital PCR
Absolute Quantitation



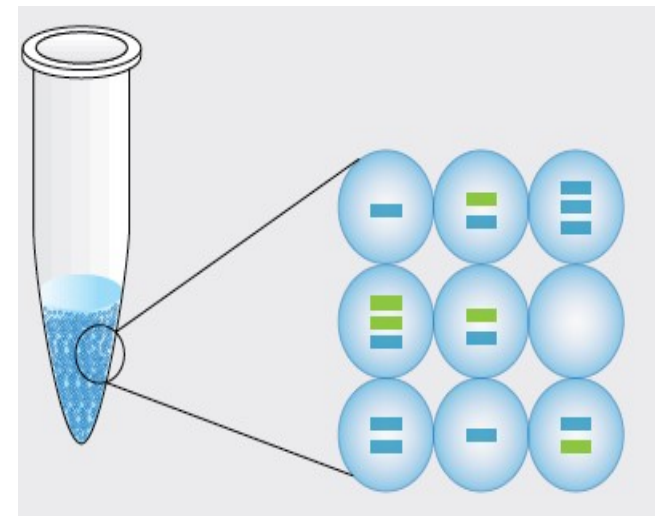
Droplets Enable Thousands of Digital Measurements



Bulk:
one
measurement

Droplets:
many thousands of
discrete measurements

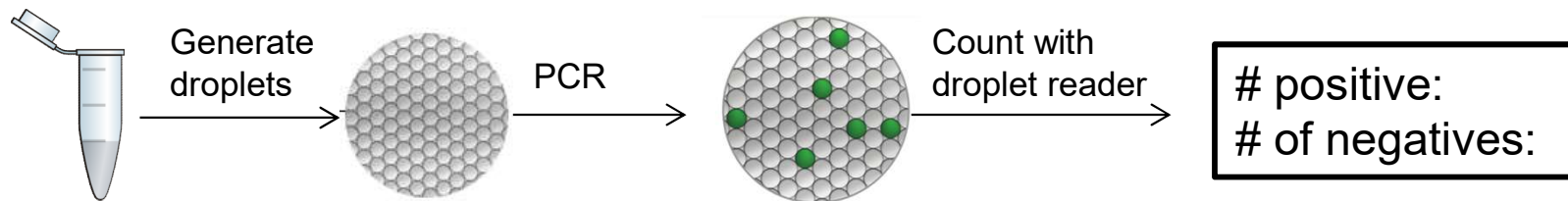
- the sample is separated into **a large number of partitions** and the reaction is carried out in each partition individually





Key Points About Droplet Digital PCR

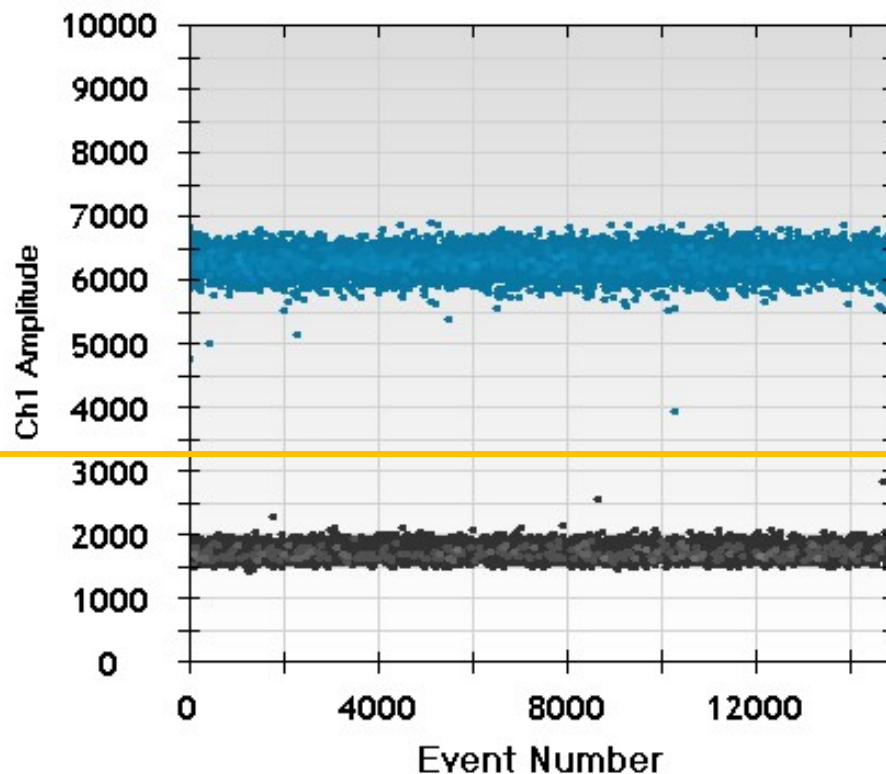
- Each droplet is an isolated reaction vessel
 - All PCR reagents are contained in each droplet volume
 - In any given droplet, target may or may not be present
- Readout:
 - Number of droplets with target (positives)
 - Number of droplets without target (negatives)
- This is an **endpoint assay**
 - Only +/- matters. 0 or 1





Droplet Readings Converted to a Digital Signal

- Positive droplets contain at least one copy of target DNA (cDNA)
- Positive droplets have increased fluorescence vs. negatives
- Software measures the number of positive and negative droplets per fluorophore per sample



