Chapter 1.

What is Digital PCR?

ABSOLUTE PRECISE QUANTIFICATION

Chapter 1.
What is Digital PCR?

OPTOLANE Technologies, Inc.

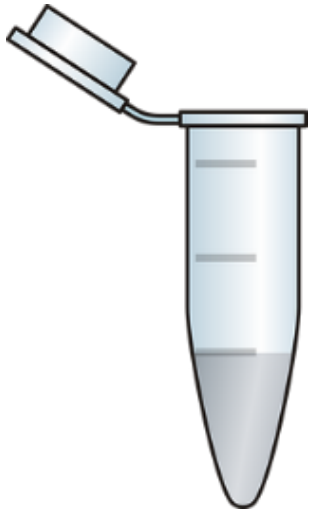
DIGITAL “Signals or data expressed as series of the digits 0 and 1, typically represented by values of a physical quantity such as light intensity.”

PCR “Polymerase chain reaction (PCR) is a method widely used in molecular biology to make many copies of a specific DNA segment.”

DIGITAL PCR (dPCR) is a quantitative PCR method that provides a sensitive and reproducible way of measuring the amount of DNA or RNA present in a sample.

WHAT IS DIGITAL PCR?

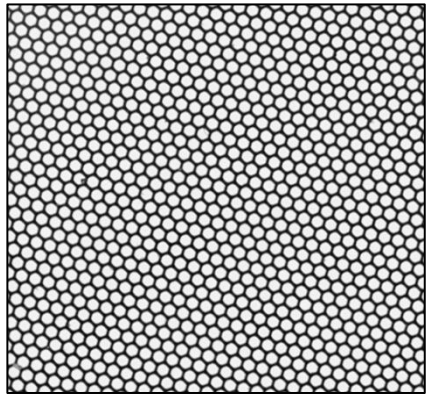
PCR REACTION THAT IS PARTITIONED



One sample

One measurement

Partitioned PCR reactions are independent, single amplification events



One partitioned sample

Thousands of discrete measurements

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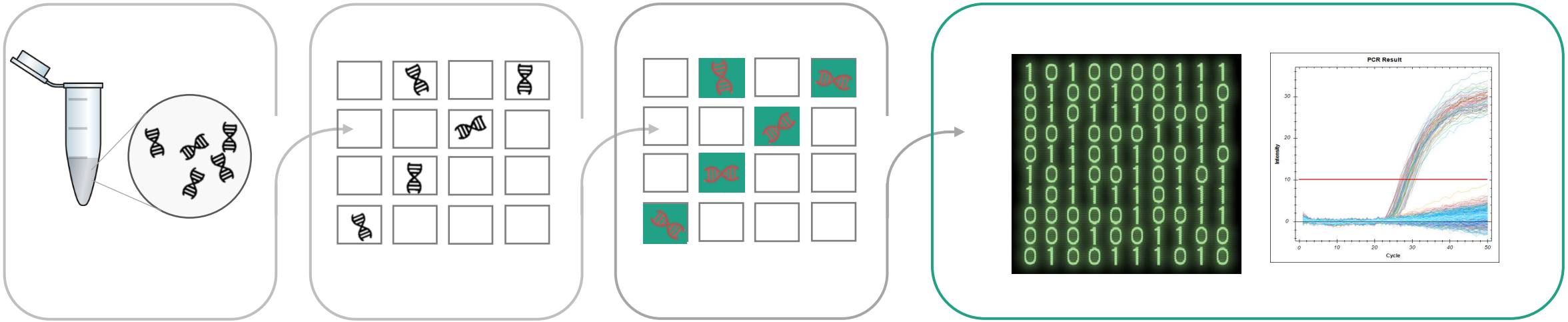
PRINCIPLE OF DIGITAL PCR

PCR REACTIONS THAT ARE DIGITALIZED



Sample prep

cfDNA, Viral DNA & others

Distribution

Sample partitioned in many well

Amplification

Target PCR product

No PCR product

Fluorescent detection & Analysis

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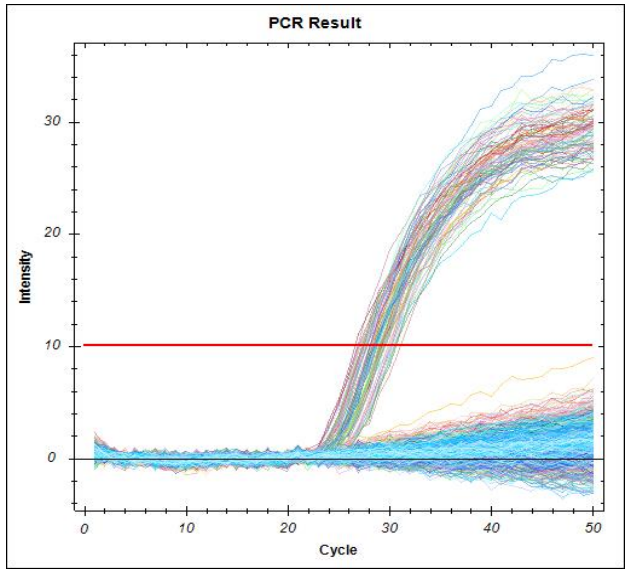
PRINCIPLE OF DIGITAL PCR

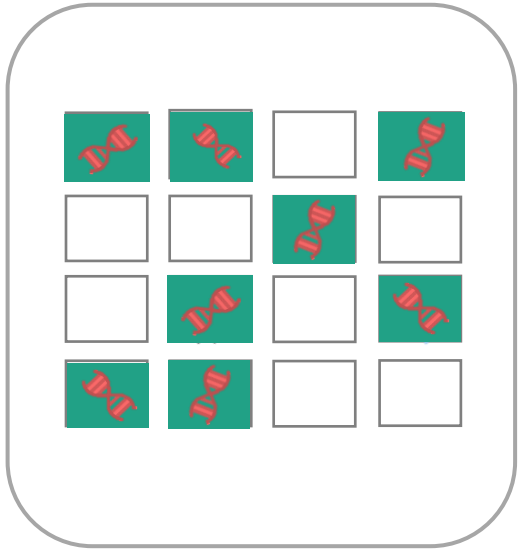
MICROWELL READINGS CONVERTED TO DIGITAL SIGNALS

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



Each positive counted as 1

Each negative counted as 0



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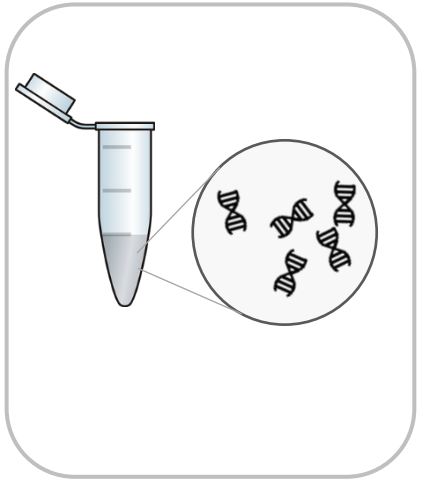
PRINCIPLE OF DIGITAL PCR

SAMPLE PREPARATION



Sample prep

- Phenol-chloroform method for DNA extraction
- Enzymatic DNA extraction method
- Silica- (spin)Column based DNA extraction method
- DNA extraction using anionic resins
- Magnetic bead method of DNA extraction
- CsCl density gradient DNA extraction method

