We advance science and save lives, together.

QX Continuum Droplet Digital PCR System

Bio-Rad Laboratories

微滴式數位PCR

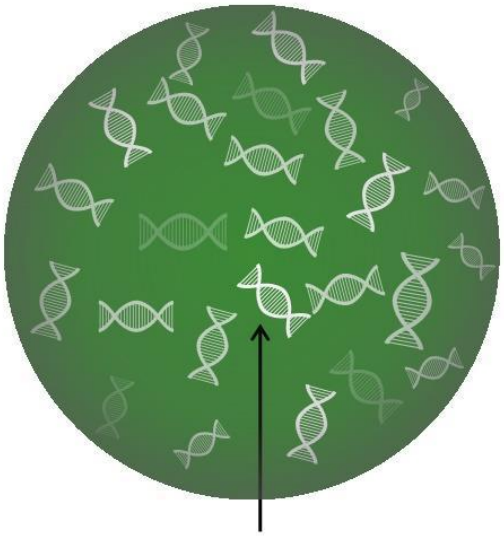
Ryan Chuang
2026/11/12

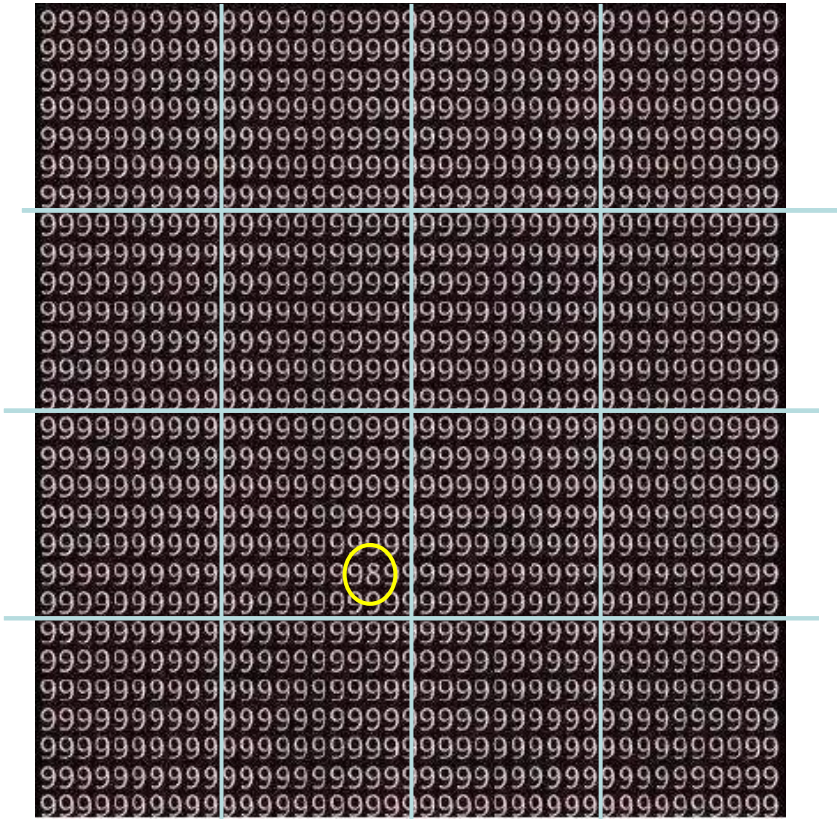


Digital Biology Center

正茂生物科技股份有限公司
Genmall Biotechnology Co., Ltd.

Challenge-How to find the target DNA?





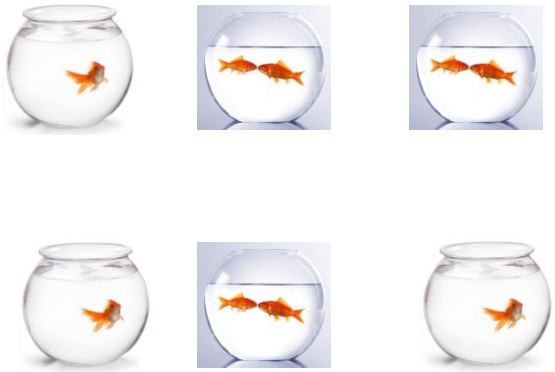
Partition can help!

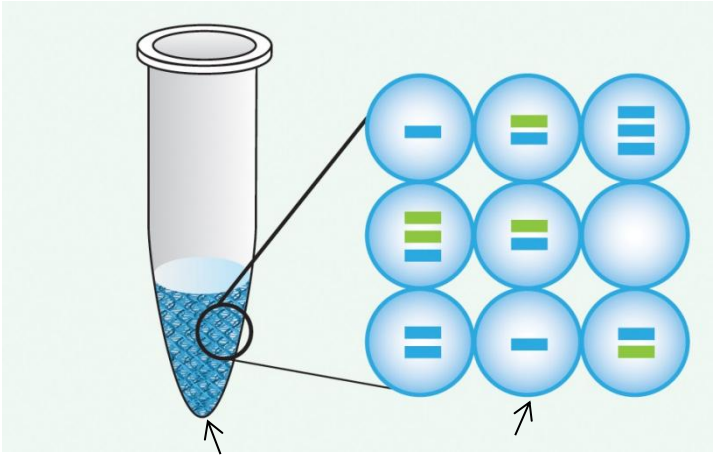
What is Digital PCR?



PCR reaction that is partitioned.







Many thousands
of discrete measurements

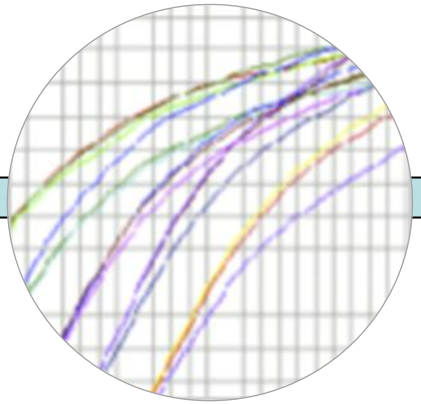
Generations of PCR

1st Generation



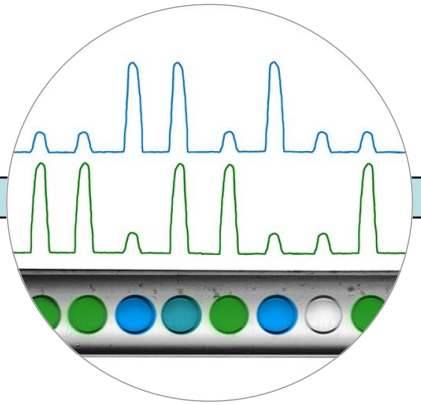
Gel Electrophoresis
(qualitative)

2nd Generation



Real-Time PCR
(indirect quantification)

3rd Generation

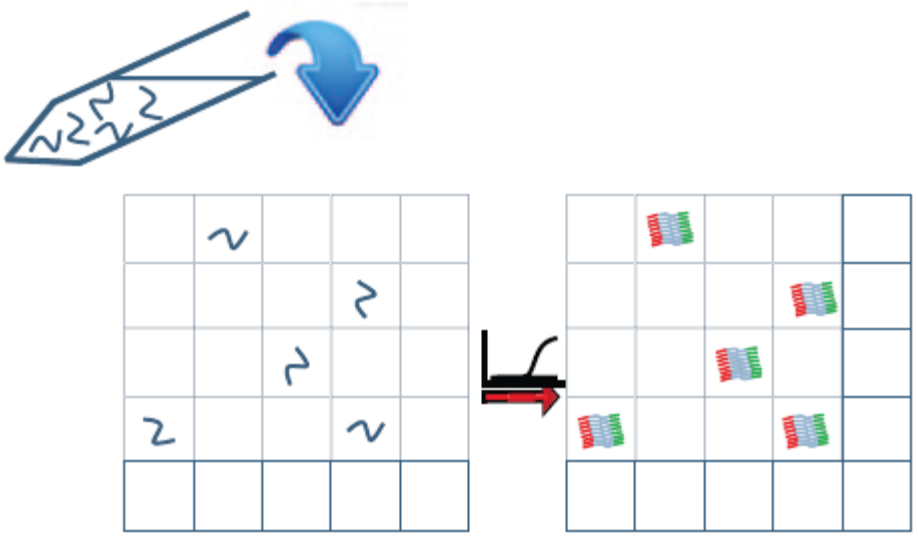


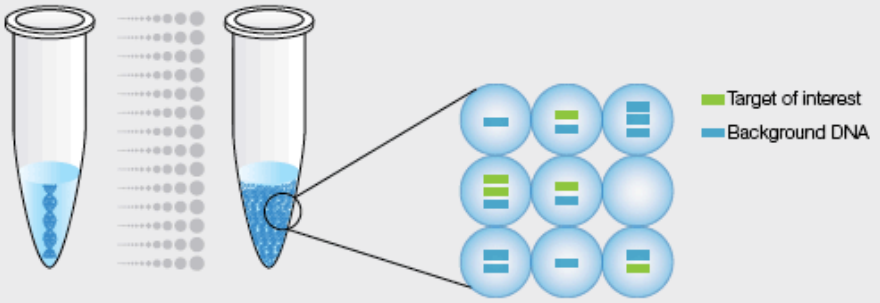
Droplet Digital PCR
(absolute quantification)

- End point (0's or 1's)
- Less sensitive to PCR efficiency
- No standard curve
- More tolerant to PCR inhibitors

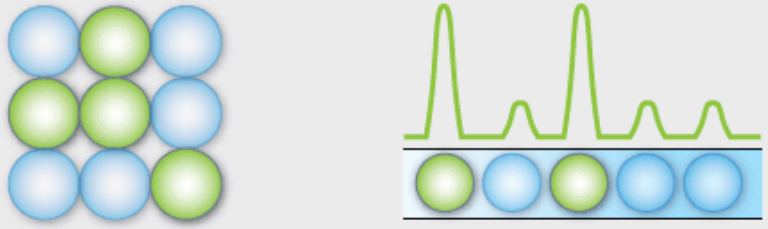
ddPCR improves precision, sensitivity and reproducibility

dPCR Principle





The sample is partitioned into 20,000 droplets, with target and background DNA randomly distributed among the droplets.



After PCR amplification, each droplet provides a fluorescent positive or negative signal indicating the target DNA was present or not present after partitioning. Each droplet provides an independent digital measurement.