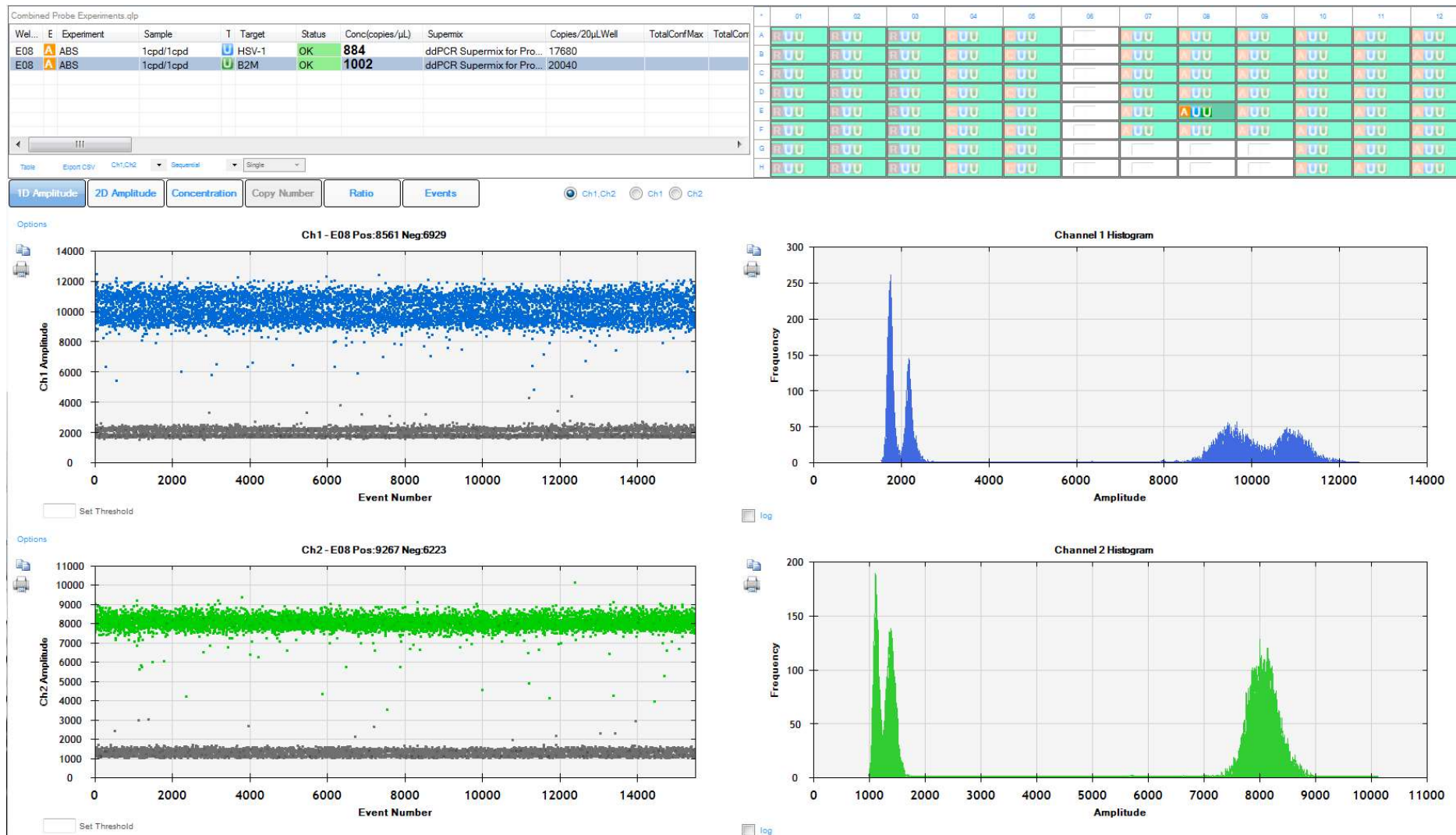
Each positives counted as 1

threshold

Each negatives counted as 0

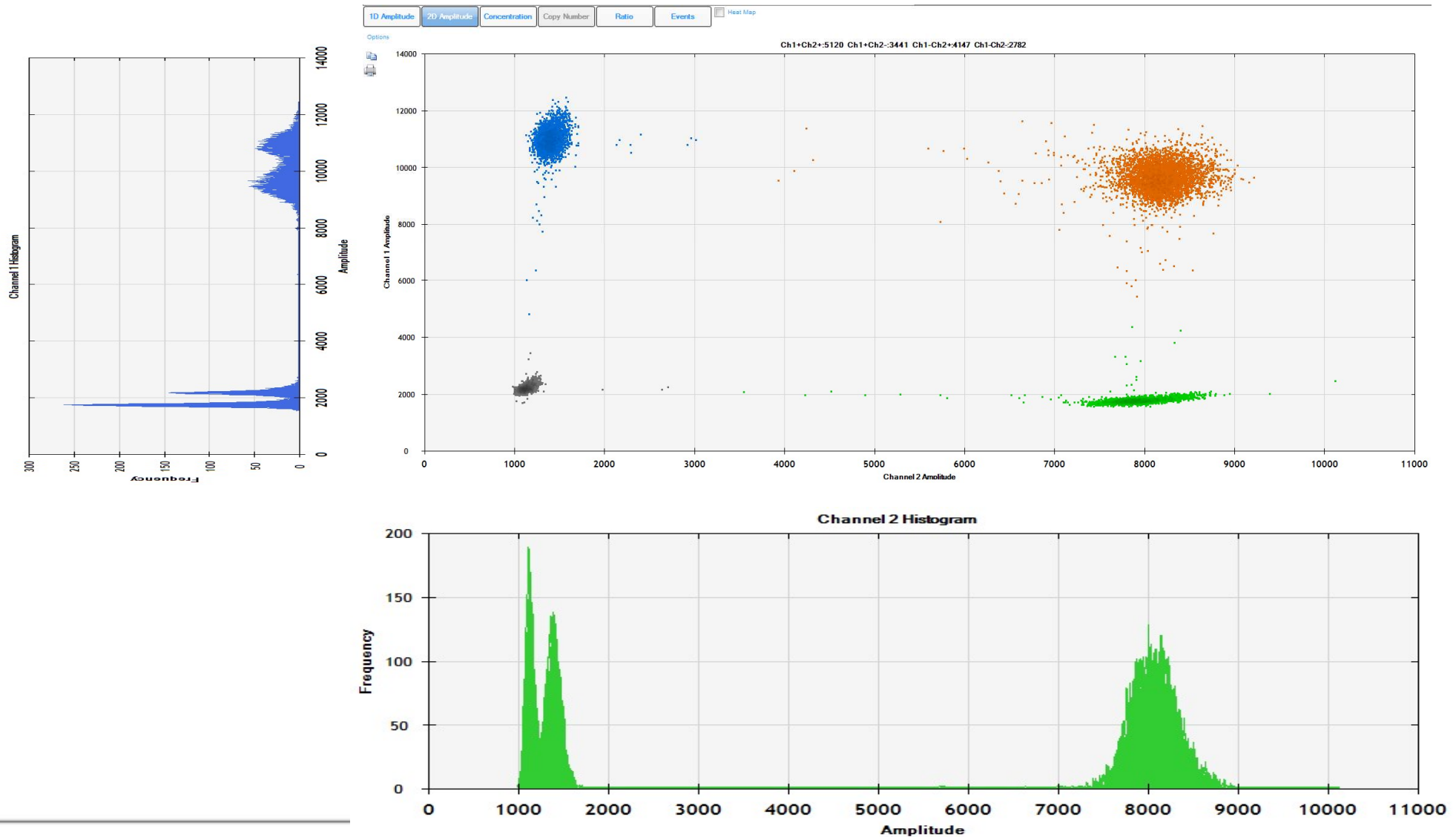


1D Amplitude





2D Amplitude (scatter plot)





Key Advantages of Droplet Digital PCR

1) Unparalleled precision (+/- 10%)

→ Detect differences as small as 0.14 Cq!

2) Enrichment of Rare Events in high backgrounds

→ Detect down to 0.001% or better

3) Absolute quantification means no standard curve or $\Delta\Delta Cq$

→ Easy to compare results from different runs or samples

