STILLA

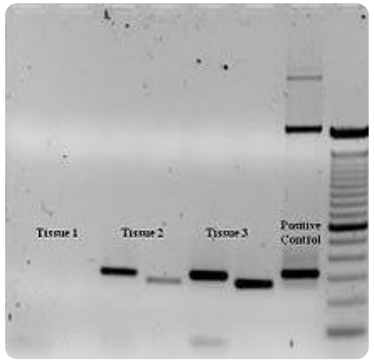
DISCOVER THE SIMPLICITY OF

DIGITAL PCR

NEXT GENERATION OF PCR
Digital PCR

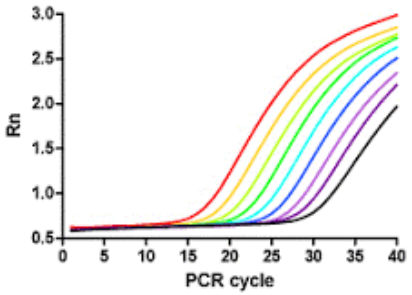
OUR MISSION:
MAKE DIGITAL PCR A LAB COMMODITY

PCR



Amplify Target DNA

Quantitative PCR

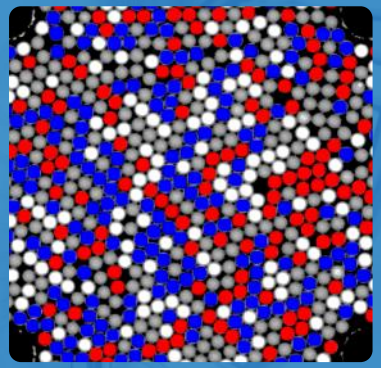


Relative quantification

Real-time with standard curves

Ubiquitously spread method

Digital PCR



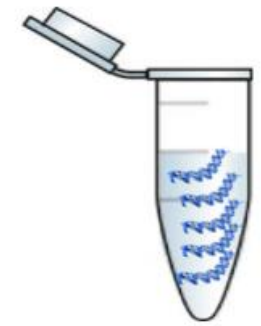
Absolute quantification

No standard curve (endpoint PCR)

Increased sensitivity

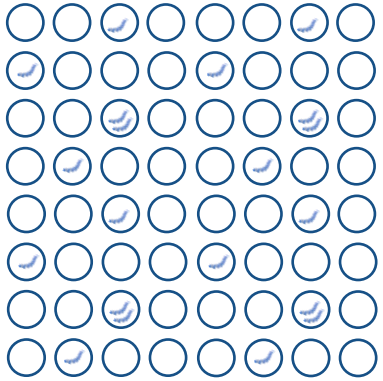
PRINCIPLE
OF DIGITAL PCR

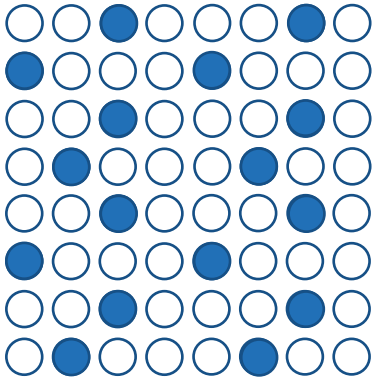
PARTITIONING



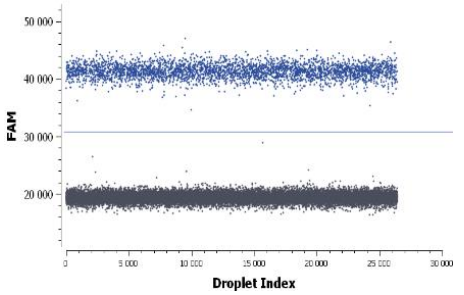
Wild-type DNA

PCR





READING & ANALYSIS



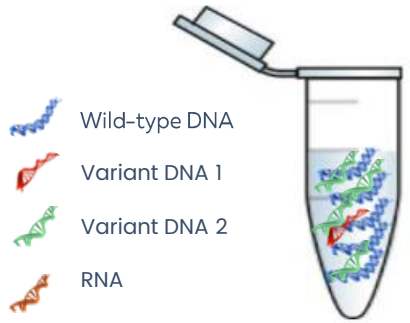
RESULTS
2636 cp/µL with **2.2 %** uncertainty