"X" target copies

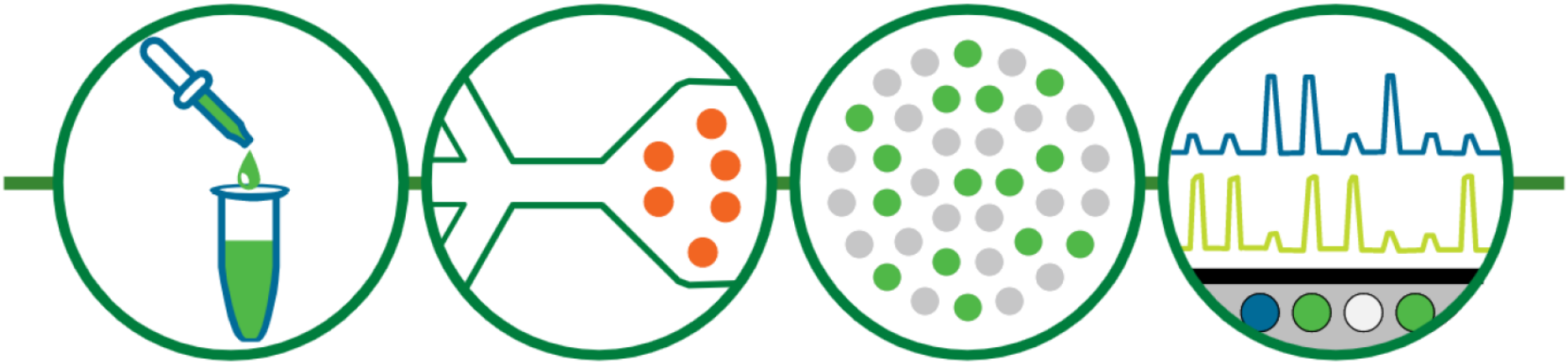
Positive and negative droplets are counted for the sample and the software calculates the concentration of target DNA as copies per microliter.

- End point (0's or 1's)
- Less sensitive to PCR efficiency
- No standard curve
- More tolerant to PCR inhibitors

ddPCR improves precision, sensitivity and reproducibility

Droplet Digital PCR Work Flow

Overall Droplet Digital PCR Assay workflow is also same

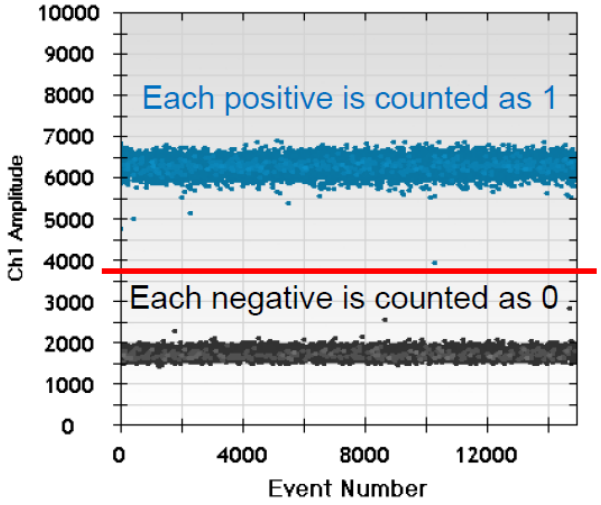


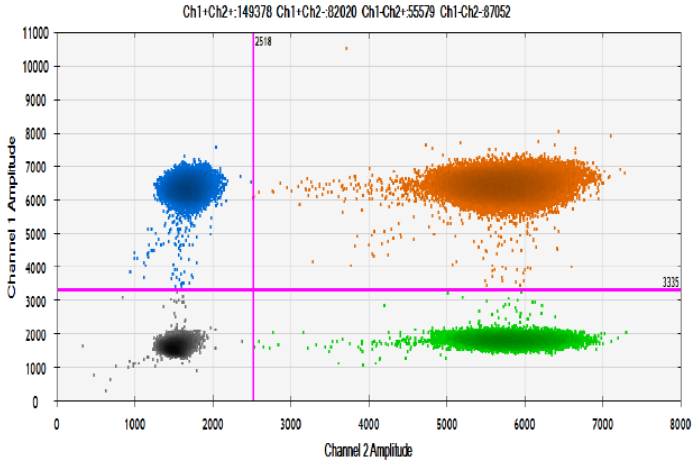
Preparing the Sample	Partitioning	Thermal Cycling the Partitions	Analyzing the Partitions
• Reaction set up is equivalent to qPCR	• Sample is partitioned into thousands of droplets.	• Each droplet behaves as an individual PCR reaction.	• Fluorescence = Positive (1) • No Fluorescence = Negative (0)

Our new portfolio offers more choices across customer needs: automation, multiplexing, throughput, sensitivity…

Droplet Fluorescence Converted to a Digital Signal

- Positive droplets contain at least 1 copy of target DNA (cDNA)
- Positive droplets have increased fluorescence vs. negative droplets
- Software measures the number of positive and negative droplets per fluorophore per sample

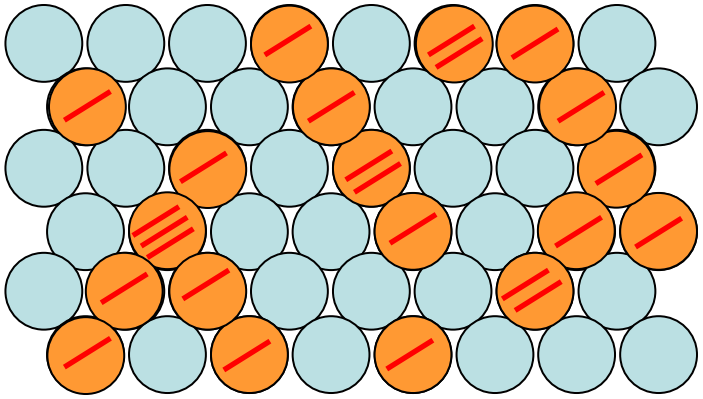


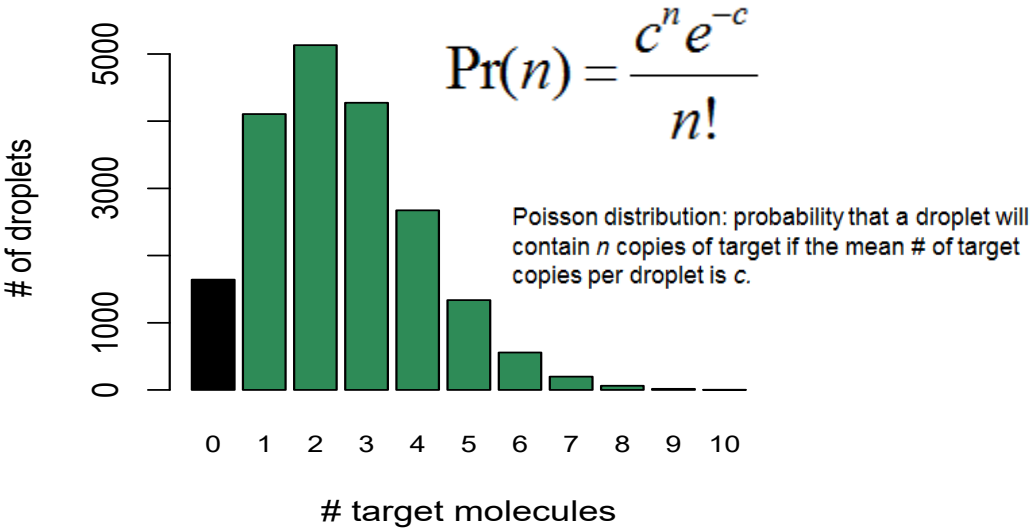


2-D Cluster Plot

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Beyond Limiting Dilution: High Target Concentration (with ddPCR)







Siméon Denis Poisson
(1781-1840)

"X" target copies

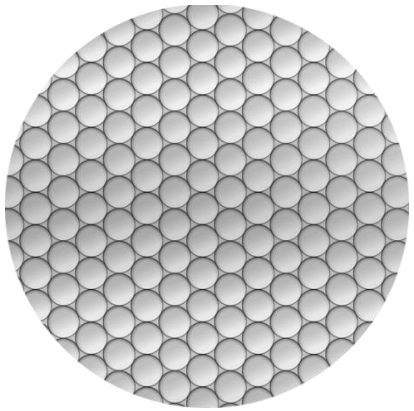
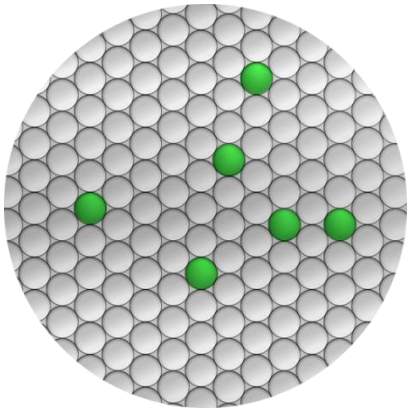
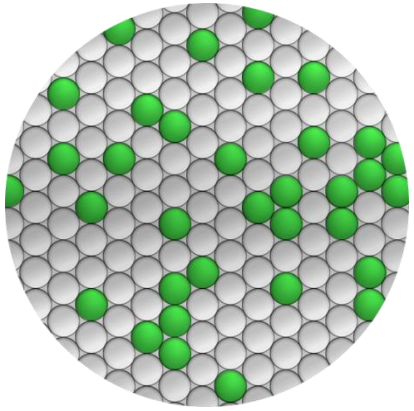
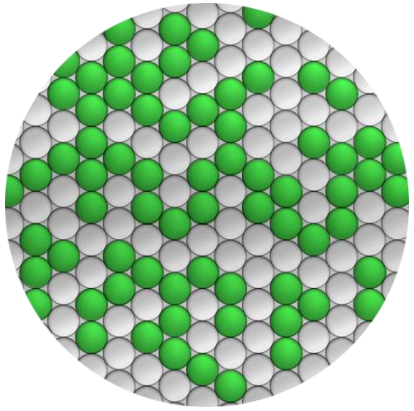
Modeling as Poisson

Copies per droplet = –ln (1 – p)

where p = fraction of positive droplets **and** (1 – p) = fraction of negative droplets.

Note: At 1 target copy/droplet, 37% negatives!