4) Much more reliable at low input concentrations

→ Maintain confidence at Cq > 32

5) Less sensitive to efficiencies and inhibitors

→ Easy to compare data from run to run and gene to gene



Bio-Rad QX200™ Droplet Digital PCR System

Droplet Reader



Droplet Generator



Automated Droplet Generator

Reagents

Life Science Group |



Consumables

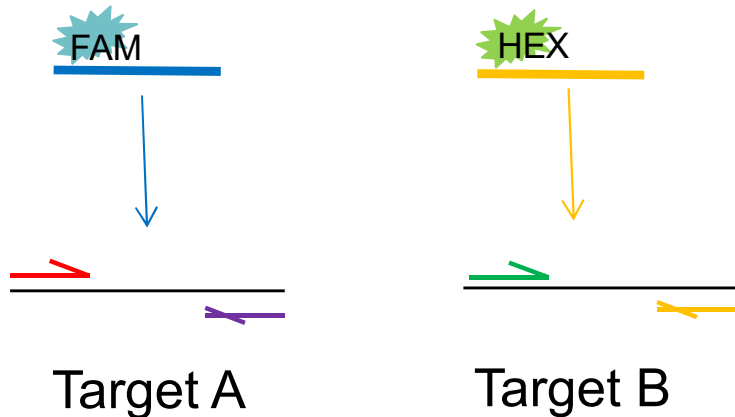




Normal Assays can be used (eg. Taqman)

Rare Sequence Detection (RSD)

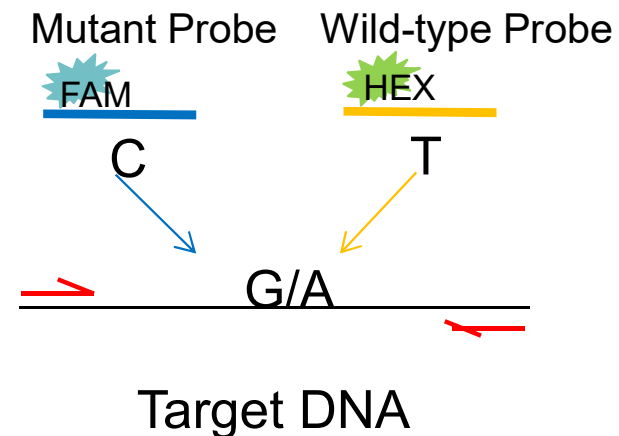
→ *analysis of sequences with NO relation to their background*