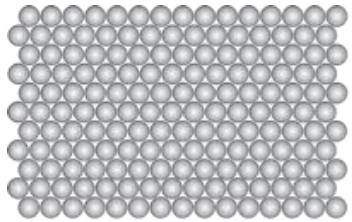
POISSON STATISTICS

$$\frac{N_{pos}}{N_{tot}}$$

PRINCIPLE
OF CRYSTAL DIGITAL PCR™

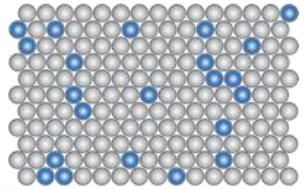
PARTITIONING

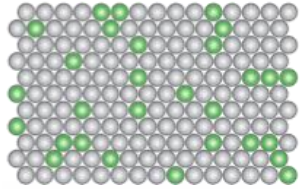


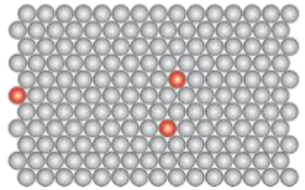


Droplet crystal:
Self-assembled
array of droplets

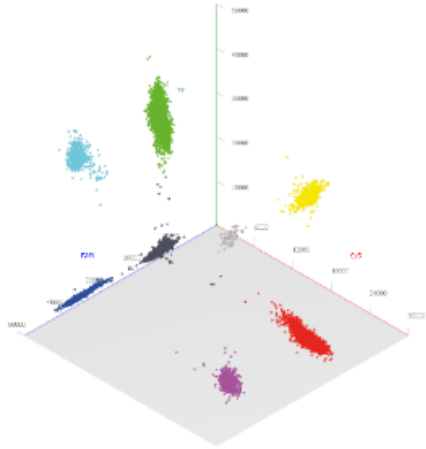
PCR







READING & ANALYSIS



C = 102 cp/µL
C = 152 cp/µL
C = 8 cp/µL

2 Parameters for accurate quantification in dPCR:

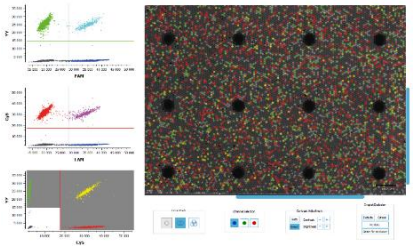
- Number of droplets
- Size of the droplets

THE UNIQUE FEATURES
OF THE NAICA™ SYSTEM

Sapphire Chip
(consumable)

Naica Geode

Naica Prism3



Crystal Miner
(software)

An easy-to-use and integrated solution for digital PCR

Fast time-to-result (2h30)

Reliable multiplex assays with 3-color detection

"We are extremely satisfied with the Naica System, which fully answers our needs in terms of precision and reproducibility for liquid biopsy testing."

Dr. Ludovic LACROIX
Dir. Translational Research /
Institut Gustave Roussy

AT THE HEART OF OUR INNOVATION:
THE SAPPHIRE CHIP

A unique and patented
partitioning technology: **droplet crystals.**

Sapphire Chip
pre-filled with oil

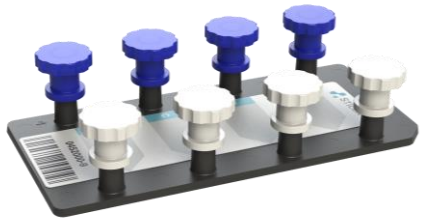
Input volume	**25 µL**
Droplets per sample	**~ 30 000**
Droplet volume	**0.59 nL**
Number of samples	**4 / chip**
LOD	**0.2 cp/µL**
Dynamic range	**5 logs**

Droplet crystal:
Self-assembled
array of droplets

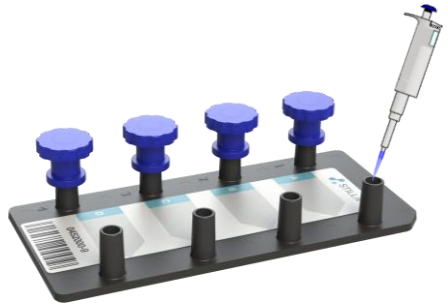
Stilla Technologies – 1 Mail du Professeur Georges Mathé,
94800 Villejuif – www.stillatechnologies.com