Counting Positives to Estimate Target Concentration
(Correct by Poisson)

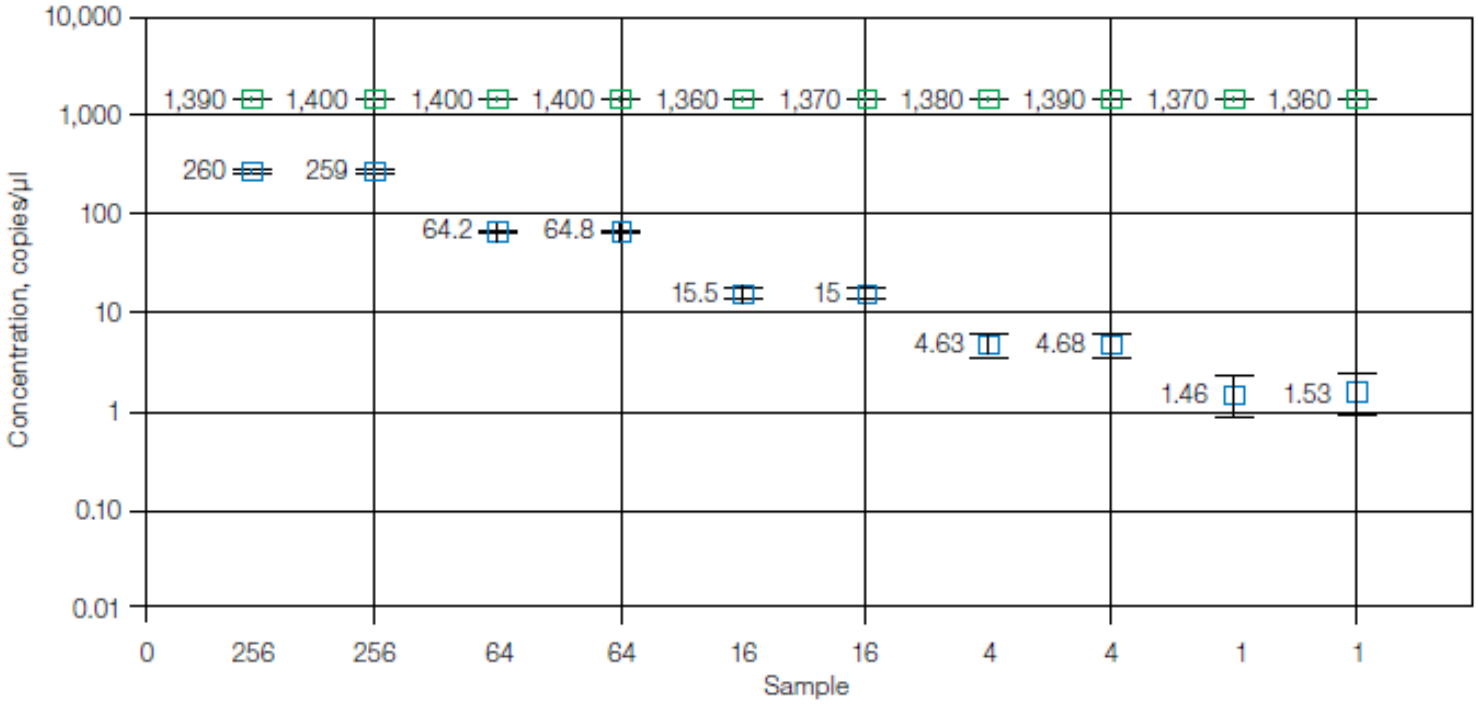
Sample 1	Sample 2	Sample 3	Sample 4
			
<u>NO</u> targets	Low concentration	Medium concentration	High concentration
$p=0$ *positive*/143 *total*	$p=6/143$	$p=34/143$	$p=70/143$
	Poisson corrected **6.2**/143	Poisson corrected **38**/143	Poisson corrected **96**/143



Siméon Denis Poisson (1781-1840)

BIO-RAD

Excellent Reproducibility and Linearity Across Concentrations and Instruments

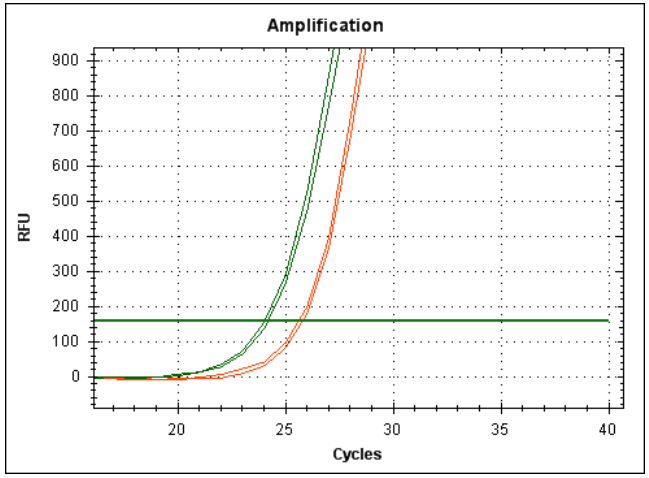


- Readout: copies/µl
- Dynamic range: 1–100,000 copies/well (~330 ng human genomic DNA)

Benefits of Droplet Digital PCR

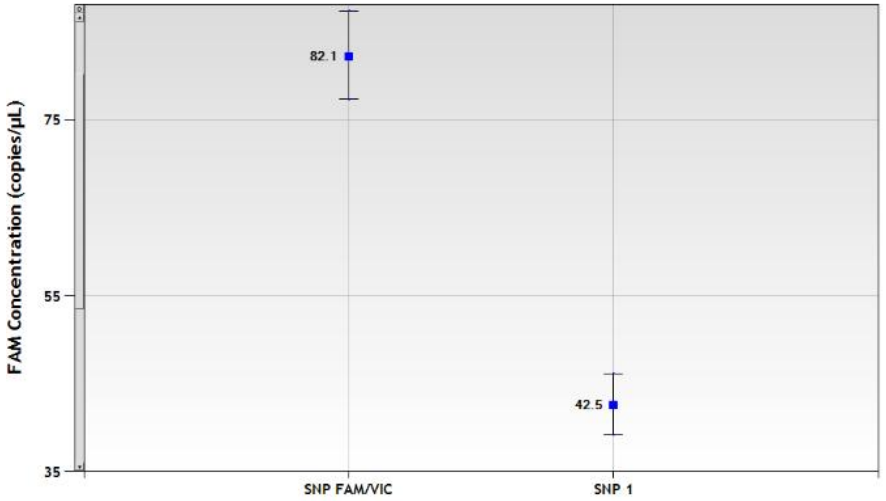
Detecting Small Fold Changes

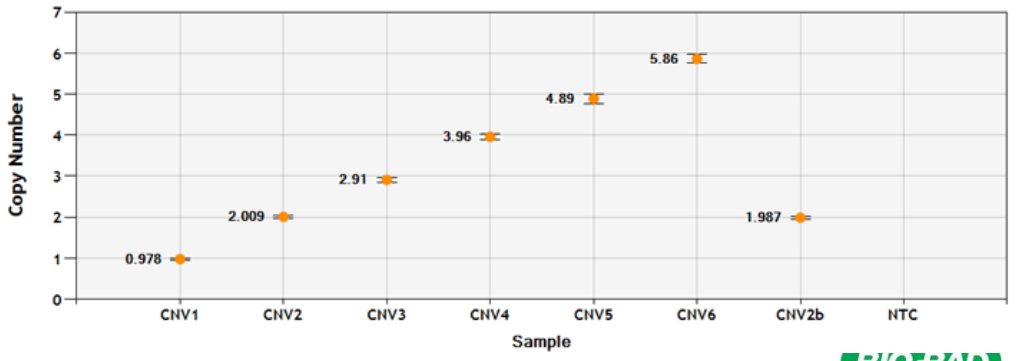
A 2-fold change measured by real-time PCR



Content	Cq	Cq Std. Dev
Unkn-14	26.99	0.110
Unkn-14	26.84	0.110
Unkn-15	25.47	0.115
Unkn-15	25.31	0.115