- Microbial genomes (virus, bacteria, yeast)
- Environmental studies
- GMO

Rare Mutation Detection (RMD)

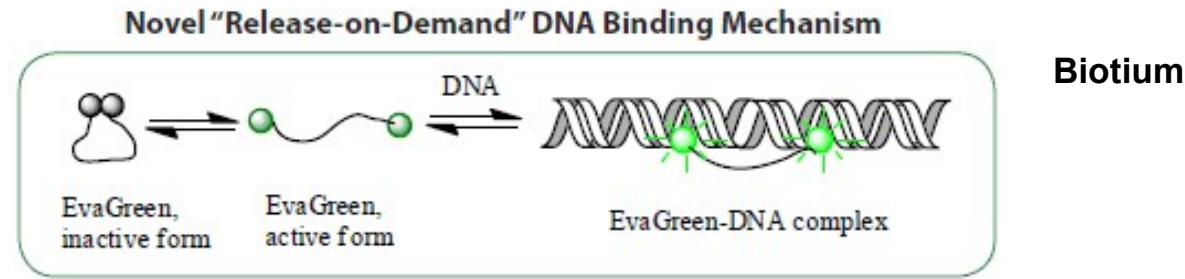
→ *analysis of sequences closely related to the background DNA (wild-type DNA)*



- Cancer mutations
- Prenatal diagnosis
- Transplanted organs



EvaGreen® Dye dsDNA Detection Capability



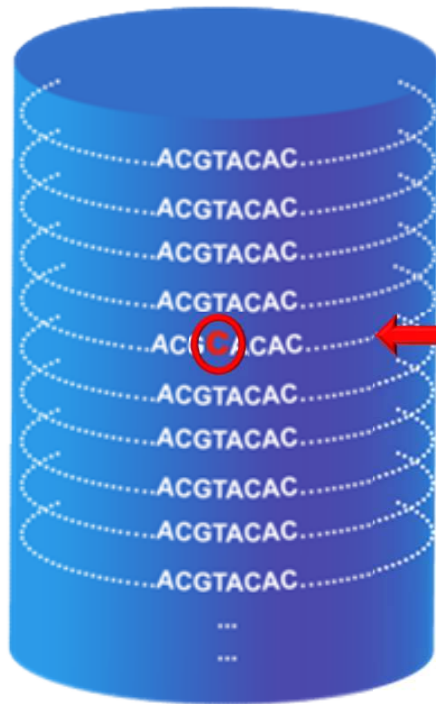
- **No preference for GC- or AT-rich sequence**
- **Less PCR inhibition than SYBR Green and lower tendency to cause nonspecific amplification**
- **Tolerated at a higher concentration = brighter signal**
- **Good stability**
- **Safety:**
 - dye is impenetrable to both latex gloves and cell membranes.
 - dye is non-cytotoxic and non-mutagenic at concentrations used in the lab.



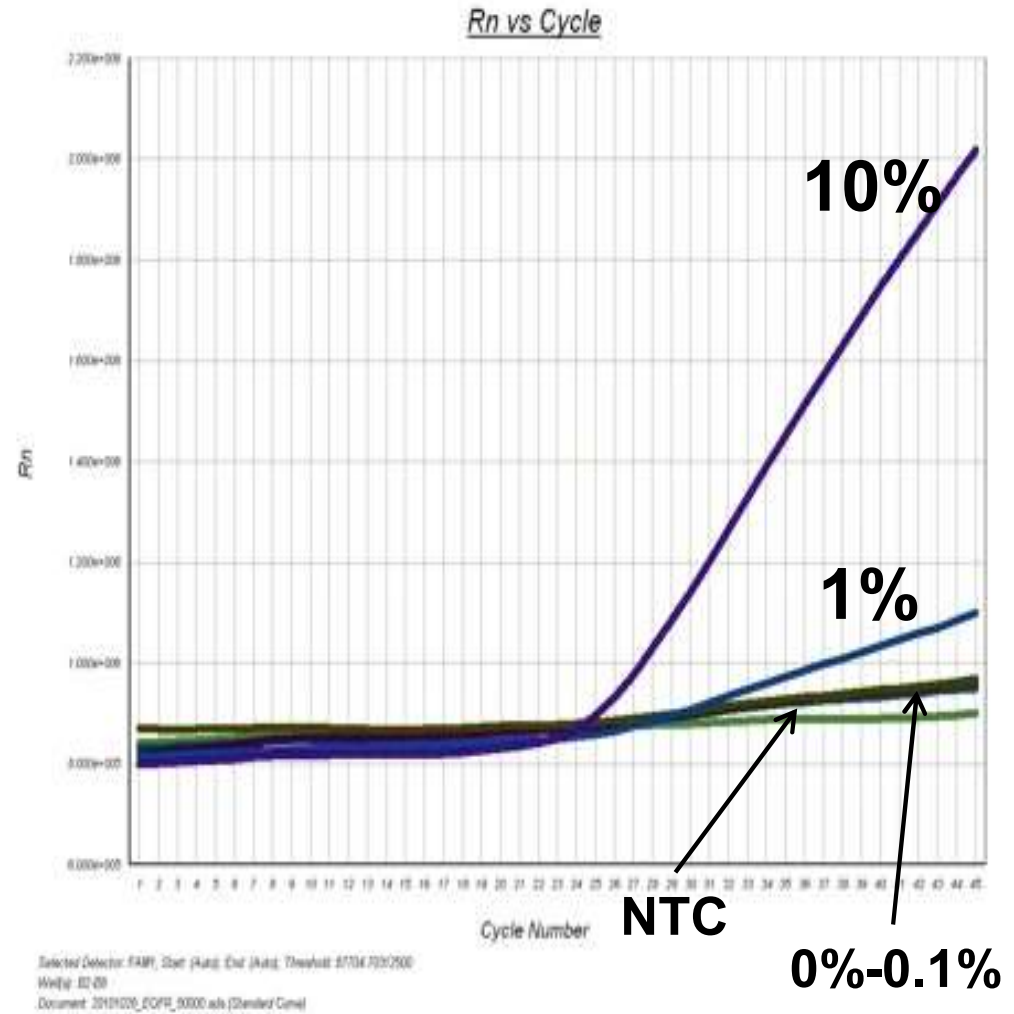
Droplet Partitioning Increases Mutant Abundance

