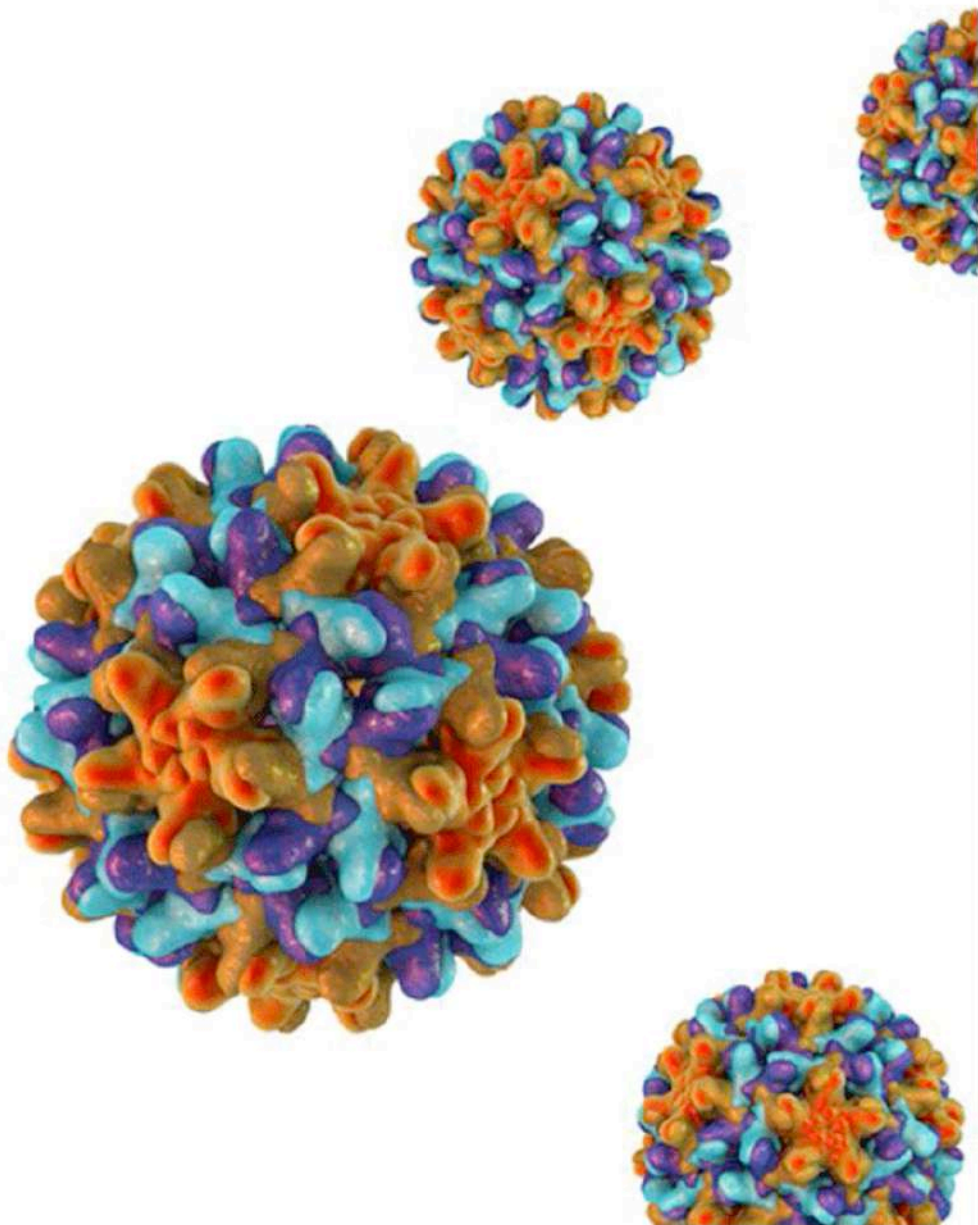




HBV RNA multiple target assays for dPCR and the Digital LightCycler® System

Aaron Hamilton, Research Leader, Infectious Disease Research

7 September 2023 | public use



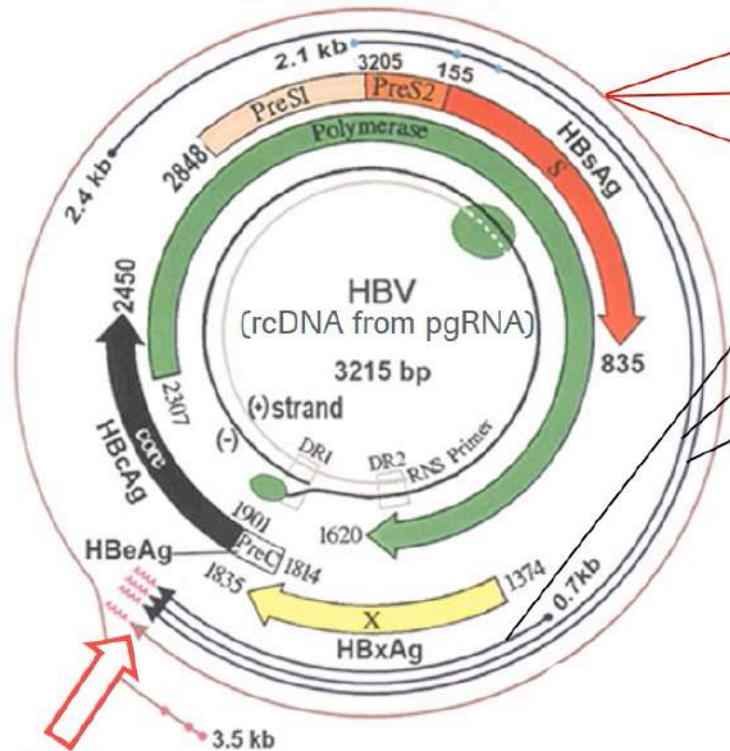
Assessing different HBV RNA forms

Leveraging the power of digital PCR

Overview of the Digital LightCycler[®] System

HBV-RNA Biomarker Assays—Multiple Targets