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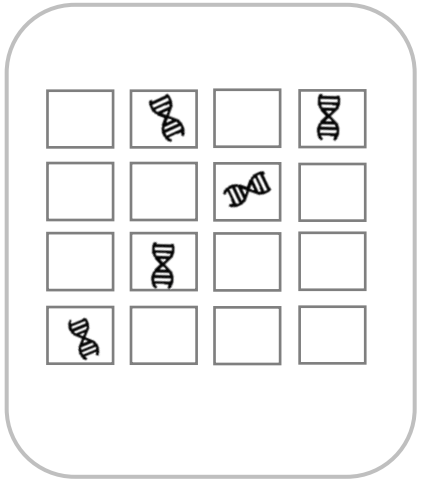
PRINCIPLE OF DIGITAL PCR

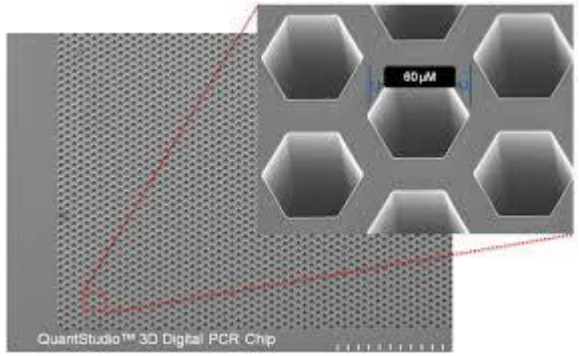
SAMPLE DISTRIBUTION



Distribution

a. Separation type

b. Droplet type



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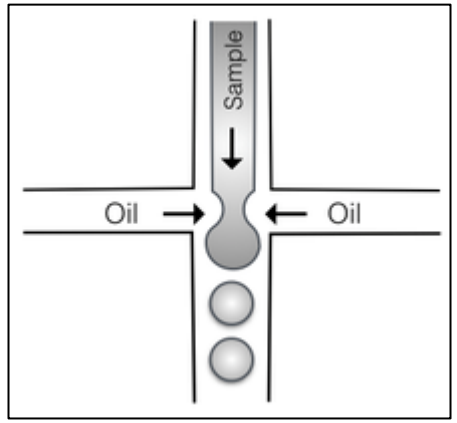

b. Droplet type

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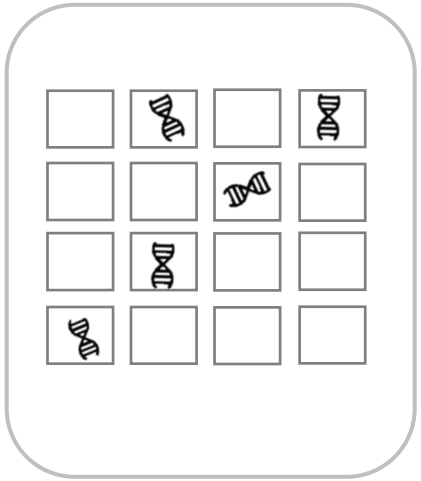
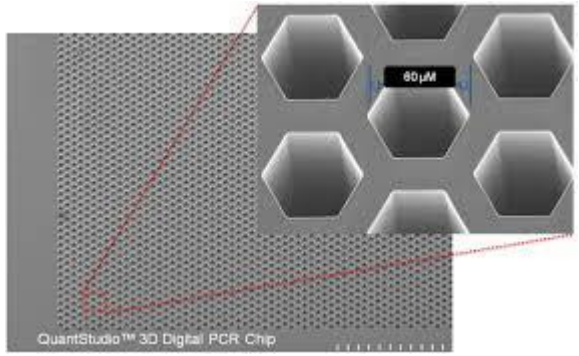
PRINCIPLE OF DIGITAL PCR

SAMPLE DISTRIBUTION



Distribution

- **Separation type**
- Droplet type



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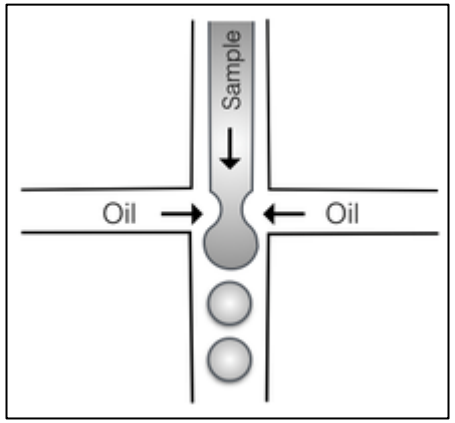

b. Droplet type

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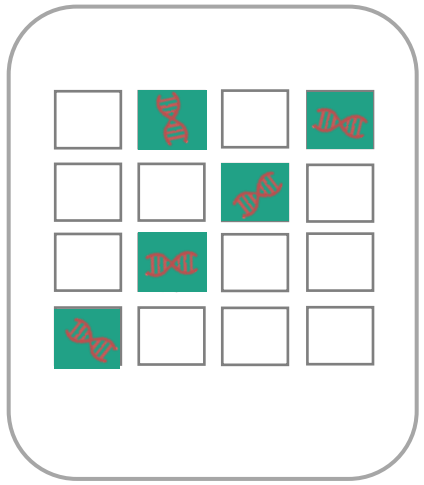
PRINCIPLE OF DIGITAL PCR

AMPLIFICATION



Amplification

- End-point PCR
- Real-time PCR

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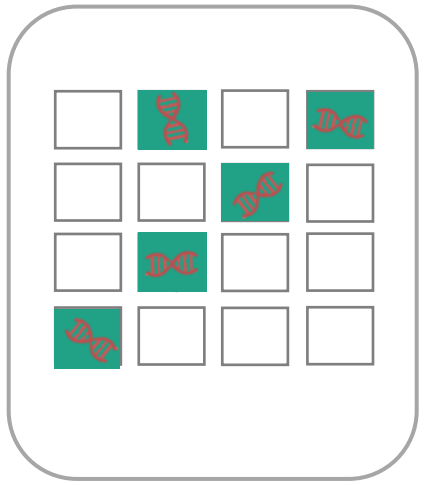
PRINCIPLE OF DIGITAL PCR

AMPLIFICATION



Amplification

- End-point PCR
- **Real-time PCR**

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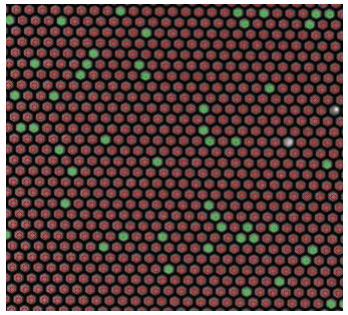
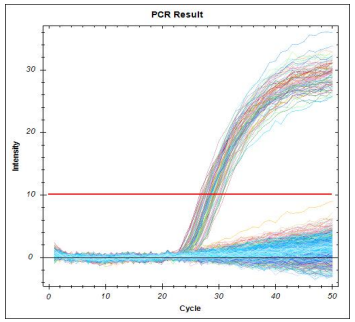
PRINCIPLE OF DIGITAL PCR