Bulk Sample – 20 μ L

40,000 wildtype molecules
40 mutant molecules



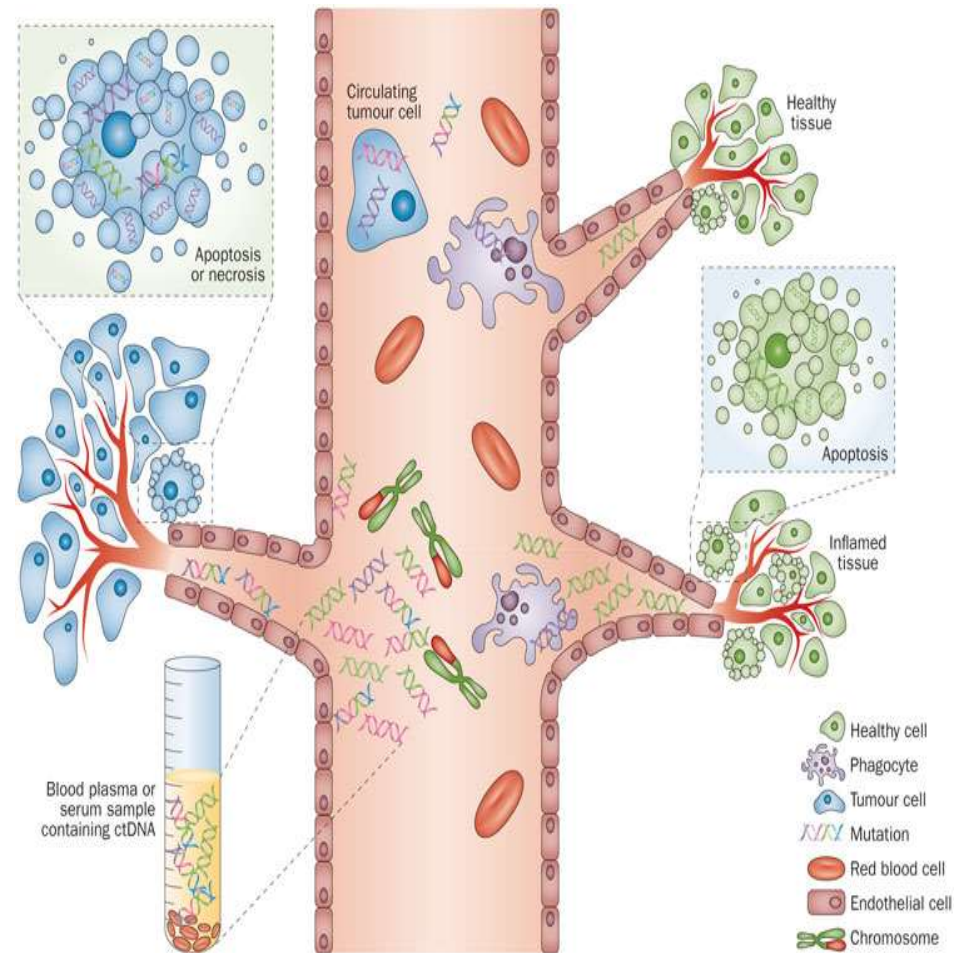
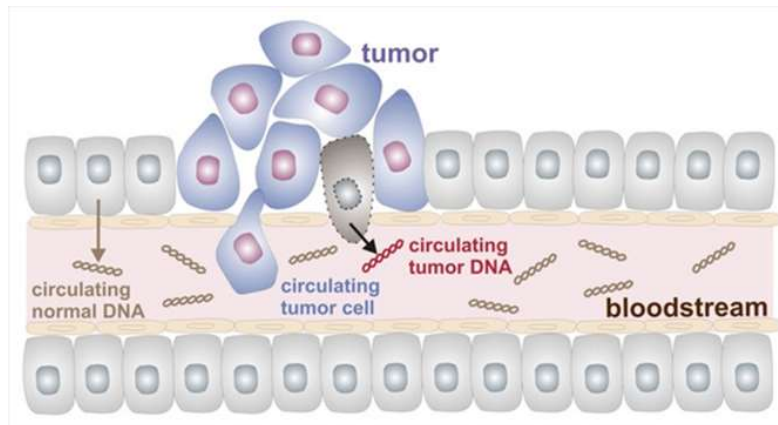
Mutant abundance 0.1%