STEP 1

PREPARE THE SAPPHIRE CHIP – 5 MIN



UNPACK SAPPHIRE CHIP



PIPETTE 25 µL OF PCR MIX

SEAL INLET PORT WITH CAP

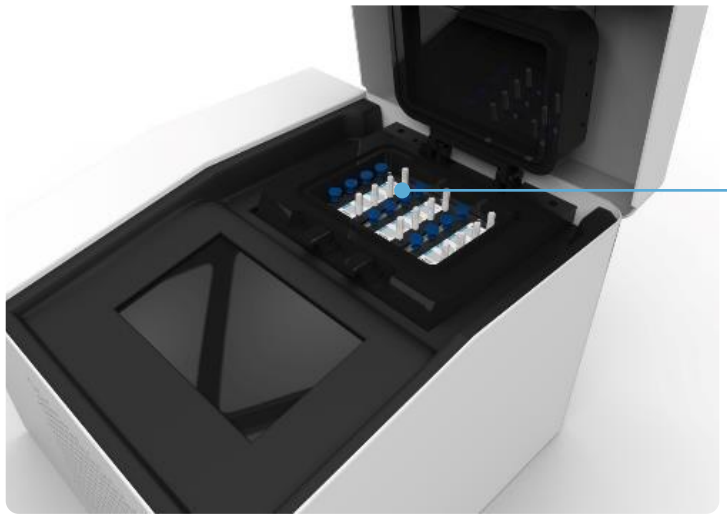
COMPATIBLE MIXES AND CHEMISTRIES:

Use with Quanta BioSciences PCR and RT-PCR Mix with no ROX

With TaqMan® Probes, add Fluorescein as reference dye

Stilla Technologies – 1 Mail du Professeur Georges Mathé,
94800 Villejuif – www.stillatechnologies.com

STEP 2
PARTITION & AMPLIFY – 2H10



STEP 2.1 – PARTITION

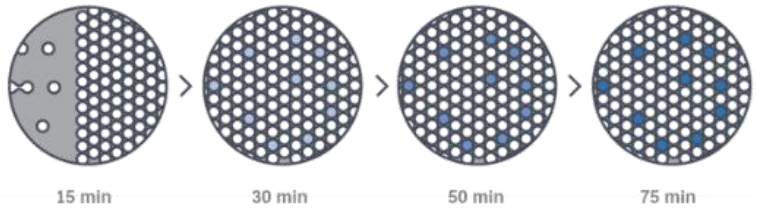
Charge chips into the Geode

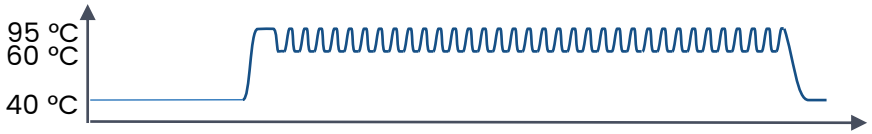
- 1-3 chips and 1-12 samples/run
- ~30,000 partitions/sample

Contactless fluid injection

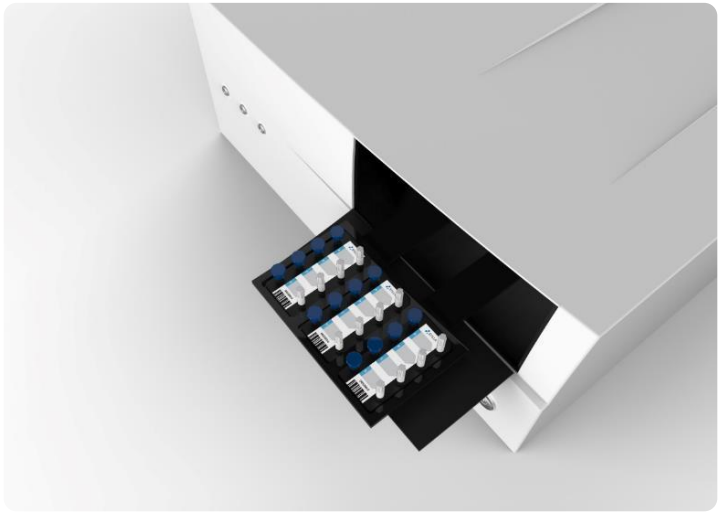
STEP 2.2 – AMPLIFY

Standard cycling conditions:



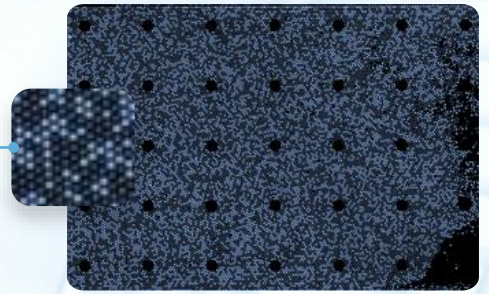


STEP 3
DETECT – 10 MIN (50s/SAMPLE)



TRANSFER CHIPS TO THE PRISM3

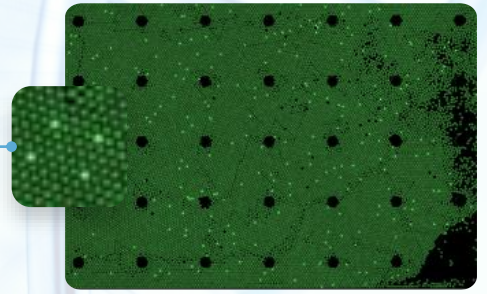
- 1-3 chips and 1-12 samples/run
- 3 color fluorescence imaging



Blue

Ex: 415-480 nm
Em: 495-520 nm

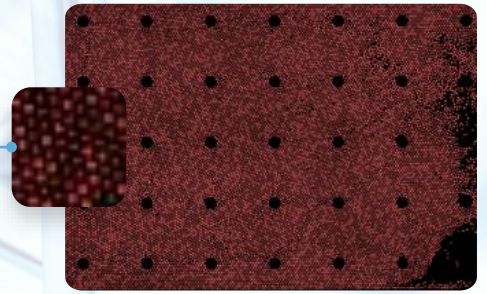
FAM…



Green

Ex: 530-550 nm
Em: 560-610 nm

ROX, HEX…



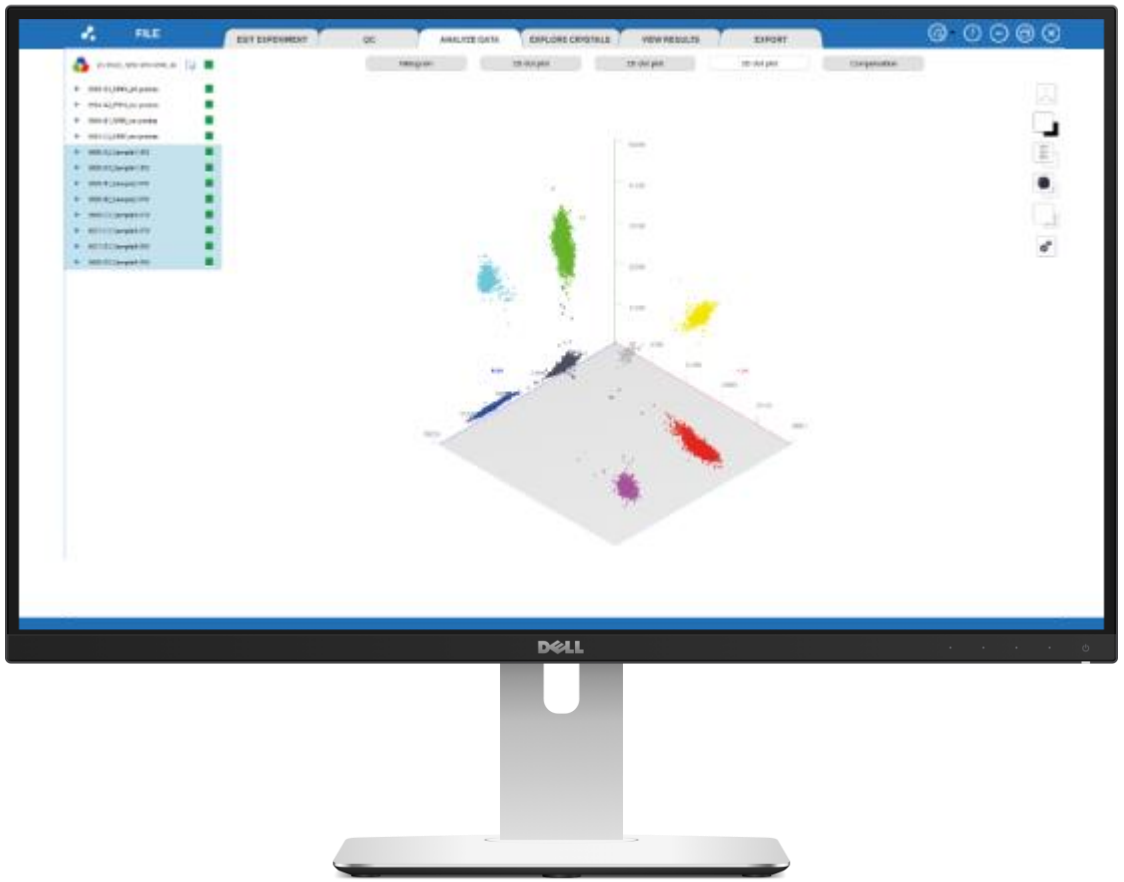
Red

Ex: 615-645 nm
Em: 655-720 nm

Cy®5…

STEP 4

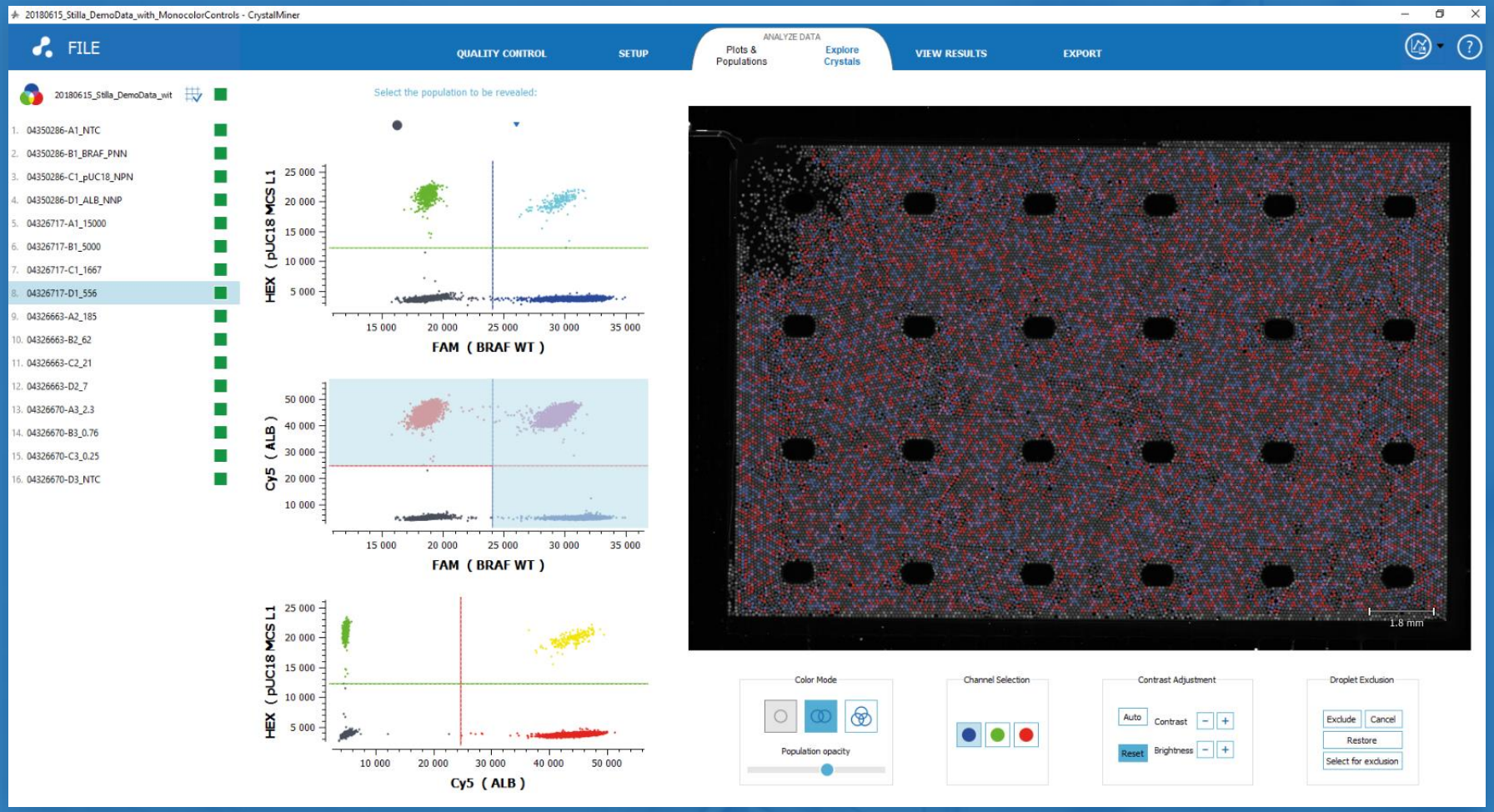
ANALYZE YOUR DATA WITH THE CRYSTAL MINER SOFTWARE