DETECTION AND ANALYSIS





Fluorescent detection & Analysis

- Laser detection
- Scanner
- Sensor

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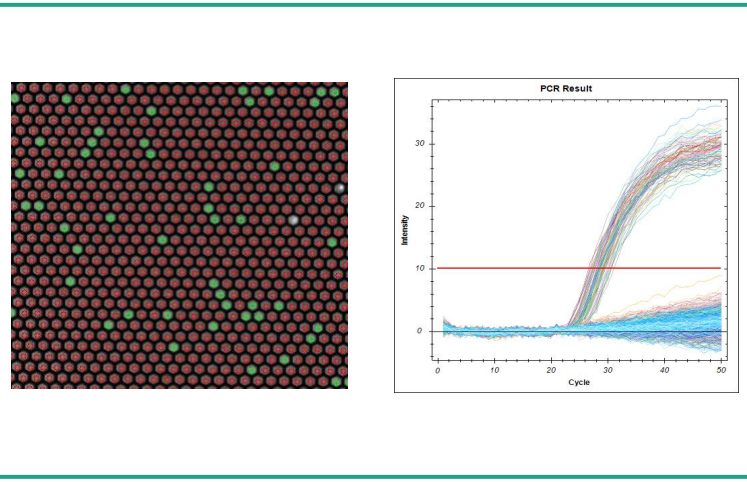
PRINCIPLE OF DIGITAL PCR

DETECTION AND ANALYSIS



Fluorescent detection & Analysis

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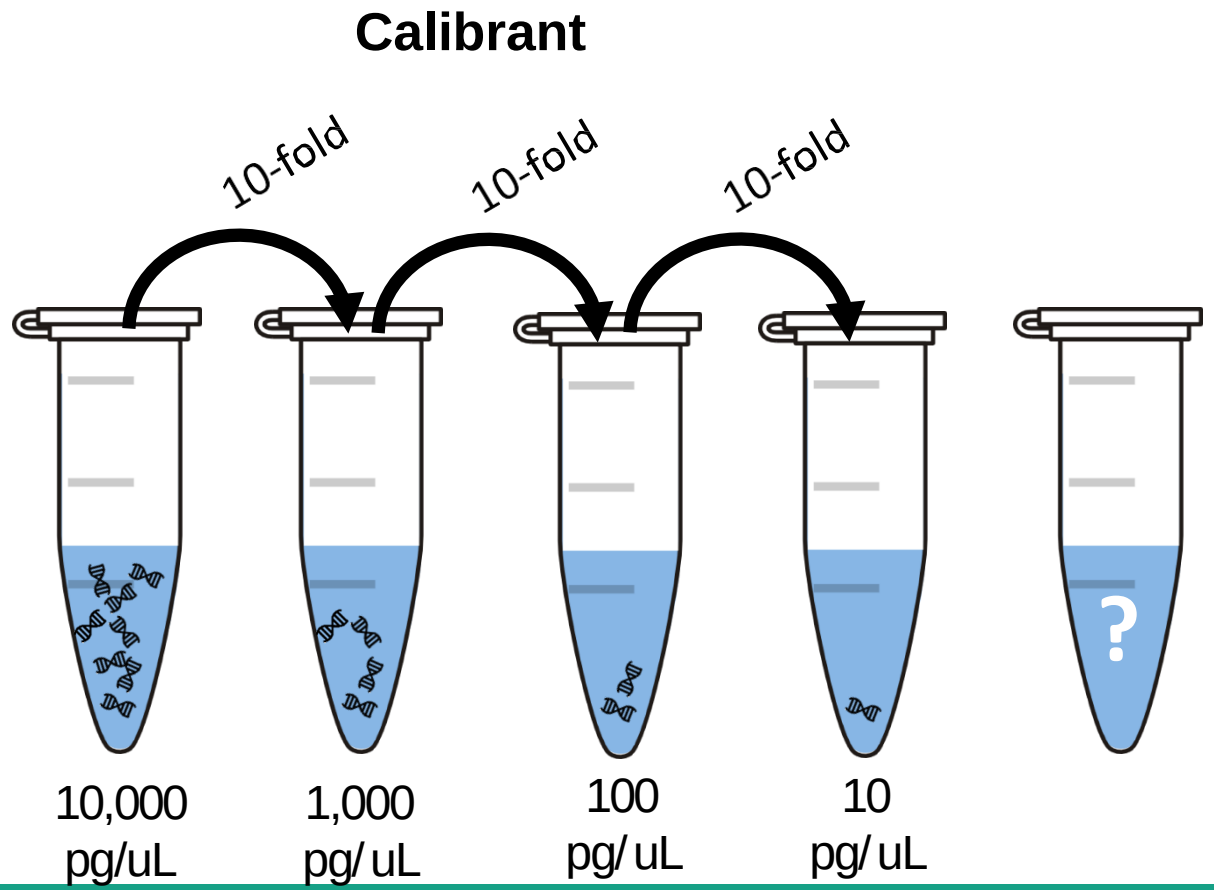
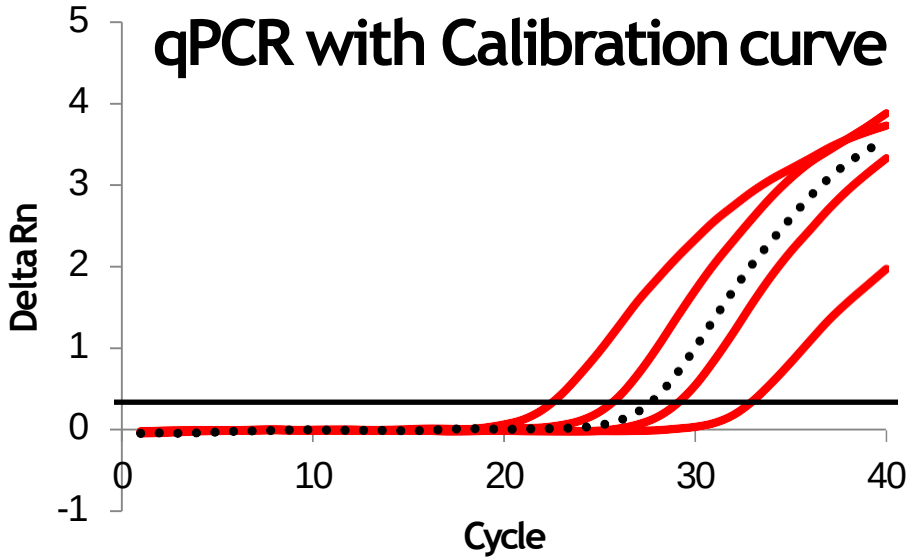
LIMITATIONS OF REAL-TIME PCR