A 2-fold change measured by ddPCR

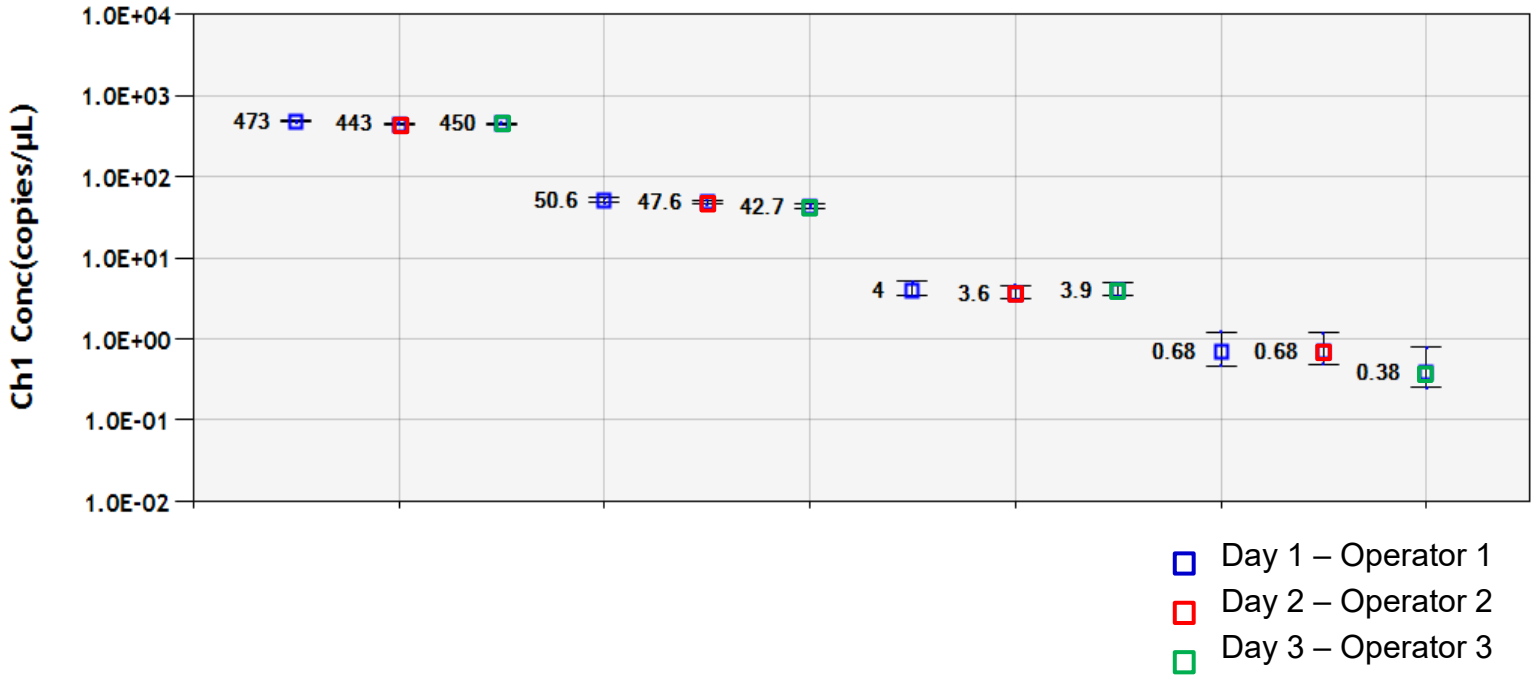




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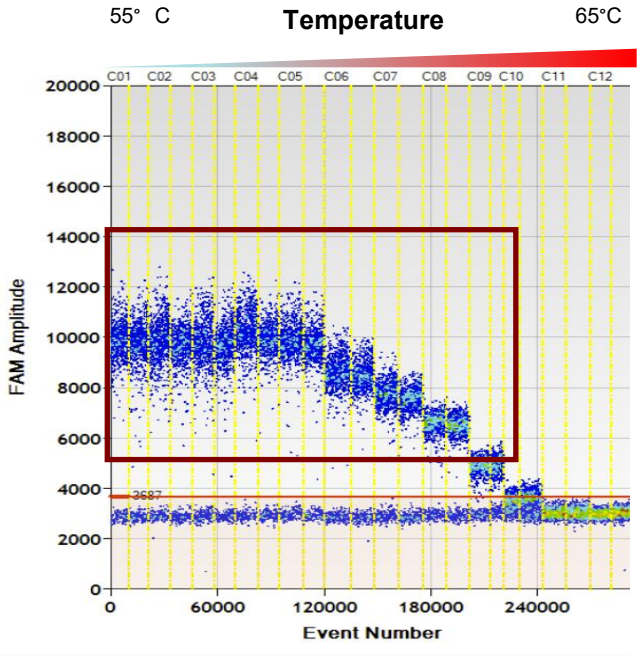
ddPCR™ reproducibility

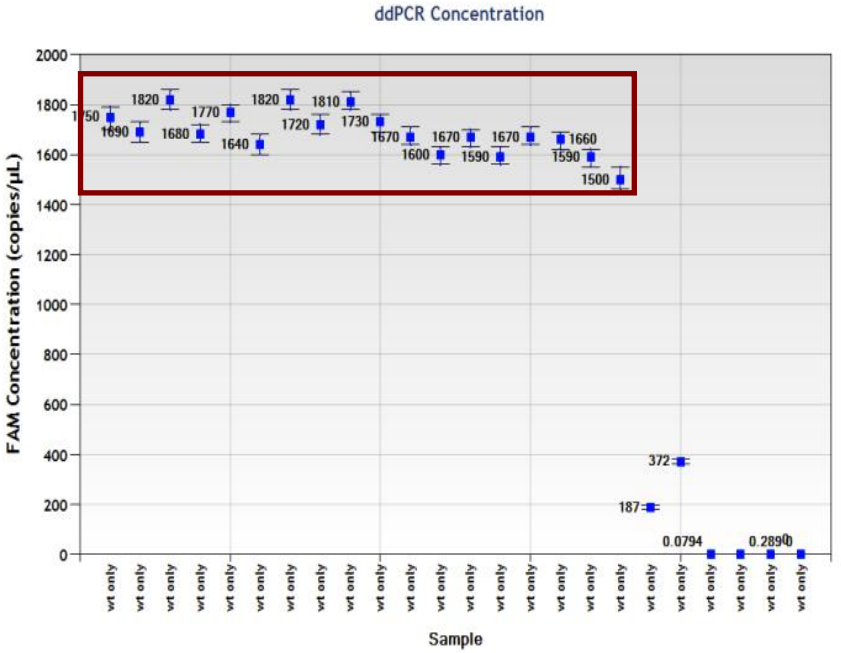
- ✓ Reproducibility trial – 3 operators in 3 different Korean laboratories using the same assay and samples obtained a high degree of data uniformity



PCR efficiency has a minimal impact on ddPCR™

- Thermal gradient mimics different PCR efficiencies
- Same concentration measured across different annealing temps





Advantages of Droplet Digital PCR

Precision

- 10% discrimination, low CVs on replicates, 95% CI

Absolute Quantification

- No standard curves required

Multiplexing

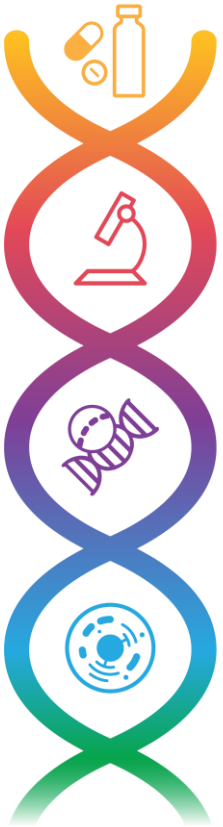
- Simple strategies to maximize sample utilization

Sensitivity

- Detection of rare targets in complex backgrounds

Tolerant of PCR Inhibitors

- Cycle to end point, partitioning



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Applications of Droplet Digital PCR

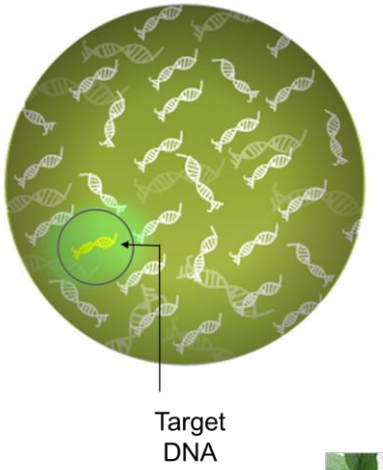
Applications of ddPCR



- **Cancer Biomarker Studies and Copy Number Variation**
  - Measure varying degrees of cancer mutations, detect rare DNA target copies, and resolve copy number variation states with superior sensitivity and resolution.

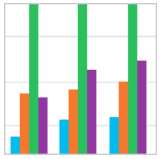


- **Pathogen Detection**
  - Employ the extreme precision of the QX200 System to quantify small fold changes in target DNA or RNA molecules in pathogen detection and monitoring.





- **Next Generation Sequencing**
  - Perform accurate quantification and qualification of NGS libraries
  - NGS data validation



- **Gene Expression Analysis**
  - Achieve reliable and reproducible measurements of small fold changes for low abundance of mRNA and microRNA and RNA variants



- **Food Testing**
  - Perform routine evaluation of genetically modified organisms (GMO) using validated ddPCR methods.



- **Environmental Monitoring**
  - Test a wide variety of environmental samples like soil and water

BIO-RAD

QXC introduction

All in ONE 並可承襲qPCR的操作習慣

PRODUCT INTRODUCTION

QX Continuum Droplet Digital PCR System delivers the **hallmark performance of ddPCR** with the **familiarity of qPCR**.

It offers affordable absolute quantification of up to four targets from a single sample well with the sensitivity to detect small changes in gene expression levels and Bio-Rad's simplest ddPCR workflow, enabling easy adoption of the technology.



特色與效能

QX Continuum ddPCR Solution

Simplicity. Performance. Flexibility.

Simplest Workflow

- All-in-one, integrated instrument delivering walkaway operation
- qPCR-like workflow, using a standard PCR plate
- Intuitive software interface for easy run setup and data analysis

Proven Performance

- Absolute quantification with the hallmark precision and sensitivity of ddPCR
- Accuracy qualified by traceable material from National Measurement Institute, Australia
- >20,000 droplets from 16uL sample, 1 - 96 samples per run

Application Flexibility

- 4-color multiplexing and up to 8 discrete thermal profiles per plate
- Access to Bio-Rad's portfolio of qPCR and ddPCR assays
- Applications: Gene Expression, Copy Number Variation, Mutation Detection, Pathogen Detection, NGS Validation, Food Safety

專用試劑耗材

QX Continuum ddPCR Solution

Instrument
- QX Continuum ddPCR System

Software
- QX Continuum On-system Software
- QX Insight Analysis Software

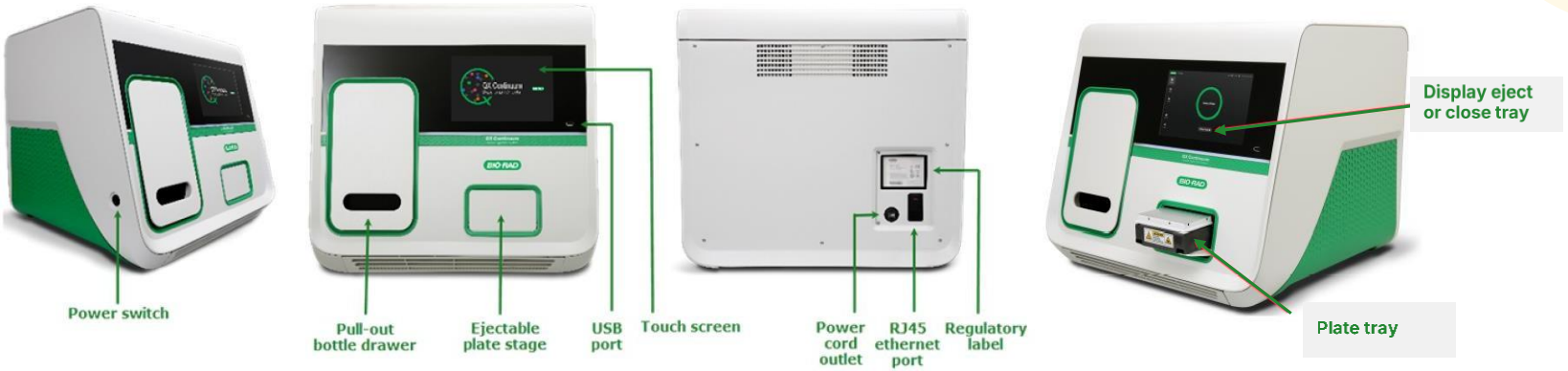
Reagents and Consumables
- QX Continuum Droplet Separation Fluid
- QX Continuum Droplet Generation Fluid
- Mineral Oil for QX Continuum
- Waste Bottle for QX Continuum
- Hard-Shell 96-Well PCR Plates
- PCR Plate Heat Seal

Supermix
- QX Continuum ddPCR Supermix for Probes



儀器外觀

System Hardware Overview



正面重要組件

Reagents
- Several plates of reagents and waste held onboard

- ✓ QX Continuum Droplet Separation Fluid
- ✓ QX Continuum Droplet Generation Fluid
- ✓ Mineral Oil for QX Continuum
- ✓ Waste Bottle for QX Continuum