Liquid Biopsies

Liquid Biopsies contain cfDNA that is **>99% wt** and **<1%** tumor DNA



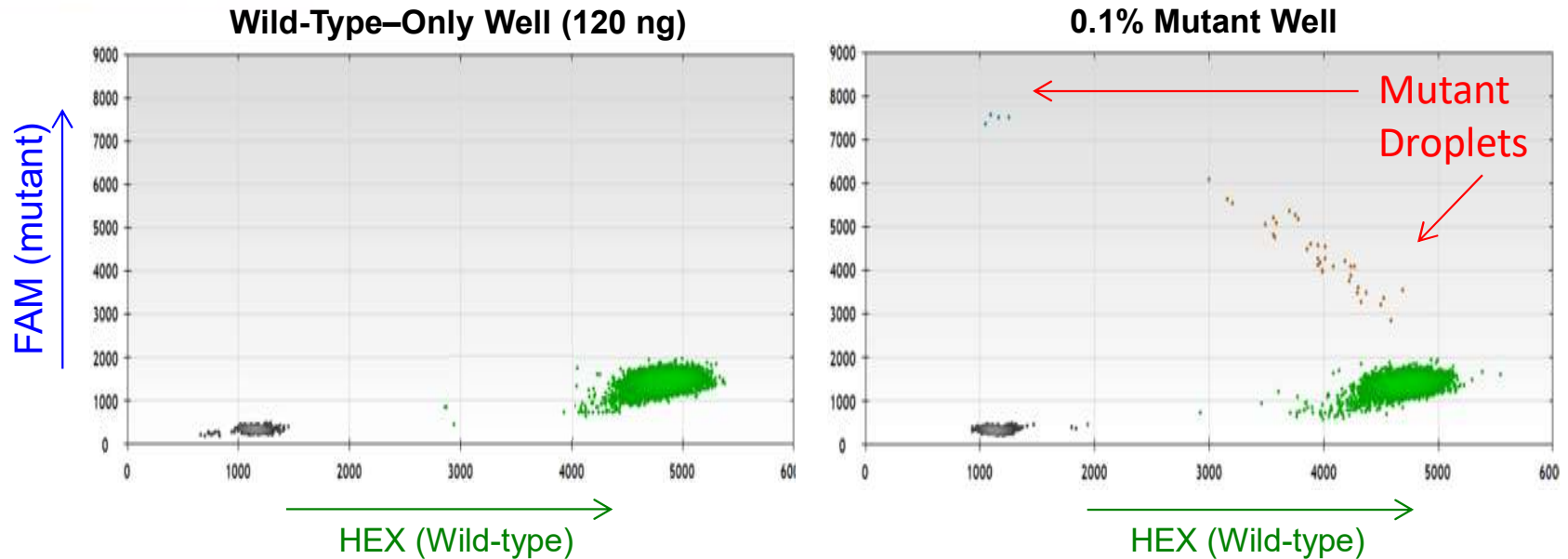
Crowley E et al., *Nat Rev Clin Oncol*, 2013.