NEED OF STANDARD CURVES AND REFERENCE SAMPLES



REAL-TIME PCR PRINCIPLE



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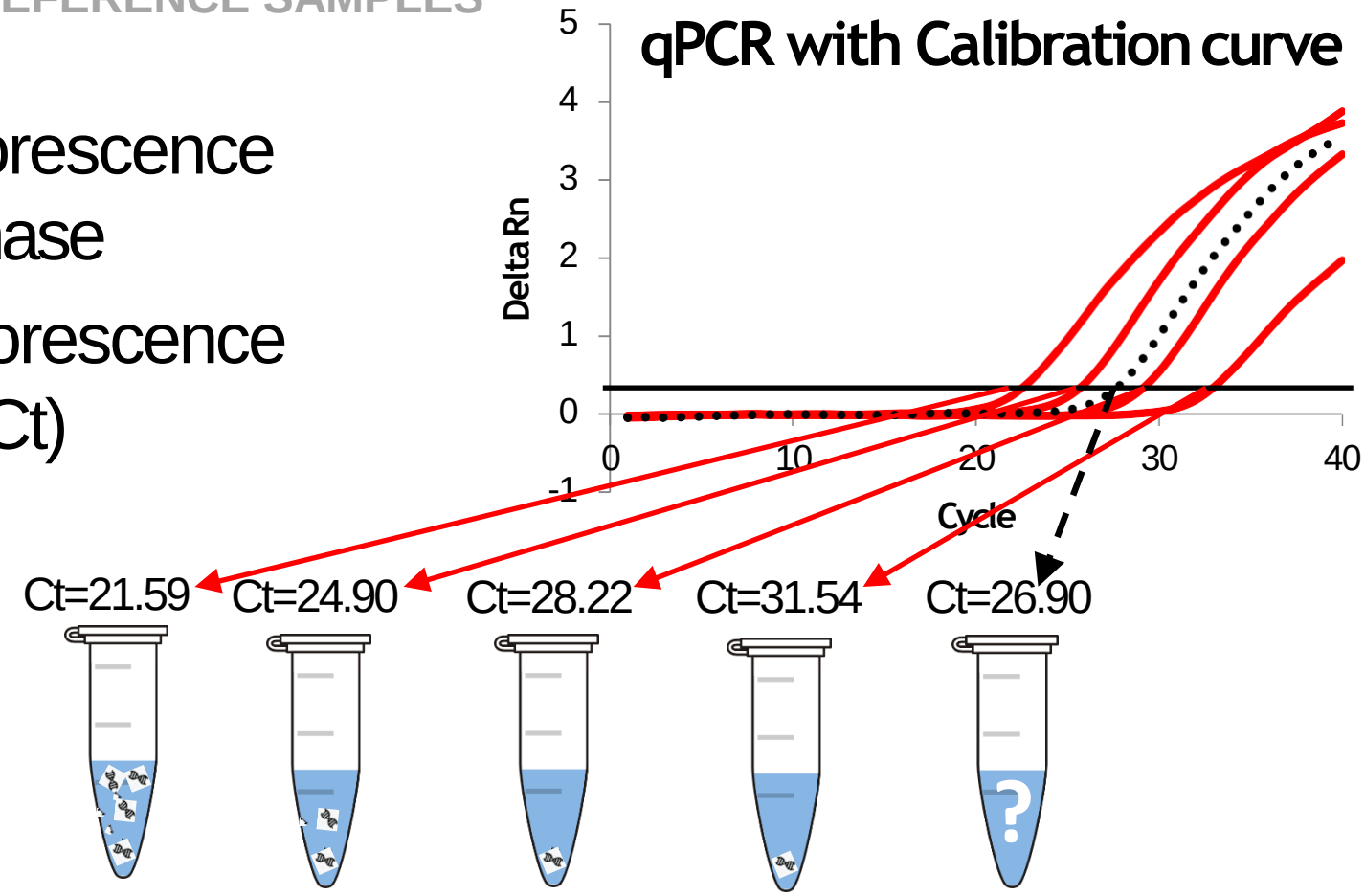
LIMITATIONS OF REAL-TIME PCR

NEED OF STANDARD CURVES AND REFERENCE SAMPLES

- Apply a threshold while florescence signal is in exponential phase
- Determine point where florescence signal crosses threshold (Ct)

REAL-TIME PCR PRINCIPLE



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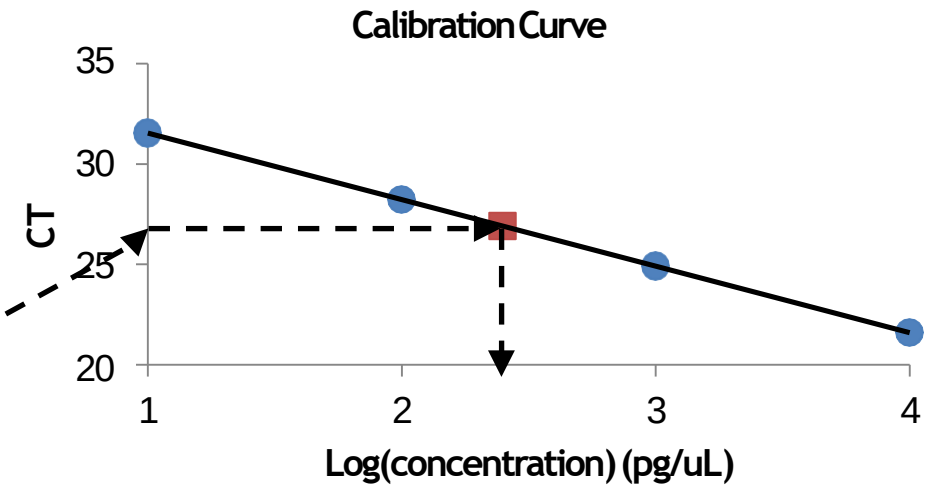
LIMITATIONS OF REAL-TIME PCR

NEED OF STANDARD CURVES AND REFERENCE SAMPLES

- Log transform concentration
- Plot Log(conc.) vs Ct

Conc. (pg/µL)	Log(conc.)	Ct
10,000	4	21.59
1,000	3	24.90
100	2	28.22
10	1	31.54
unknown	unknown	26.90



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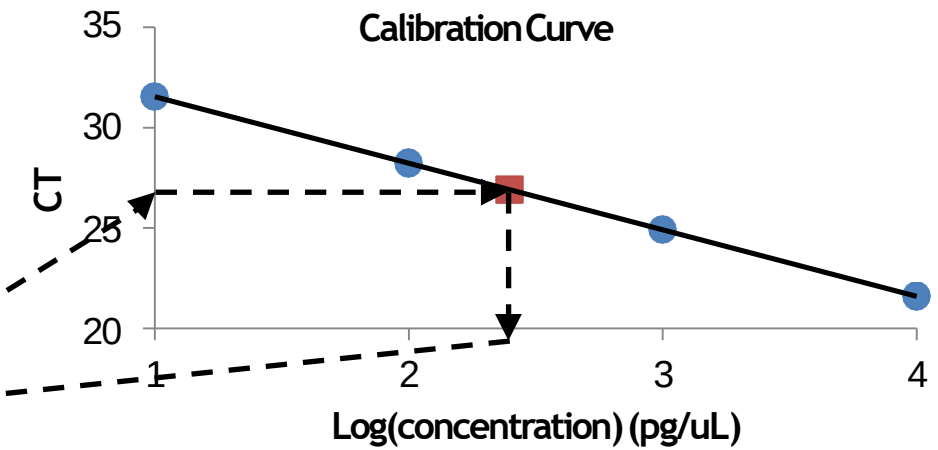