External Computer
- Plate Layout
- Analysis Package



- ✓ QX Insight Analysis Software



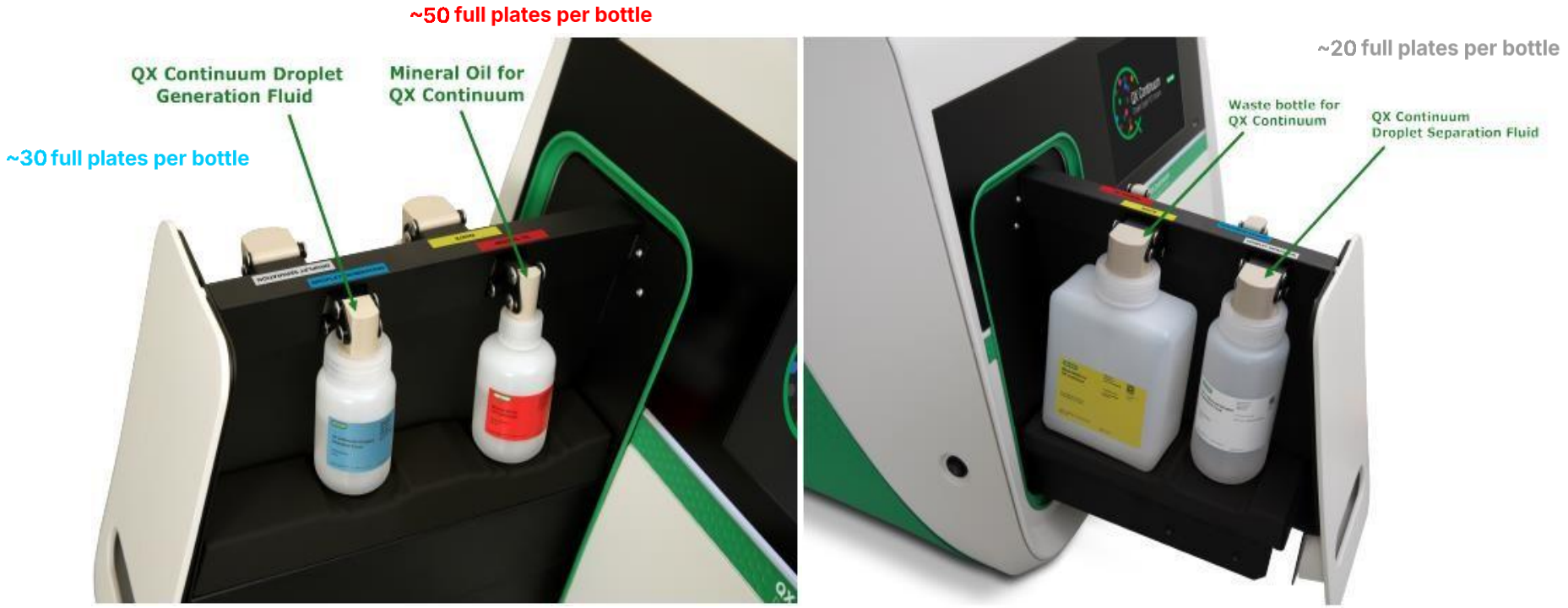
Touchscreen
- Simple interface to start and monitor runs
- USB for setup & data transfer flexibility

- ✓ QX Continuum On-system Software

Consumables
- Samples in standard 96-well PCR plate

- ✓ Hard-Shell 96-Well PCR Plates
- ✓ QX Continuum ddPCR Supermix for Probes

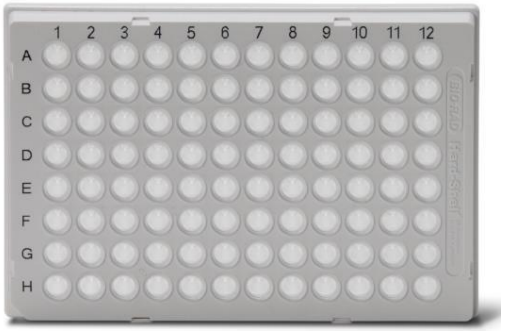
依顏色區分關鍵上機用油



Color-coded labels identify the correct slot for each bottle. The label color on the bottle drawer matches the label color on the bottle.

指定96well & 熱封鋁膜





Hard-Shell 96-well PCR Plate
(HSP9601)



Note: PX1 PCR Plate Sealer and Pierceable Foil Heat Seal are needed to seal the plate.

耗材編號

QX Continuum Consumables and Accessories



Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/clear (HSP9601)

Pkg of 50, white shell/clear well PCR plate



PCR Plate Heat Seal, foil, pierceable (1814040)

Pkg of 100, foil seals for use with PX1 PCR Plate Sealer



PX1 PCR Plate Sealer (1814000)

PCR plate sealer, includes heat sealing instrument, plate support blocks

試劑編號與介紹

QX Continuum ddPCR Supermix for Probes



200 reaction size (Catalog #12019028)

2 tubes of 100 reactions each (2x0.4 mL tube volume)



500 reaction size (Catalog #12019141)

5 tubes of 100 reactions each (5x 0.4 mL tube volume)

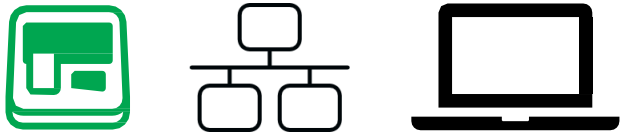


2,500 reaction size (Catalog #12019133): Made to order (MTO) at launch

5 tubes of 500 reactions each (5 x 2 mL tube volume)

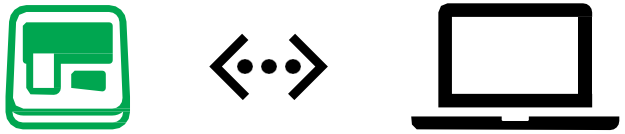
多元化儀器控制方式

Multiple Connection Options



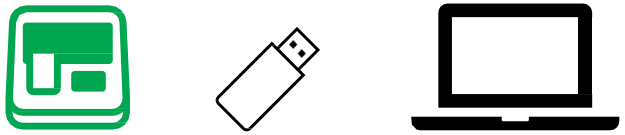
Networked

- Recommended configuration
- QX Continuum connected to lab network
- PC/Laptop connects to QX Continuum via TCP/IP



Direct

- QX Continuum & PC/Laptop connected directly via ethernet cable



Offline

- QX Continuum not connected to network
- USB Drive used to transfer data to/from QX Insight

上機軟體 & 分析軟體

Powerful Software

Intuitive & Simple Workflow



QX Continuum Software

- On-instrument touchscreen
- Main Features
  - Instrument operations
  - Reagent & waste management



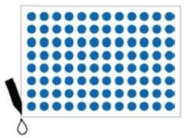
QX Insight Software

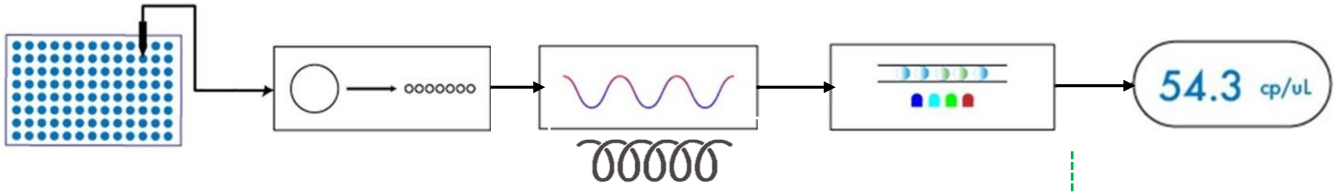
- Off-instrument software
- Main Features
  - Experiment setup
  - Data analysis

工作流程

All-In-One, Walk-away Operation

Setup | **Droplet Generation → Thermal Cycling → Droplet Detection** | **Analysis**





QX Insight SW



- Set up experiment
- Transfer plate map

QX Continuum HW & SW



- Load sample plate
- Start run and walk away

QX Insight SW



- Analyze data
- Export data

樣品前處理與注意事項

0. Prepare Sample plate



QX Continuum ddPCR Supermix for Probes is **a 4x concentrated**, ready-to-use reaction mix containing all components — except primers, probe(s), and template — required for probe-based Droplet Digital PCR (ddPCR).

Table 2. Example of a multiplex copy number variation (CNV) assay.

Component	Volume per Reaction, µl	Final Concentration
4x ddPCR Supermix for Probes	4	1x
20x target 1 primers/probe (FAM)	0.8	900 nM/250 nM
20x target 1 primers/probe (HEX)	0.8	900 nM/250 nM
20x target 1 primers/probe (ROX)	0.8	900 nM/250 nM
20x target 1 primers/probe (Cy5)	0.8	900 nM/250 nM
Diluted restriction enzyme (see DNA digestion section)	1	2–5 units*
Sample	Variable	Up to 16.5 ng/µl**
RNase-/DNase-free water	Variable	—
Total volume	**16**	**—**

* For DNA ≤3.3 ng/µl, restriction enzyme digestion may not be necessary, and no wait is needed for digestion. The reaction mixture can be loaded into the QX Continuum System immediately.

** Maximum input should be ≤264 ng per reaction.

DNA Digestion Improves ddPCR Performance

- Fragmentation before droplet generation improves:
  -Accuracy (separates tandem copies) CNV assay
  -Lower viscosity
  -Better template accessibility (>3.3 ng/µl) loading>50ng
- Use restriction enzymes:
  -Do NOT cut target/reference
  -Methylation-insensitive
  -Prefer 4-base cutters / high-fidelity enzymes
  -Validated enzymes: HaeIII, MseI, AluI, HindIII, CviQI
- Perform digestion directly in ddPCR setup

Important: Use **only** the supermixes from Bio-Rad that are specifically designed and approved for use with the QX Continuum Droplet Digital PCR System. Do **not** use other supermixes, including QX supermixes, since they can harm the instrument.