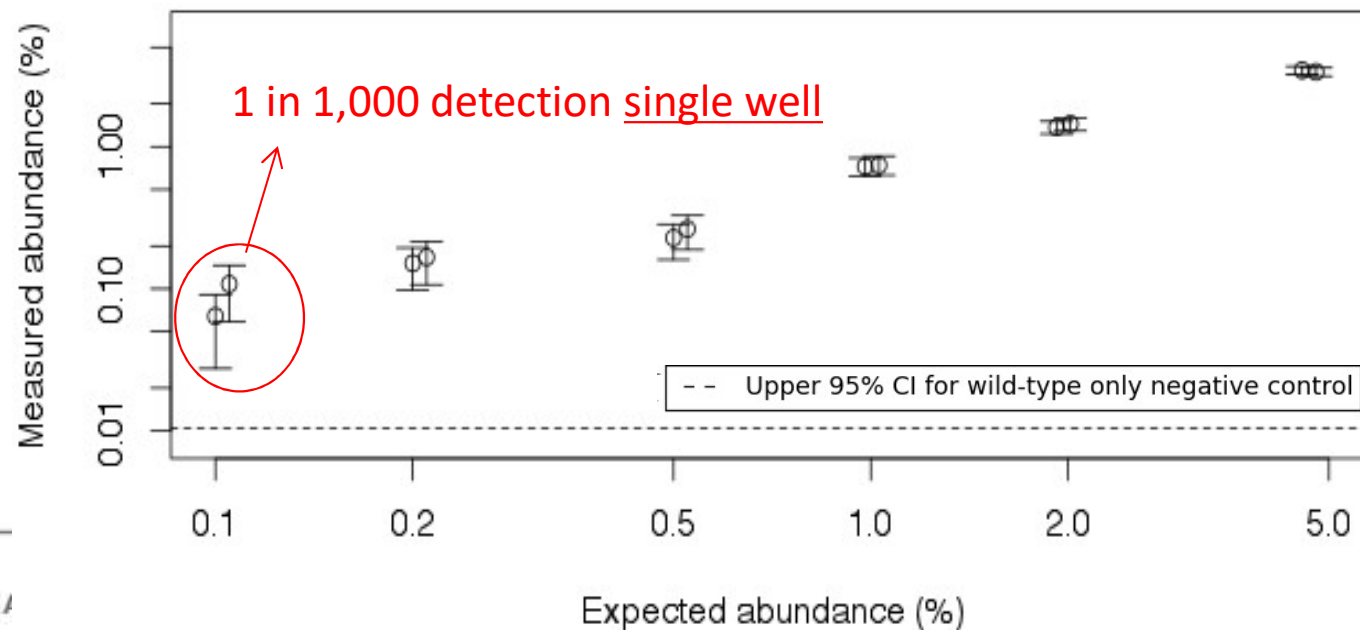
Example Validation Data: *BRAF* V600E

14

2-D
Amplitude
Plot



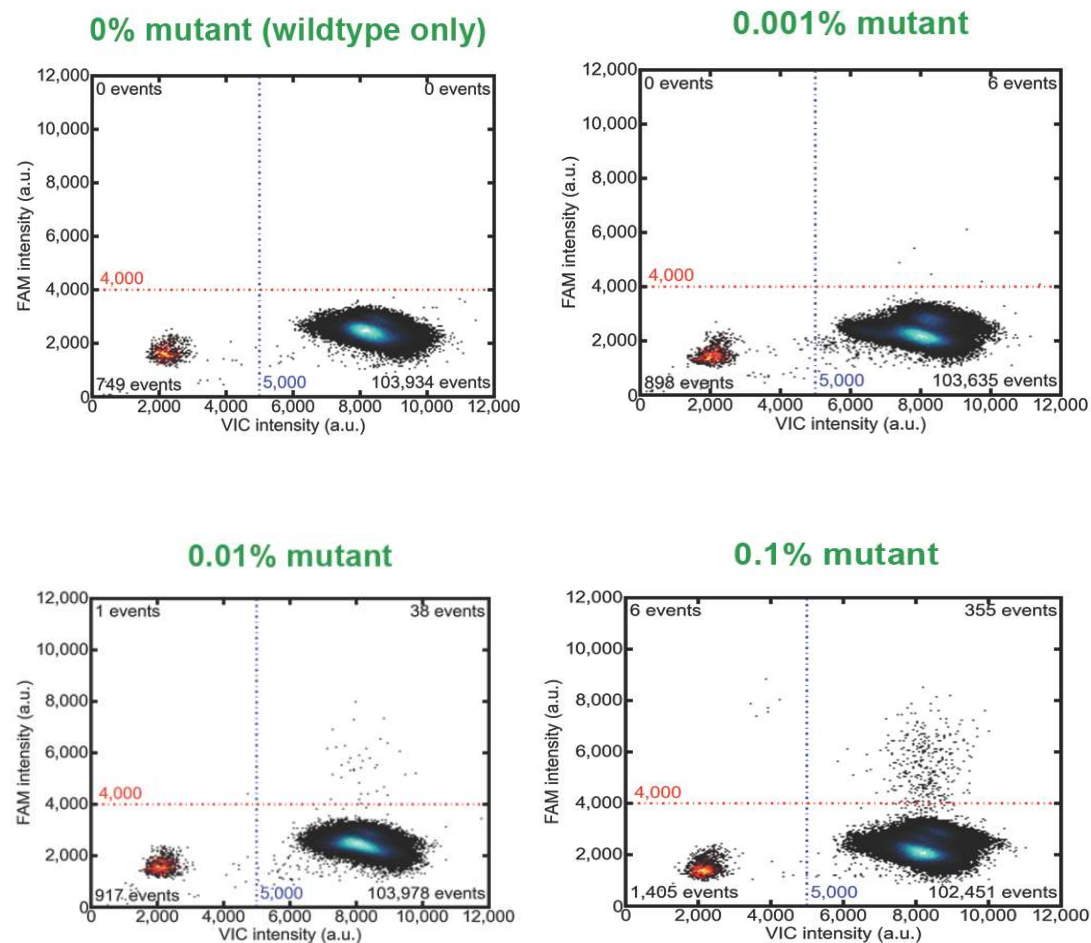
Limit of
Detection
Plot



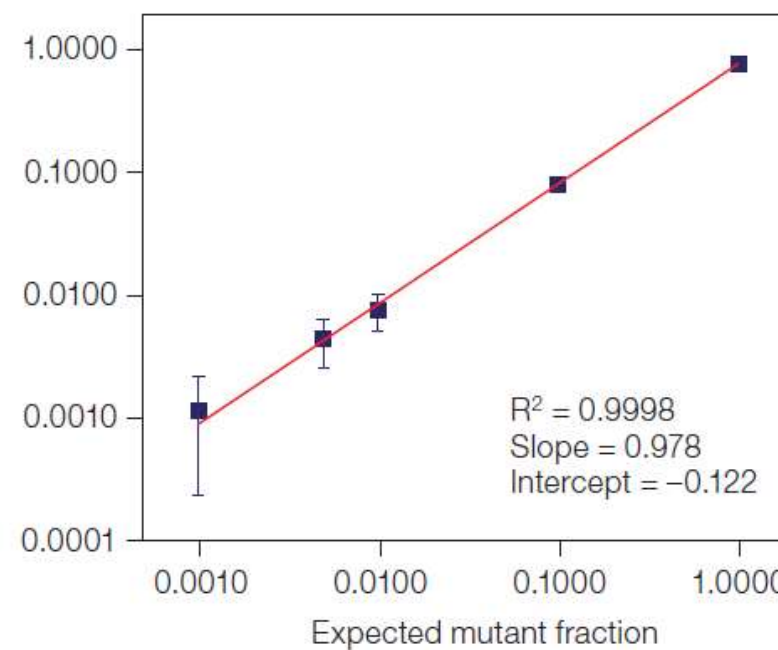


Measuring RMD: ddPCR vs. qPCR

ddPCR detects *BRAF* V600E down to **0.001%**



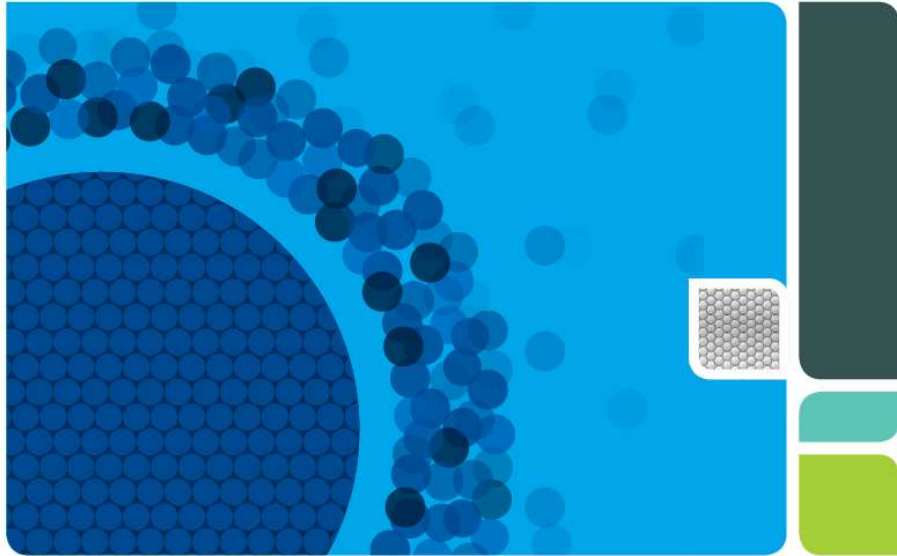
ddPCR





Learn More At Our Apps and Techs Pages

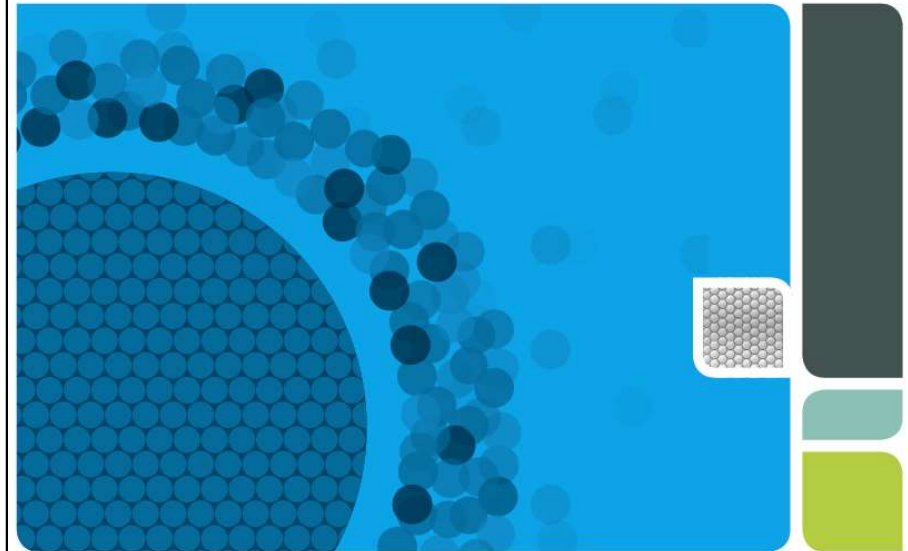
Droplet Digital PCR



Droplet Digital™ PCR
Applications Guide

BIO-RAD

Droplet Digital™ PCR



Rare Mutation Detection
Best Practices Guidelines

BIO-RAD



Applications

Common Applications

- Rare mutation detection
- Copy number variation analysis
- Nucleic acid quantification and detection (viral, pathogen, genetically modified organism, etc.)
- High-resolution gene expression
- Proximity studies (phasing)
- NGS library quantification

Additional Droplet Digital PCR Applications

- Allele-specific gene expression
- microRNA research
- Methylation studies
- Haplotyping
- TRAP assays
- Genome editing



Key Advantages of ddPCR

Absolute quantification

- No standard curve is needed

Precision

- Low variance on replicates

Sensitivity

- Detection of rare targets in complex backgrounds

Tolerant to PCR Inhibitors

- End-point PCR

QX200 Droplet Digital PCR System:



Thank you



Questions?