Circularizing HBV RNA forms



HBV RNA form	
cap	3.5 kb pgRNA
cap	3.5 Kb PreC RNA
cap	Spliced RNAs
cap	0.7 Kb HBx
cap	2.1 Kb HBs
cap	2.4 Kb HBs
cap	trRNAs (alternate polyA)
cap	Fusion RNAs (various possible)

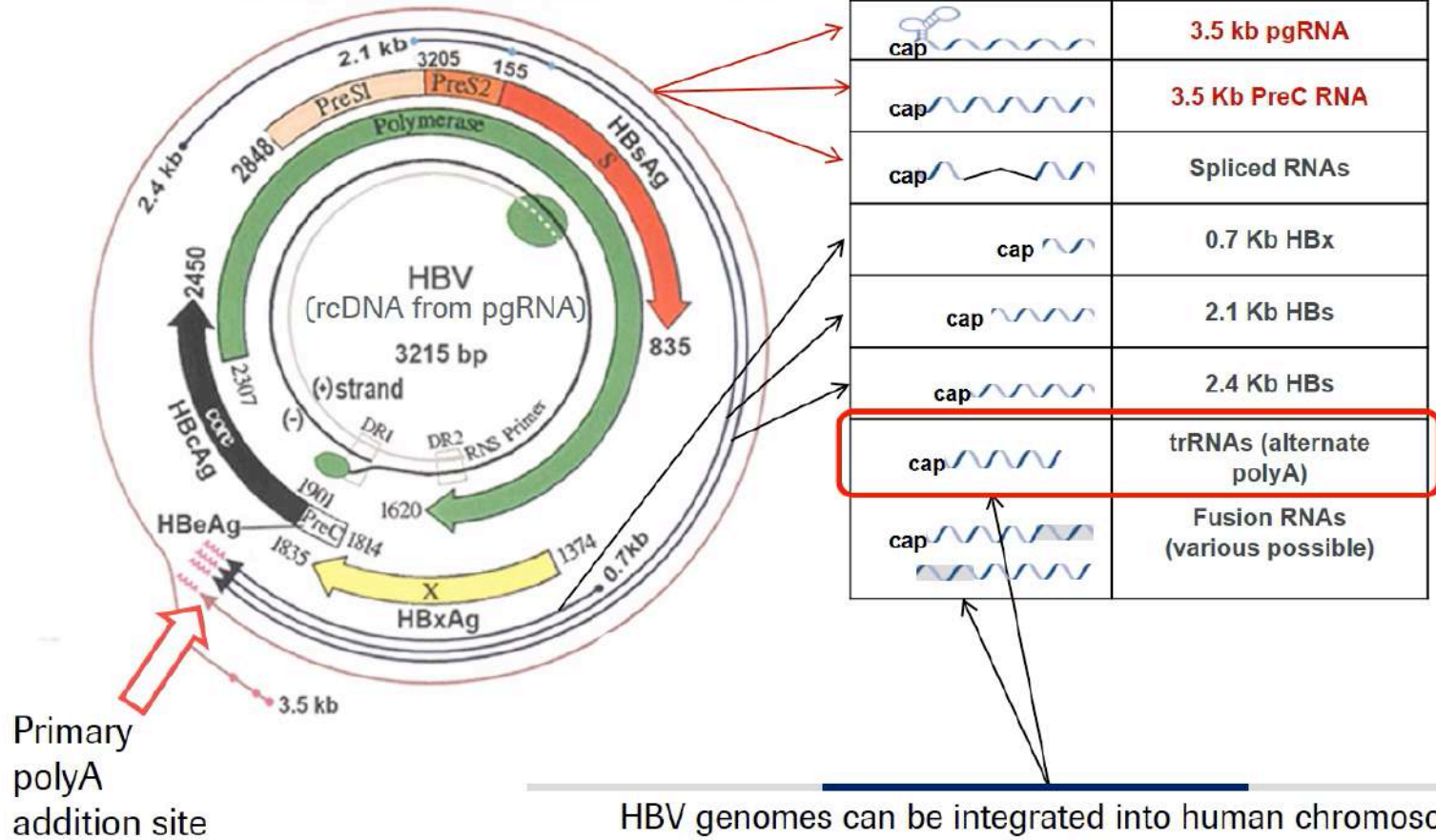
IA and RUO target

Primary polyA addition site

HBV genomes can be integrated into human chromosomes
However none of these can make the complete pgRNA