設定溫度 & 盤面規劃

1. Set-up Experiment (QX Insight)

Thermal Profile Setup

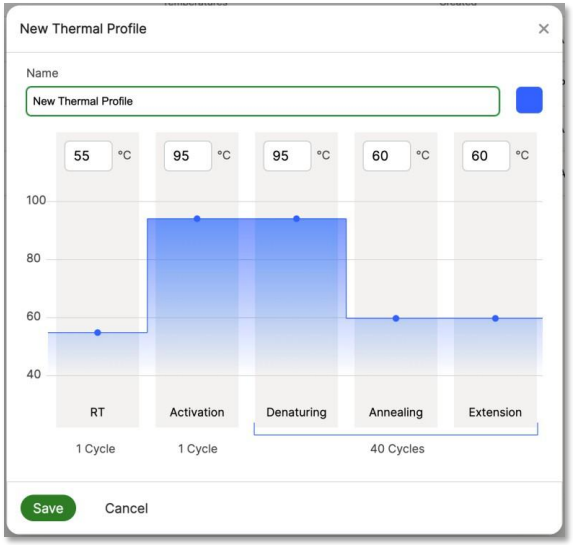
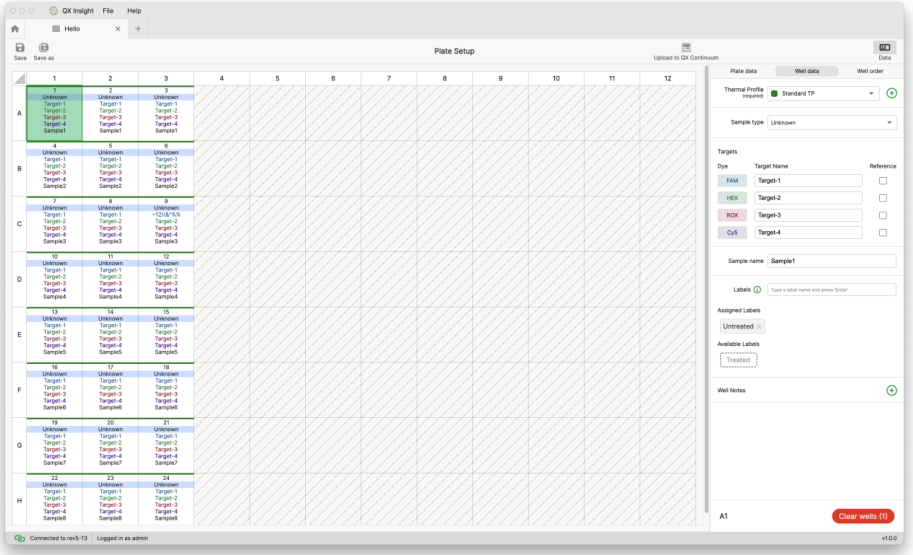


Plate Setup



BIO-RAD

封膜 & 放置孔盤 & 設定

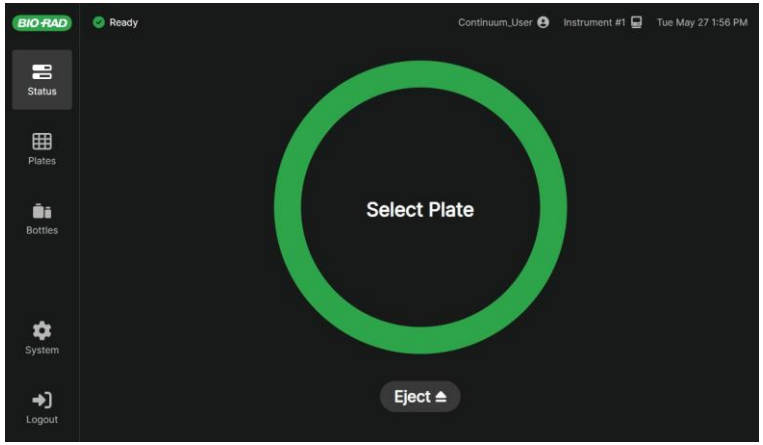
2. Load Sample Plate



Load Sample Plate



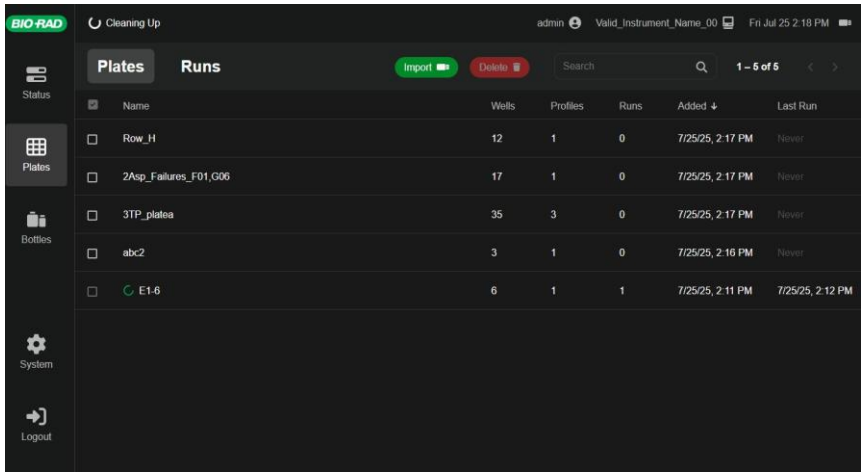
Select Plate



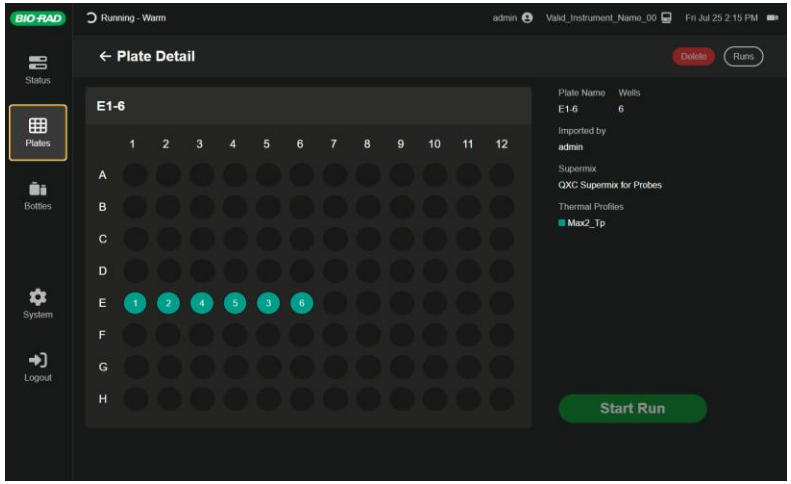
點選已上傳的protocol & Run

3. Select Plate and Start Run (QX Continuum Software)

Plate Selection



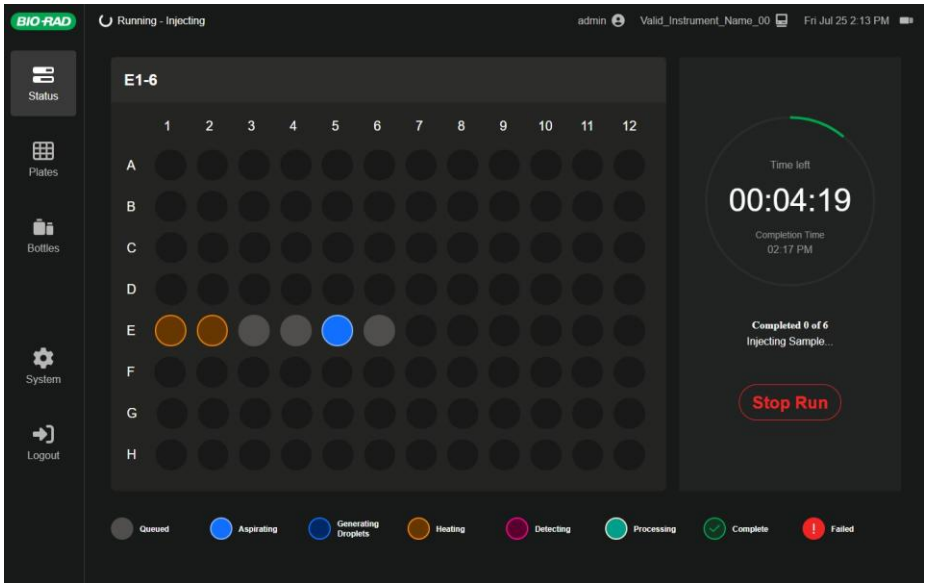
Start Run

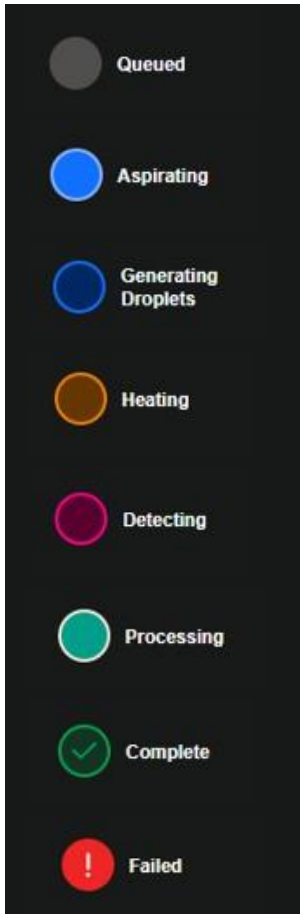


依顏色標示每個well 現行狀態

4. Walk Away or Monitor Run Status on Screen (QX Continuum Software)

Run Status







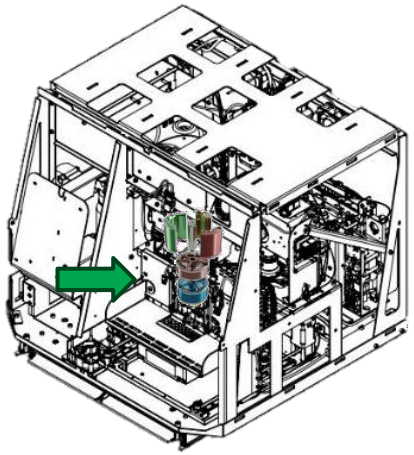
BIO-RAD

獨特的加熱模式

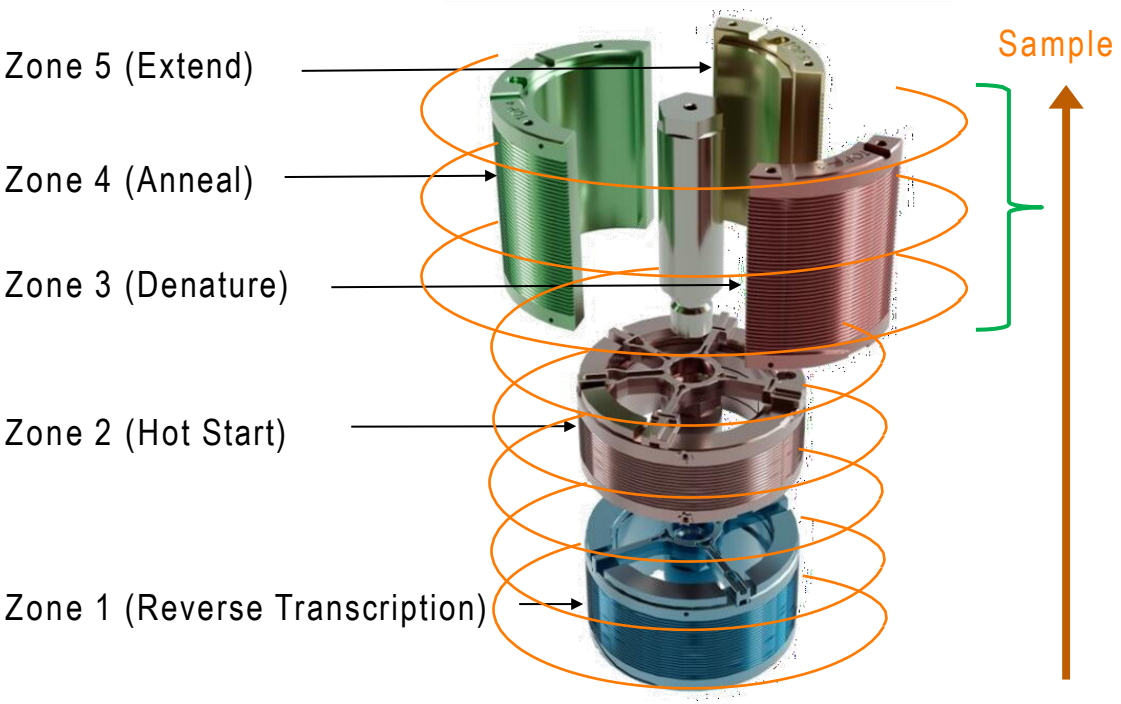
4. Run in Progress (behind the scenes)