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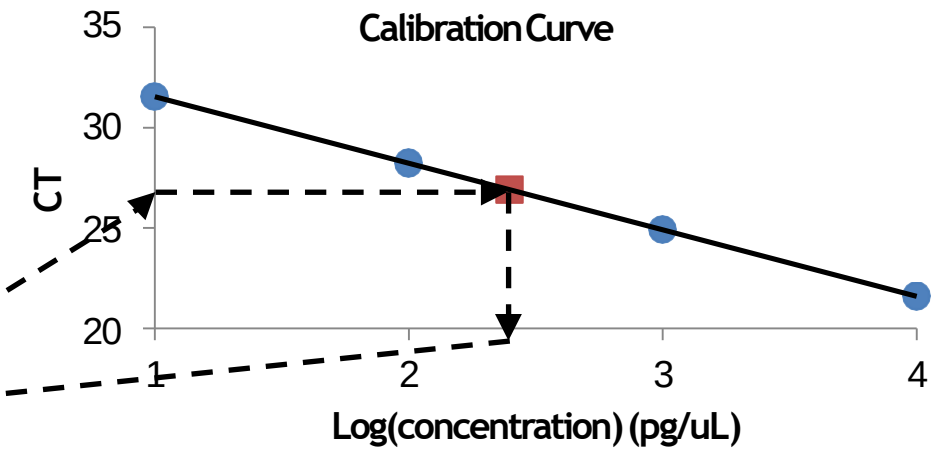

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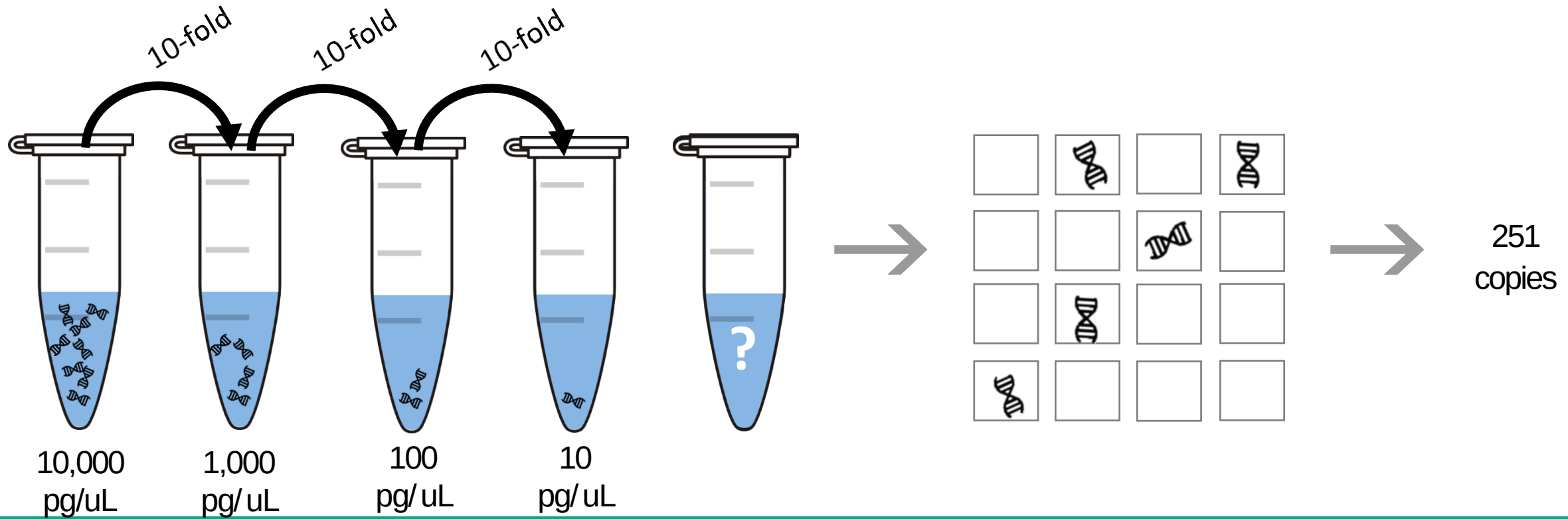
1. ADVANTAGES OF DIGITAL PCR

DIGITAL PCR

NO NEED OF STANDARD CURVES AND REFERENCE SAMPLES

Ca rant



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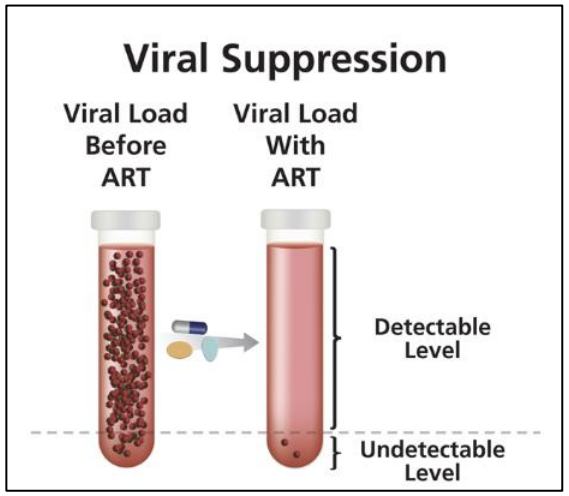
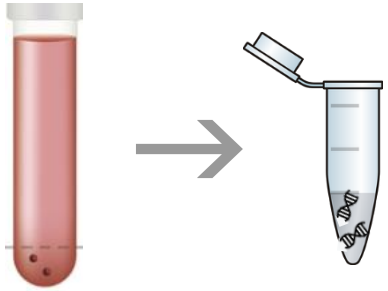
3. ADVANTAGES OF DIGITAL PCR

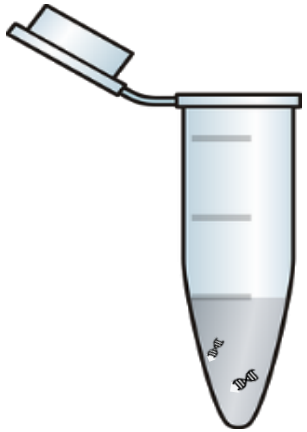
GREATER SENSITIVITY FOR LOW VIRAL LOAD TITER