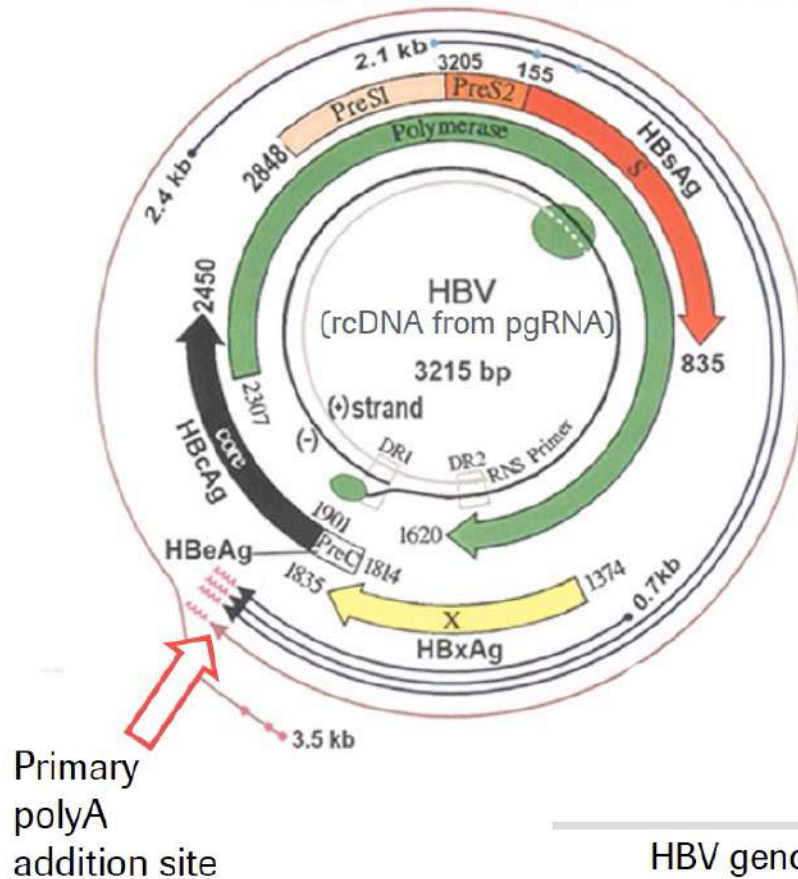
HBV-RNA Biomarker Assays—Multiple Targets

Circularizing HBV RNA forms



HBV-RNA Biomarker Assays—Multiple Targets

Circularizing HBV RNA forms



New Biomarkers

- Resolve mixtures of HBV RNA molecule types in circulation
- Characterize RNA from compartments other than viral particles
- Track shifts in RNA forms during disease or treatments
- Distinguish integrated vs cccDNA derived HBV RNA

HBV genomes can be integrated into human chromosomes
However none of these can make the complete pgRNA

Assessing different HBV RNA forms

Leveraging the power of digital PCR

Overview of the Digital LightCycler[®] System

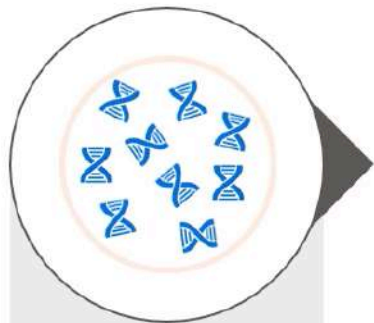
Principle of digital PCR

New-biomarker multiple-target HBV RNA assays have been designed for digital PCR

Partition

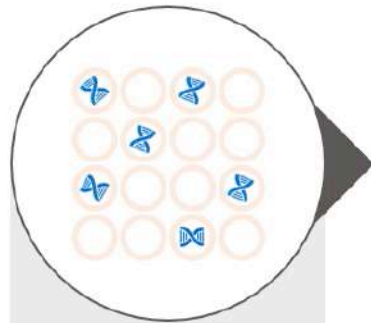


Absolute quantification



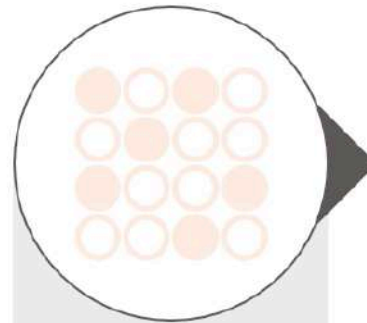
Prepared PCR reaction

Master mix & sample (nucleic acid)