Unique Thermal Cycler Design

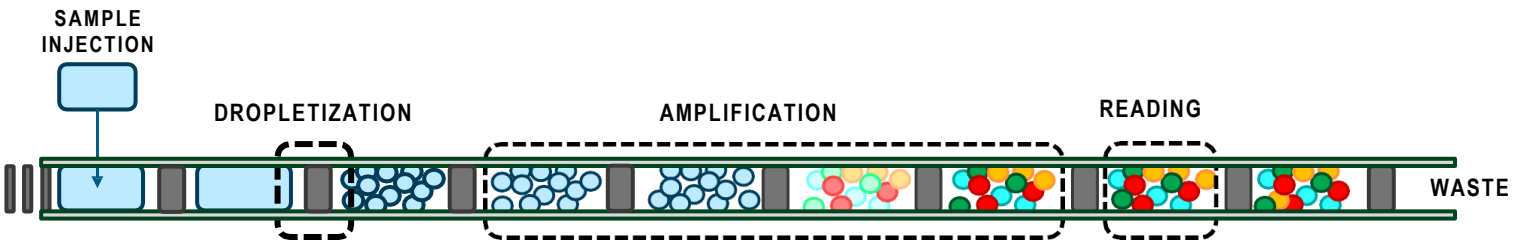
- 5 zones
- Fixed time for each zone
- Up to 8 independent thermal profiles per plate



40 rounds = 40 cycles



儀器工作示意圖

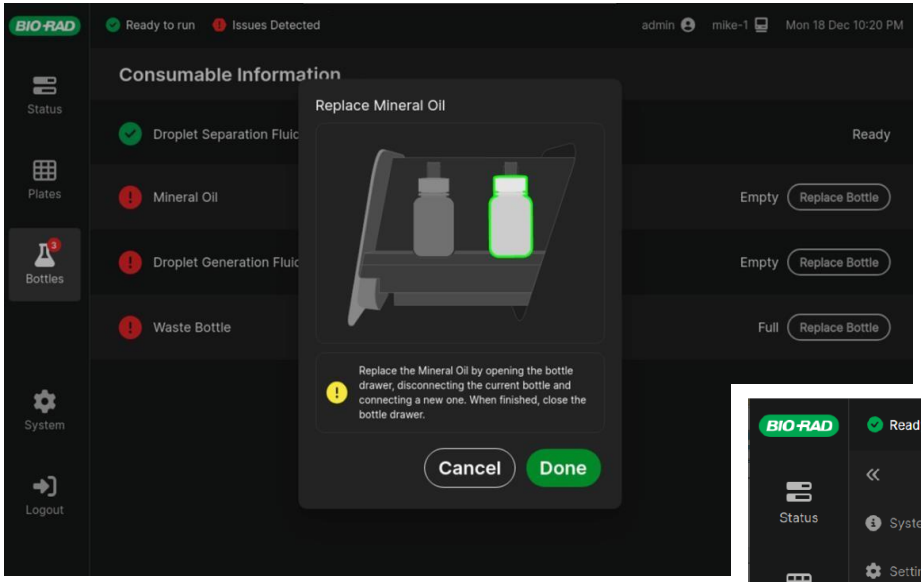


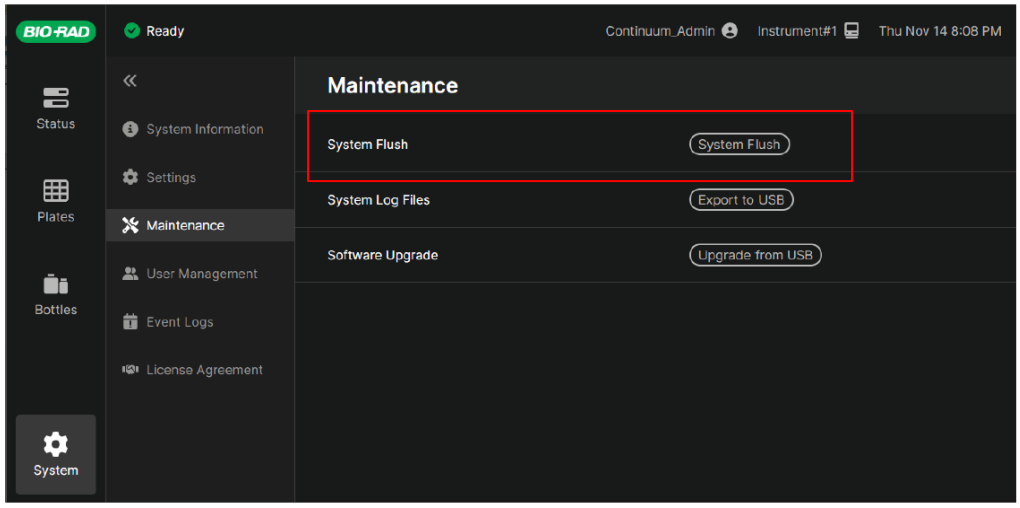
In a **single, continuous conduit of fluid** (connected by through junctions) aqueous sample boluses—equally spaced by a **heterogenous mix of immiscible fluid**—are **dropletized, amplified, and read** to provide **precise quantification** of nucleic acid targets in **four channels**.