Bulk Sample - 30 uL

2 copies of HIV RNA molecules



Target abundance **very low**

Partitioned Sample – 20,000 x 0.79nl

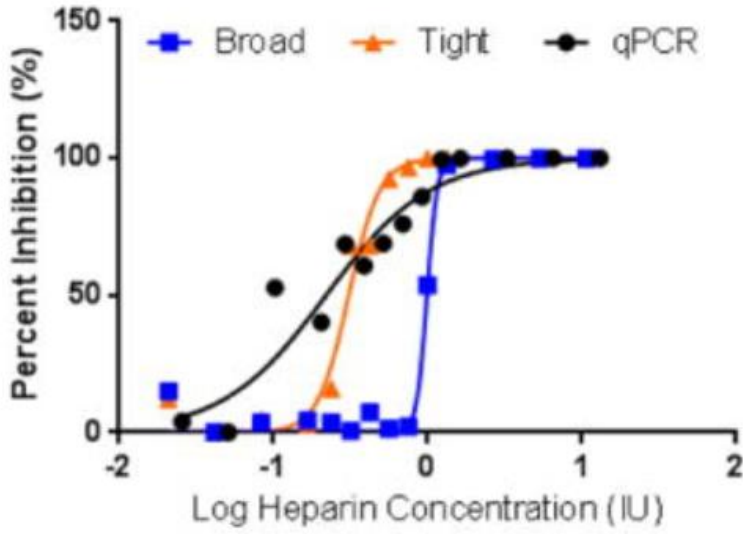
2 wells with viral RNA

Target abundance **100%**

19,998 wells w/o viral RNA

4. ADVANTAGES OF DIGITAL PCR

TOLERANCE TO MINOR INHIBITORS THAT AFFECT AMPLIFICATION



Inhibition tolerance of laboratory-developed CMV qPCR and dPCR assays

"The ddPCR CMV assay is more tolerant to SDS and heparin than the qPCR assay, indicating reaction partitioning through digitization may reduce susceptibility to traditional PCR inhibitors. The data suggests that individual micro-reactions mitigate the impact of inhibitors on PCR amplification by retaining discernible positive signal even when moderate PCR inhibition is occurring in a droplet. Since PCR reactions are not partitioned in qPCR, amplification is dependent on the concentration of inhibitor in the entire reaction and will result in an increased number of amplification cycles required to reach a signal above a given threshold. In turn, this will result in inaccurate quantification of template in the original sample. "

Dingle T.C et al (2014)

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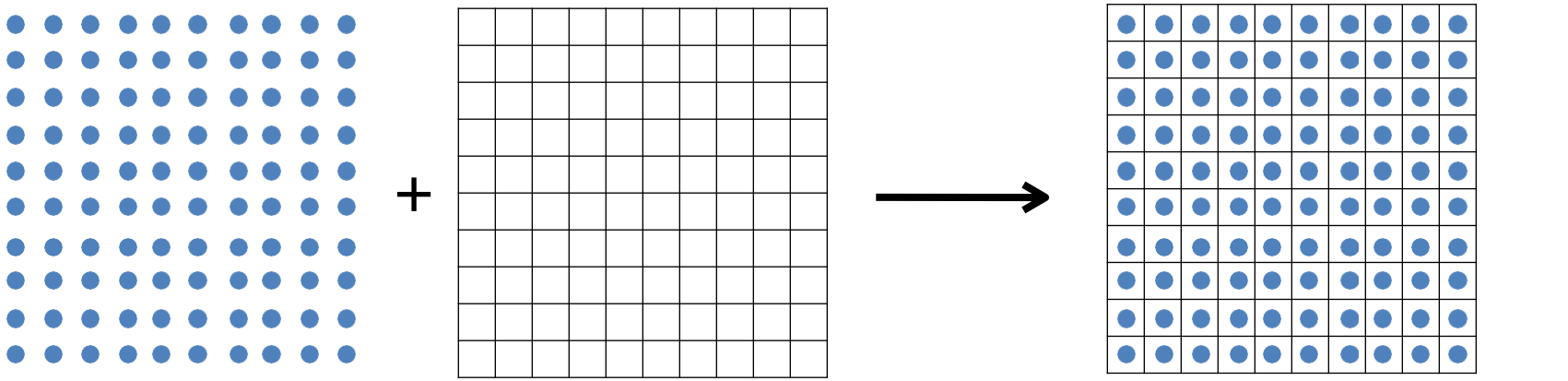
MEASURING TARGET CONCENTRATION

EVEN VS. RANDOM DISTRIBUTION

- Even distribution



100 molecules

100 wells

All 100 well have exactly one molecule

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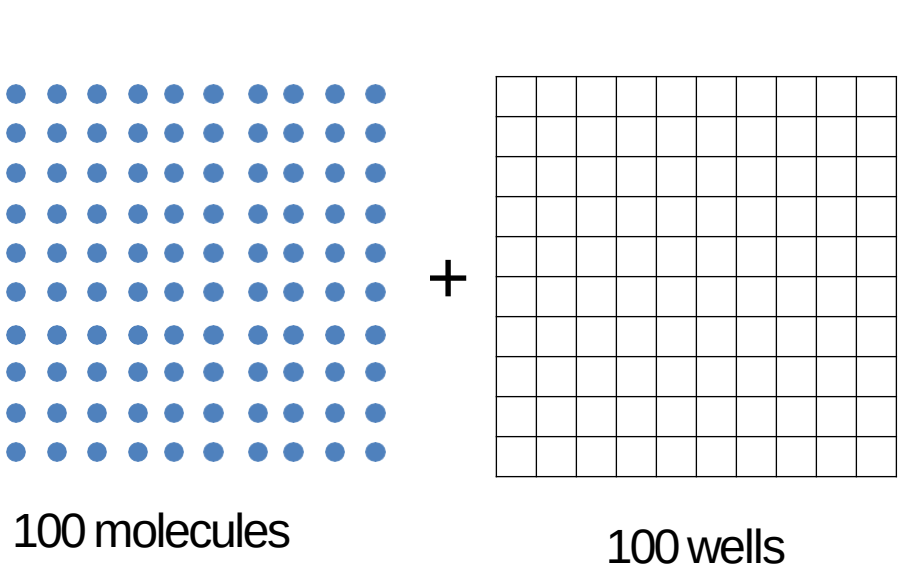
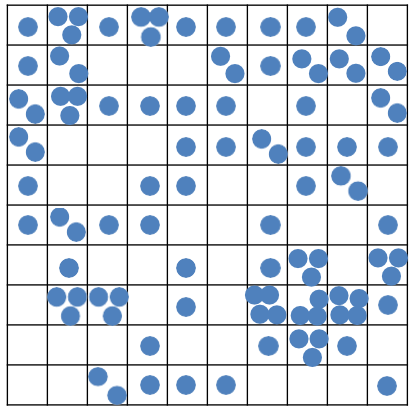
MEASURING TARGET CONCENTRATION

EVEN VS. RANDOM DISTRIBUTION

- Random distribution



One possibility



37 wells with 0 molecule
39 wells with 1 molecule
13 wells with 2 molecules
9 wells with 3 molecules
2 wells with 4 molecules
Total = 100 molecules

If we could count the individual molecules we would not need to use Poisson statistics or dPCR