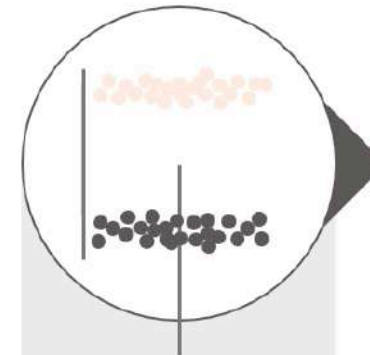


Partitioning

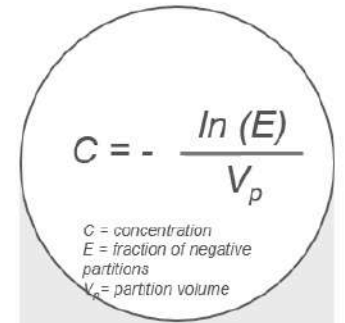
Partition sample (nucleic acid) into many chambers or partitions



Perform thermal cycling & end-point detection



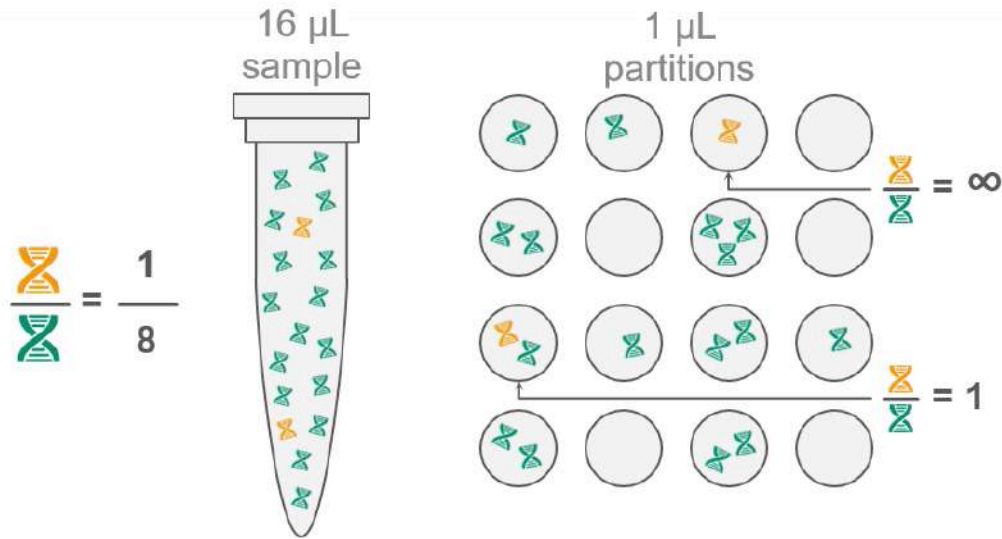
Count positives & negatives