機器維護（重要: 每月進行系統Flush）

QX Continuum Software

Reagent & Waste Management

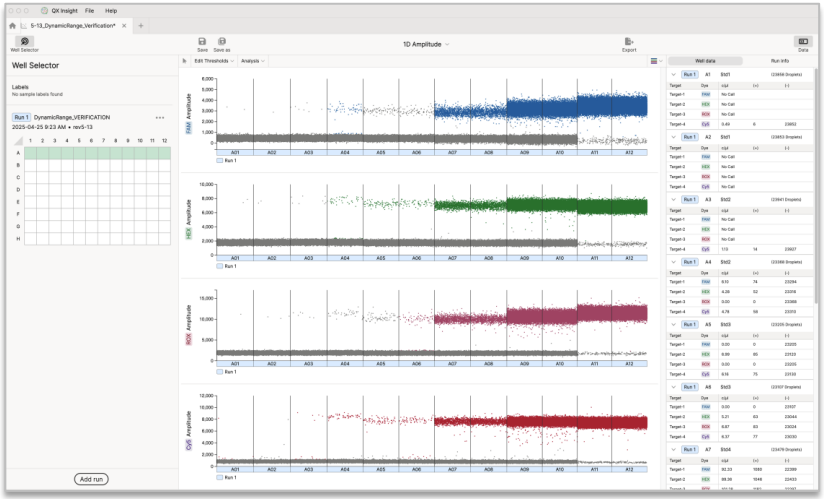




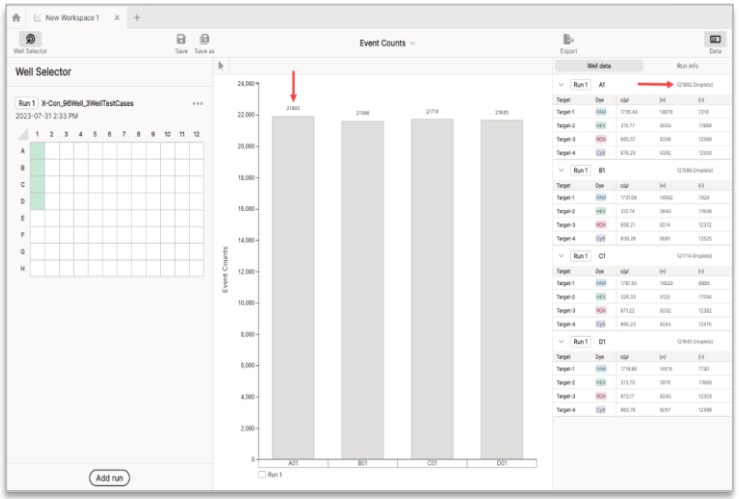
分析軟體介面

5. Data Analysis (QX Insight)

1D Amplitude



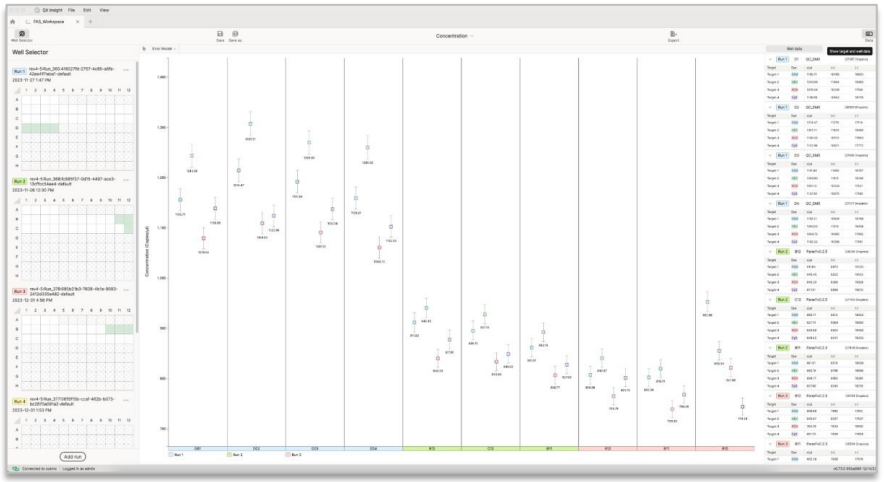
Event Counts



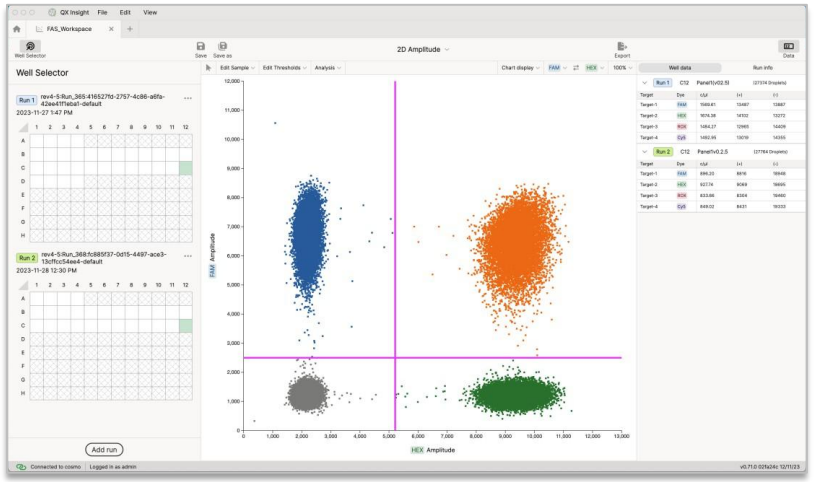
分析軟體介面-2

5. Data Analysis (QX Insight)

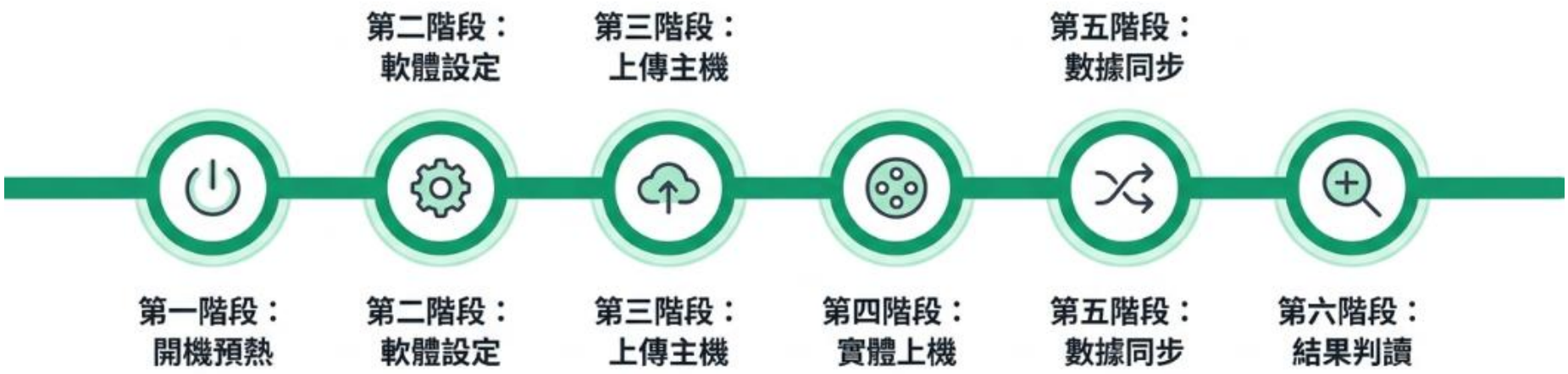
Multi-Plate Analysis – Concentration



Multi-Plate Analysis – 2D Amplitude



流程回顧


第二階段：
軟體設定
第三階段：
上傳主機
第五階段：
數據同步
第一階段：
開機預熱
第二階段：
軟體設定
第三階段：
上傳主機
第四階段：
實體上機
第五階段：
數據同步
第六階段：
結果判讀

流程回顧

第一階段：機台開機與系統預熱



電源啟動

按下主機左側電源開關。

系統登入

觸控螢幕下拉 Username，
輸入 6 位數 PIN 碼。

PIN: 123456

30 Min

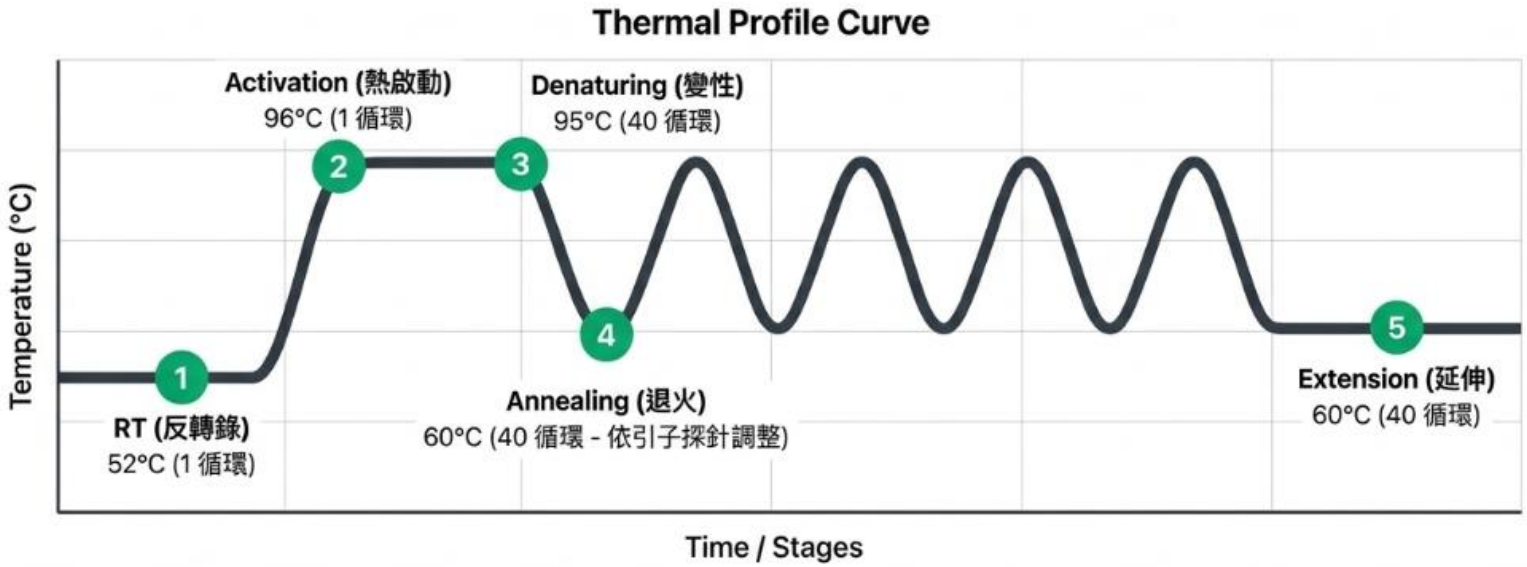
開機自檢與初始化，完全就緒前方可使用

⚠ 注意：登入後系統將持續保持登入狀態，直到手動點擊左下角 Logout。

流程回顧

第二階段 (上)：編寫溫度程序 (Thermal Profiles)

⚠ 重要先決條件：建立盤面前，必先建立溫度程序！
(單盤最多 8 組程序，運行時間最長可達 16 小時)

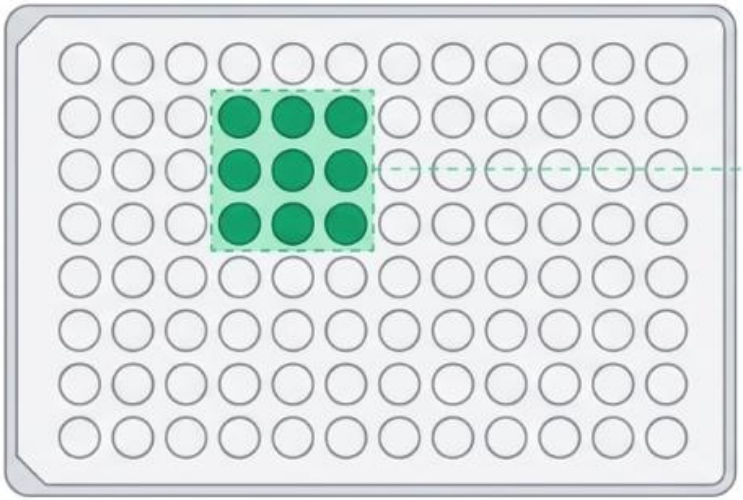


操作步驟：QX Insight → Thermal Profiles → New → 命名與指派專屬色標 → 儲存

BIO-RAD

流程回顧

第二階段 (下)：反應盤面編寫 (Plates Layout)



Step 1 選取

滑鼠框選目標孔位。

Step 2 賦值 (Well Data)

指派已建立的 Thermal Profile、輸入 Sample Name / Sample Type (如 Unknown, NTC)。

Step 3 目標

設定檢測 Targets。

Targets

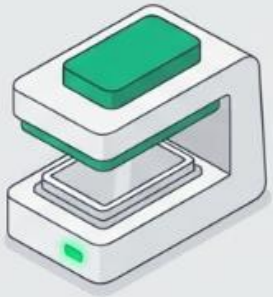
Dye	Target Name	Reference
FAM	Target-1	☐
HEX	Target-2	☐
ROX	Target-3	☐
Cy5	Target-4	☐

第三階段：盤面上傳至 QXC 主機

流程回顧

第四階段 (上)：實體上機準備與裝載

1



1. 熱封 (Seal)

將反應液加入 HSP9601 孔板（空孔保持乾燥）。覆蓋鋁膜，使用 PX1 於 180°C 熱封。

2



2. 離心 (Spin)

以 2,200 rcf 離心 2 分鐘，確實沉降氣泡。

3

務必確認 A1 孔位於左上角！

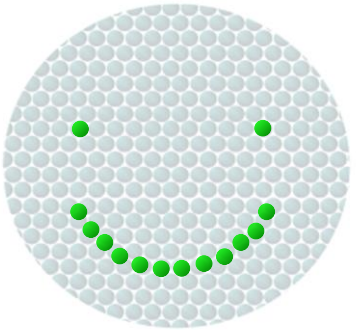


3. 裝盤 (Load)

主機螢幕點擊 Start Run 彈出托盤。水平放入孔板。

ddPCR 優勢總結

- **Sensitivity**
  - 10-1000x fold improvement over qPCR
  - Works with FFPE blood and tissues samples
  - Compatible with diverse applications



- **Absolute Quantification**
  - Quantify gene expression more precisely
  - No Standard Curve or ΔΔCq
- **Precision**
  - Measure more subtle differences in expression or mutation
  - Detect structural variants in cancers
  - Unparalleled precision (+/- 10%)
- **High/Low Throughput**
  - Flexibility to tune the system to meet user needs
  - Maximum 4 x 96 per day
  - Save $ and Time

Q&A



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