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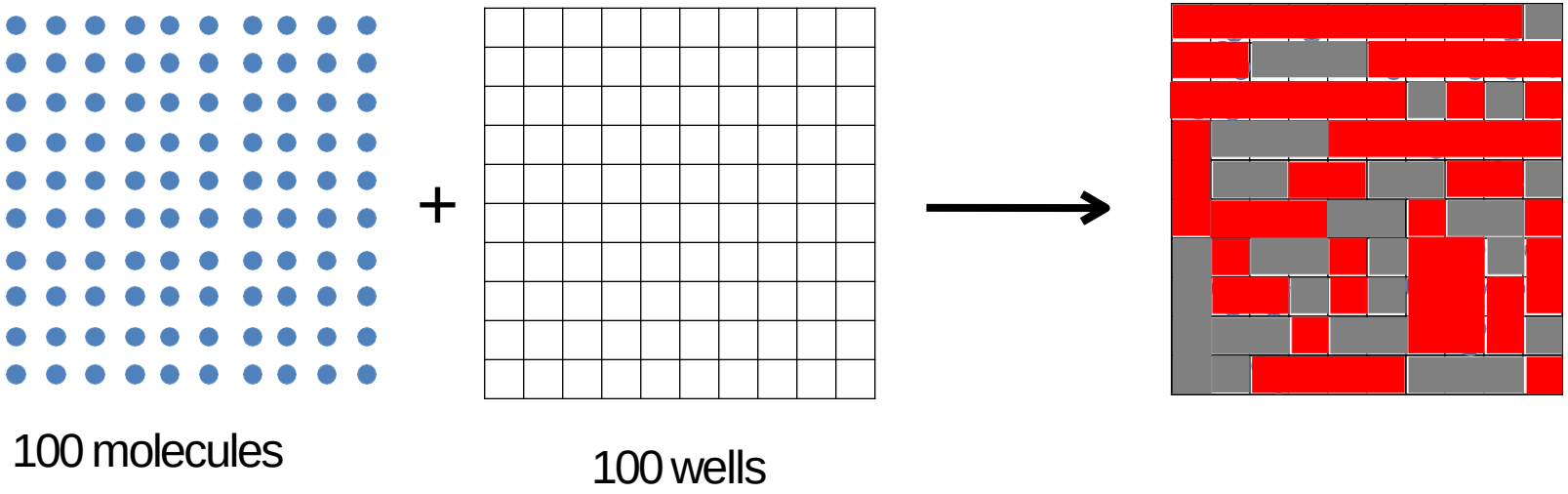
MEASURING TARGET CONCENTRATION

EVEN VS. RANDOM DISTRIBUTION

Positive well

Negative well

- Random distribution



37 wells with 0 molecule
63 wells with ≥1 molecules
Poisson calculated 99 molecules
95%CI 77 to 129 molecules

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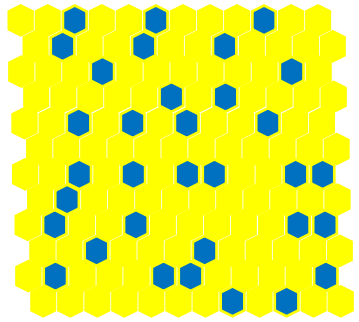
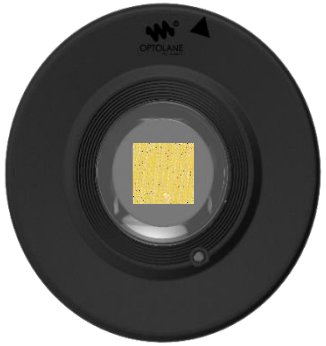
MEASURING TARGET CONCENTRATION

POISSON CORRECTED SAMPLE EXAMPLES

Sample 1



Cartridge 1



$$\text{Concentration (copy/ul)} = \frac{\text{Number of wells}}{\text{Volume of all PCR reactions}} \ln\left(\frac{\text{Number of wells}}{\text{Number of negative wells}}\right)$$

Medium concentration

P=34/144

After Poisson Correction **38**

P=16,076/17,451

After Poisson Correction **55 800**

P=16 076 /17 451

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DIGITAL PCR APPLICATIONS

POISSON CORRECTED SAMPLE EXAMPLES

LOAA(Lab On An Array) is unique digital PCR platform which can perform absolute quantitation in real-time fashion. You can see the real-time amplification curves throughout your digital process.

LOAA allows you to run all applications in the digital PCR research field.

Viral load determination

Check a numerical expression of the quantity of virus in a given volume.

Copy number variation

Confirm whether the copy number of a sequence of interest deviates from wild type and by how much.

Gene expression

Detects a lot smaller gene expression level

Single cell analysis

Can detect expression levels of multiple genes in a set of single cells

NGS library validation

Quantifies and normalizes sample variants

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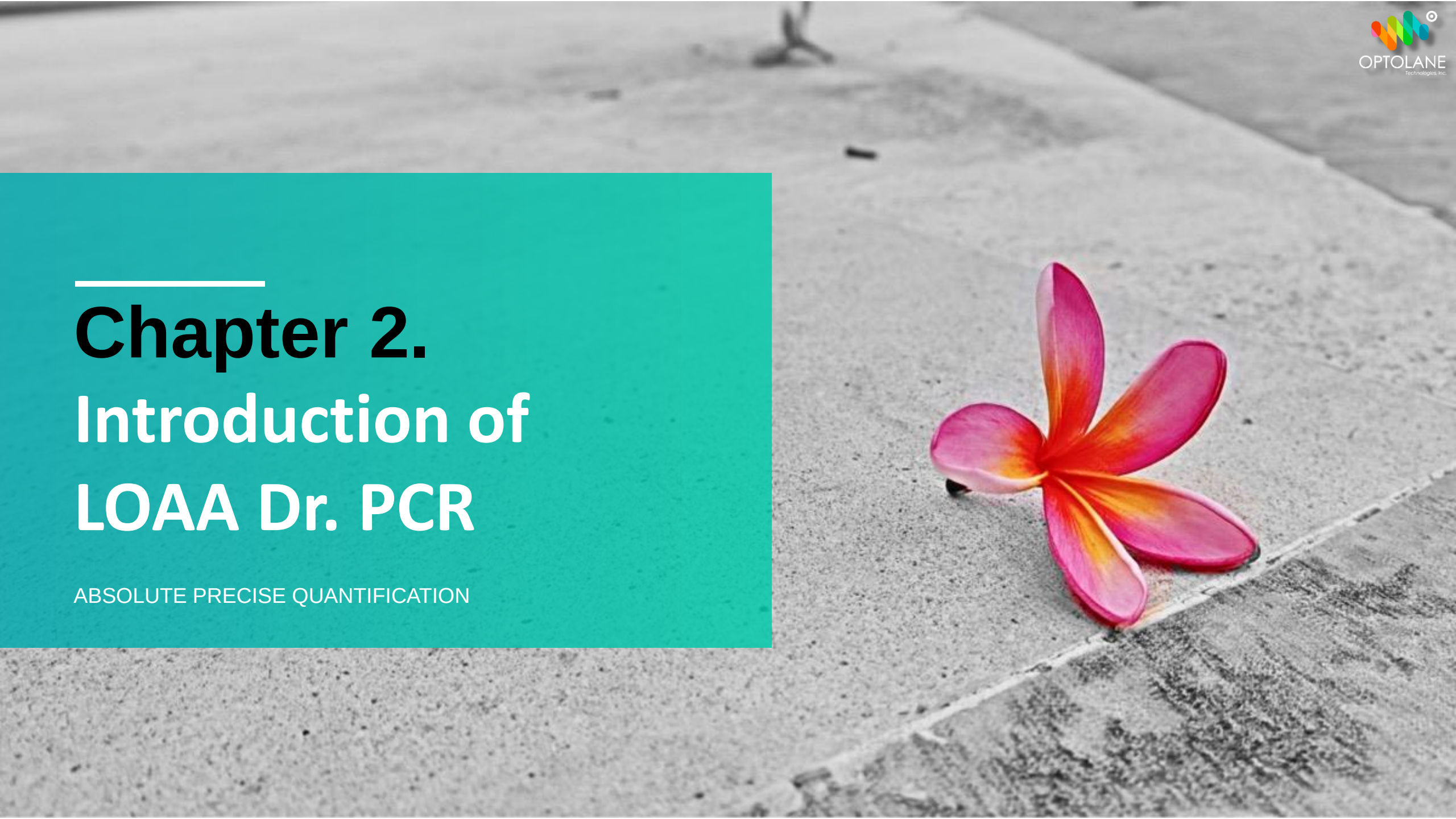
REAL-TIME PCR VS. DIGITAL PCR