Apply Poisson calculation to determine concentration

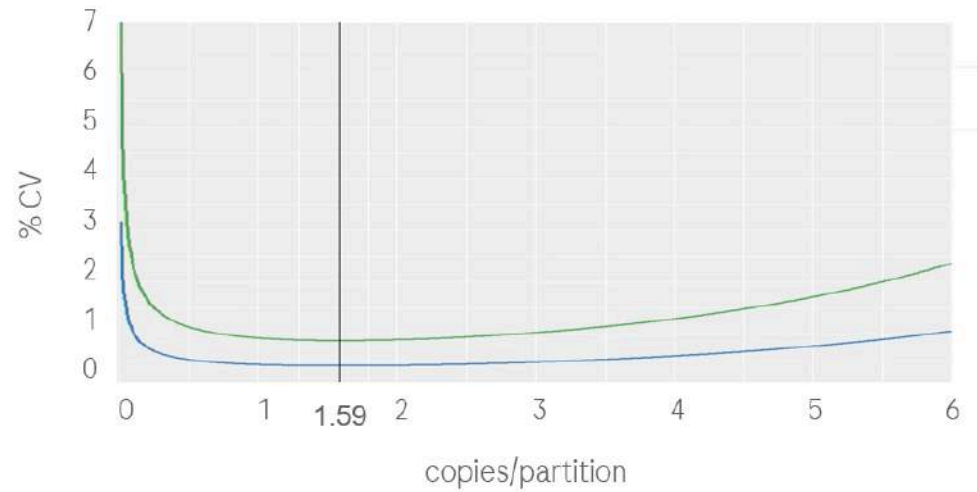
Going digital

Key advantages of digital PCR



Absolute quantitation
without need for
reference standards

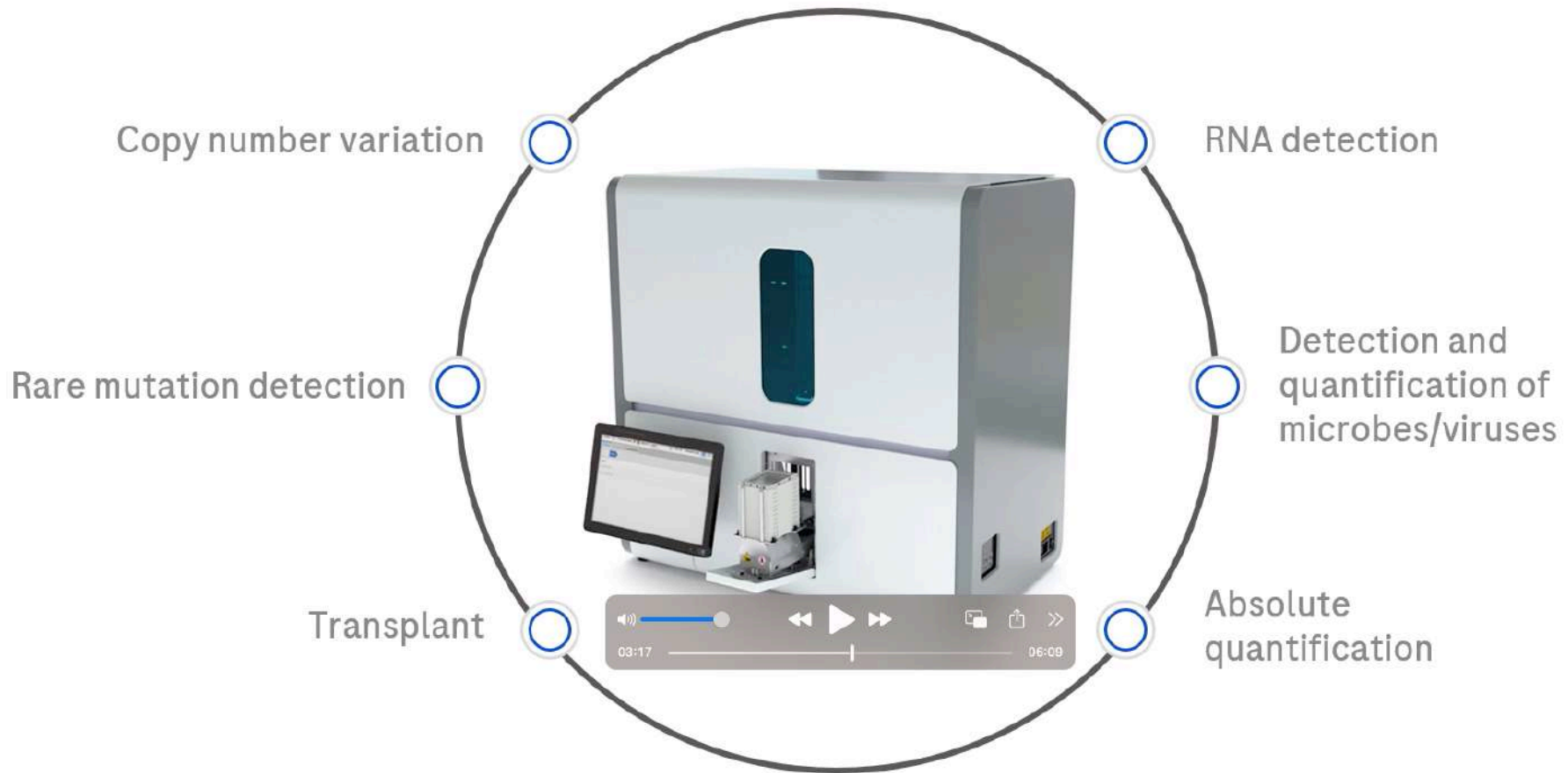
**Increased detection
sensitivity** for
low-abundance targets
due to **enrichment**



Digital PCR delivers **high
precision** (low CV) over
wide dynamic range

More partitions deliver
even higher precision

Digital PCR applications

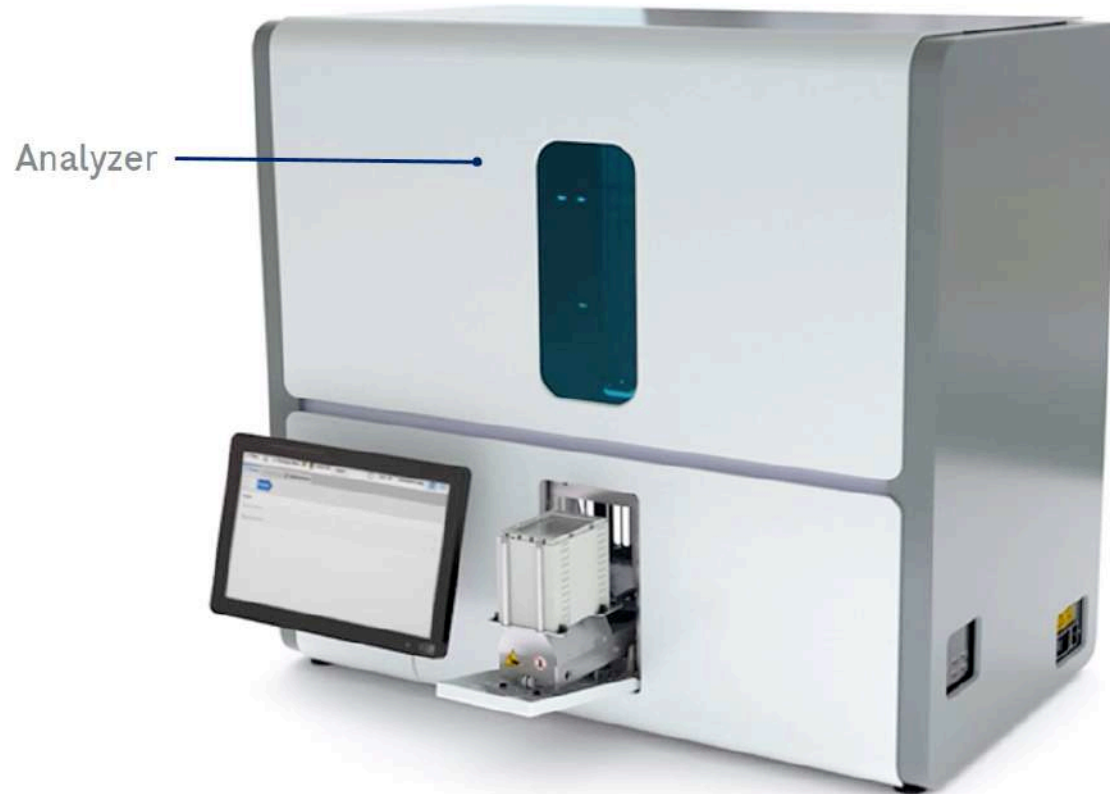


Assessing different HBV RNA forms

Leveraging the power of digital PCR

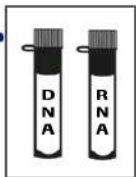
Overview of the Digital LightCycler[®] System

Digital LightCycler[®] System Components



Analyzer

Generic 5X Rxn Mix



Partitioning engine

Consumable

Partitioning fluid

Digital  LightCycler[®]₁₁

Digital LightCycler® System consumables



3 plate configurations to cover multiple applications

High sensitivity (2.2nL/partition)

~45uL, ~20k partitions

Cell-free DNA | Residual DNA testing
Microbial detection | Rare mutation detection



Universal (1.1nL/partition)

~30uL, ~28k partitions

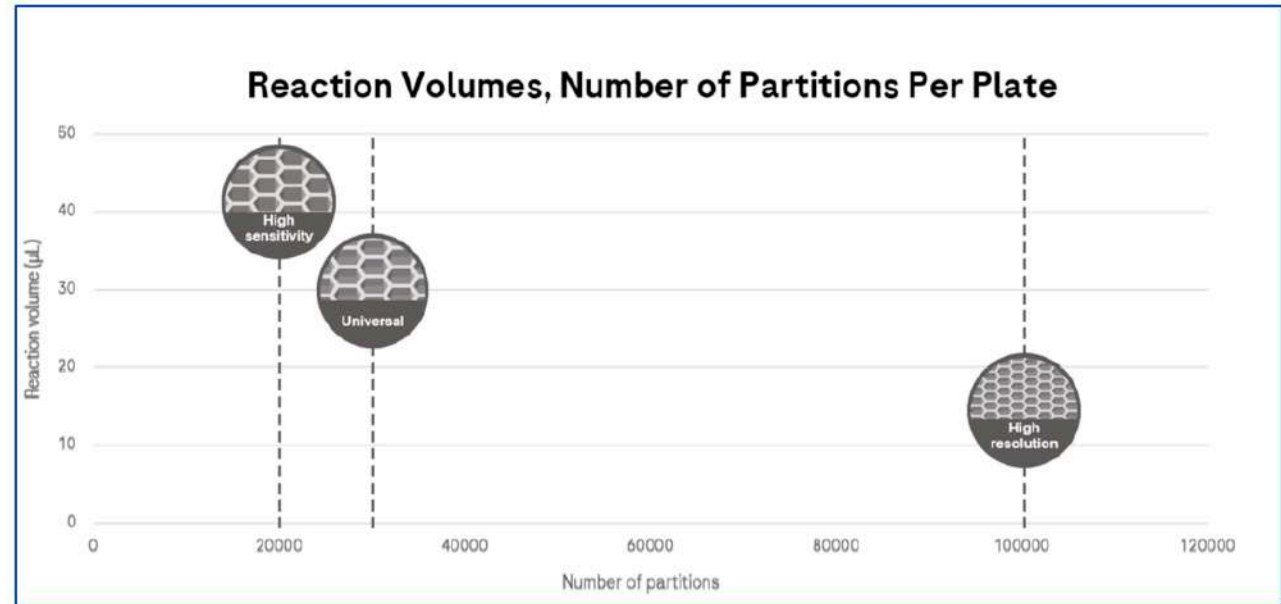
Gene expression
Absolute quantitation



High resolution (0.125nL/partition)