	Real-time PCR	LOAA (Digital Real-time PCR)
Similarities	• Compatibility with sample preps (gDNA digestion or shearing for dPCR) • Reaction components and chemistry • Compatibility with hydrolysis (Taqman) or intercalating (SYBR, EvaGreen) chemistries • Multiplexing capacity • Similar initial reaction and sample volumes • Wide dynamic range	
Quantification	Relative; standard curve	Absolute; no external calibrant needed
Acceptance	Older technology Widely accepted	New technology Gaining acceptance
Data Collection	Real-time (every cycle)	Real-time (every cycle)
Sensitivity	0,1-1% MAF	0,0005%
Specificity	Non-specificity of primers and probes ➔ problematic	Non-specific cross reactivity reduced greatly by partitioning
Reproducibility	Medium; StDev high at low concentrations	High; Low StDev



Chapter 2.
Introduction of LOAA Dr. PCR

ABSOLUTE PRECISE QUANTIFICATION

Chapter 2.
Introduction of Dr.PCR

1. Key technologies of LOAA System
2. Dr.PCR system configuration
    - 2-1. LOAA Analyzer
    - 2-2. Dr.PCR Cartridge 20K well
    - 2-3. Postman sample loader
3. Dr.PCR system benefits
    - 3-1. Digital Real-time
    - 3-2. Decentralization – simple configuration, compact size
    - 3-3. Room temperature Storage
    - 3-4. Connectivity

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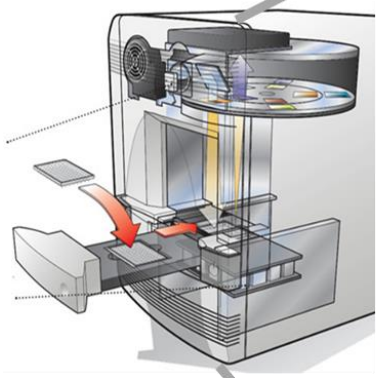
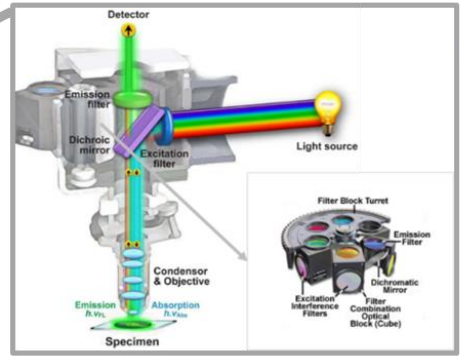
KEY TECHNOLOGIES

PCR Reaction & Fluorescence Detection on Semiconductor Chip

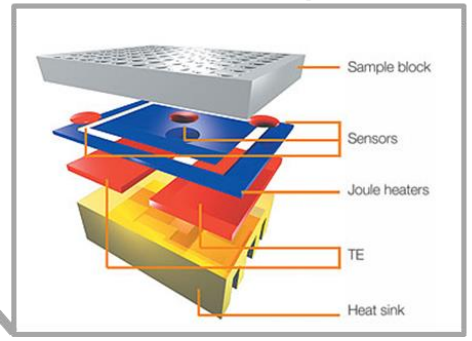
Current Real-Time PCR



Optical System

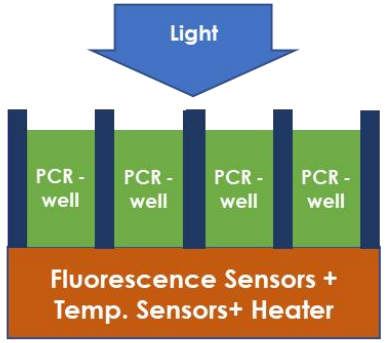


Thermal Control System



"Integration"

OPTOLANE's Real-Time PCR



MEMS well
acts like PCR tube

OPTOLANE
Technologies, Inc.

Dr.PCR SYSTEM CONFIGURATION

Simple configuration

Analyzer

Cartridge & Sample Loader

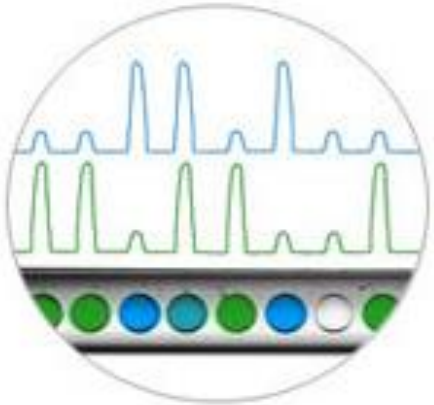
OPTOLANE
Technologies, Inc.

DIGITAL REAL-TIME PCR

More Reliable Data

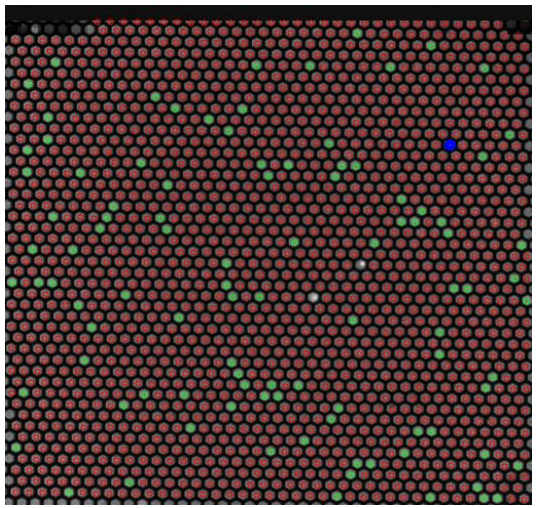
- It can tell true positive and false positive by Probe Chemistry and Real-Time Curves
- *Be able to confirm very low copy number of positive results in Liquid Biopsy*

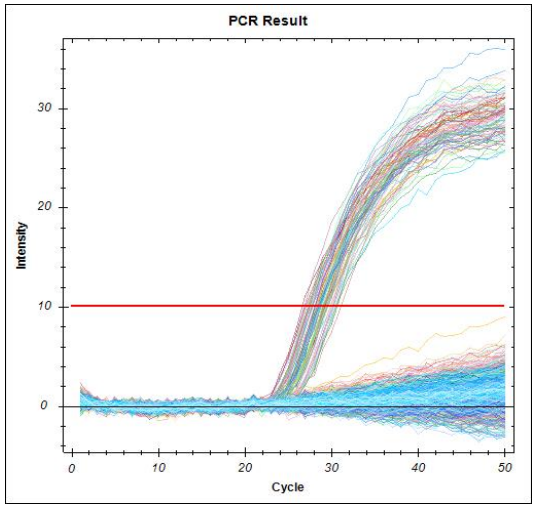
End-Point Digital PCR



Droplet Digital PCR
Absolute Quantitation

OPTOLANE Digital Real-Time PCR





Real-Time PCR

DECENTRALIZATION

Compact size, Small footprint

LOAA Analyzer
3.0 Kg

Multi Test Analyzer


Thank you for
your attention!