~15uL, ~100k partitions

Copy number variation



3 nanowell plate types (physical partitions)

8 sample lanes per plate

The Digital LightCycler® Partitioning Engine

For Intelligently Simple Sample Partitioning



Touchscreen
operation

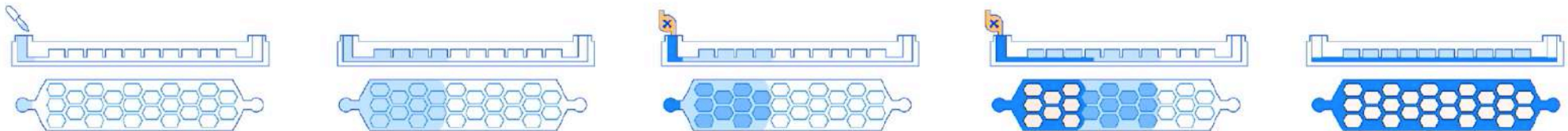
Smart liquid handling with
RFID

Standalone to accommodate multiple room
configurations



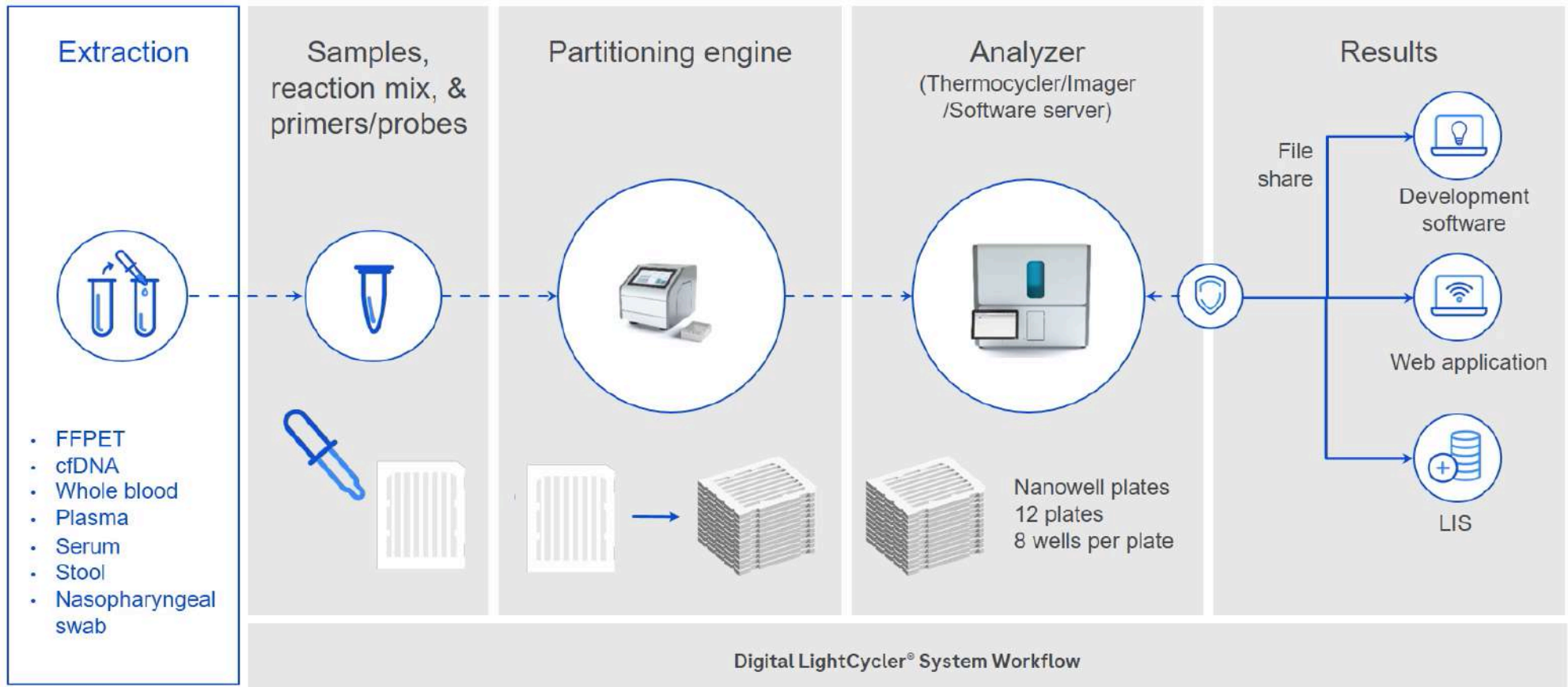
Process of partitioning fluid pushing and separating sample into the partitions

(Roche Digital LightCycler® Nanowell plate zoom-in - illustrative -)



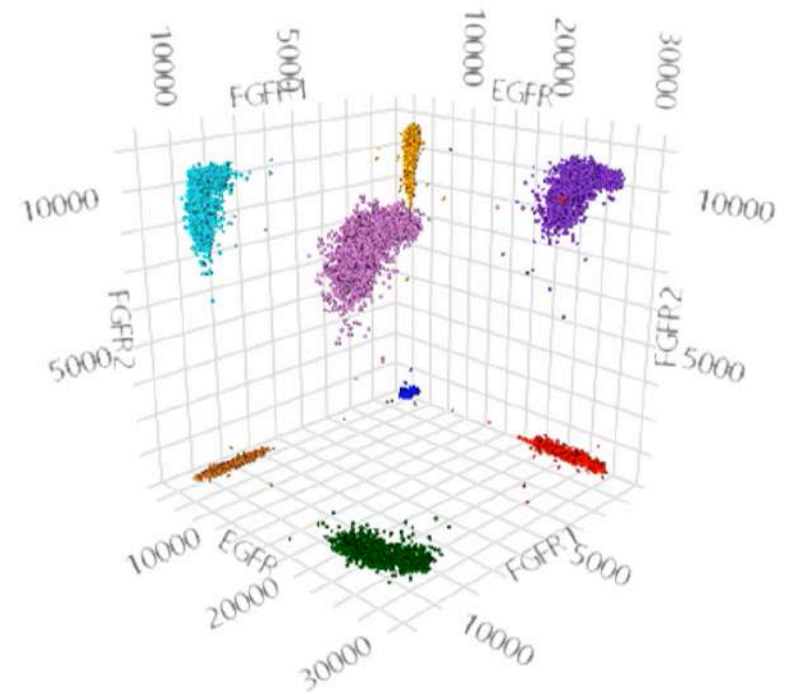
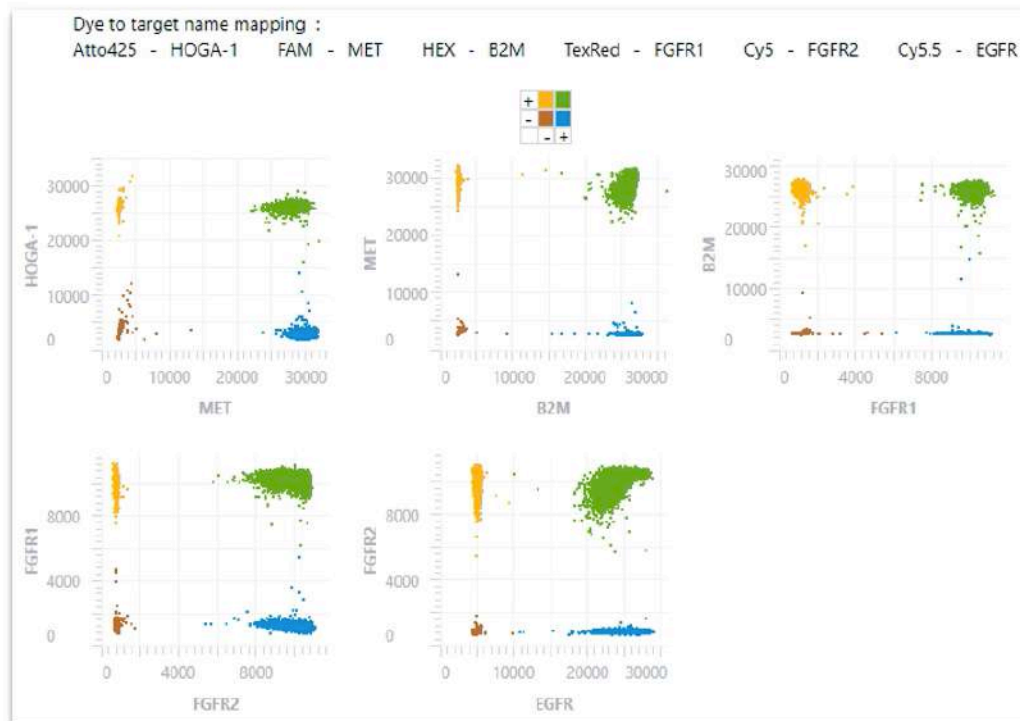
Digital LightCycler® System Workflow

A Connected System to Track the Sample Through the Workflow



6 Optical Channel Detection

2D Scatter plots facilitate manual thresholding for mutation and indel assays. 3D Scatter plots permits visualization of partition fluorescence for three targets (example data shown)



Acknowledgements

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- Fabien Zoulim



Doing now what patients need next