- User-friendly software with intuitive visuals
- Simple image analysis and data exploration
- A trustworthy quality control

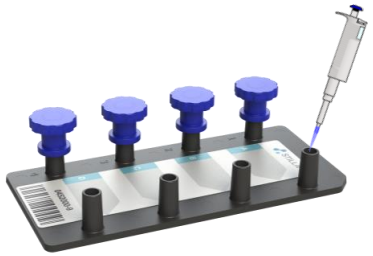
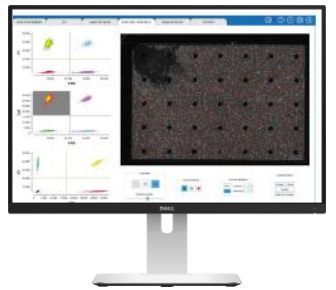
Stilla Technologies – 1 Mail du Professeur Georges Mathé,
94800 Villejuif – www.stillatechnologies.com

EXPLORE CRYSTALS



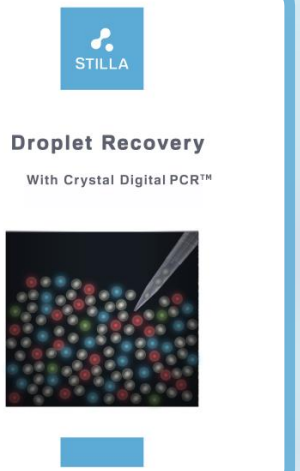
PERFORM CRYSTAL DIGITAL PCR™

IN 2H30 WITH MINIMUM HANDS-ON TIME

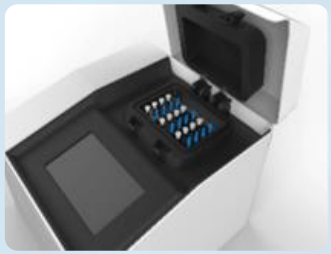
				
DESCRIPTION	Pipette 25 µL of PCR mix into the Sapphire Chips and seal with cap	Place Sapphire chip into the Geode and launch the combined partitioning and thermocycling program	Image Chips using three fluorescent detection channels	Analyze results using our intuitive Crystal Miner software
PROCESS TIME 2H30	**5 min**	**2h10 min**	**10 min**	**5 min**
HANDS-ON TIME 5 min	**5 min**	**15 s**	**15 s**	

APPLYING OUR TECHNOLOGIES BEYOND DIGITAL PCR:

DROPLET RECOVERY



01

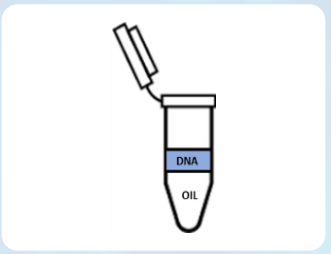


After Crystal Digital PCR, place the Sapphire chips back into the Geode and play the droplet recovery program.

02

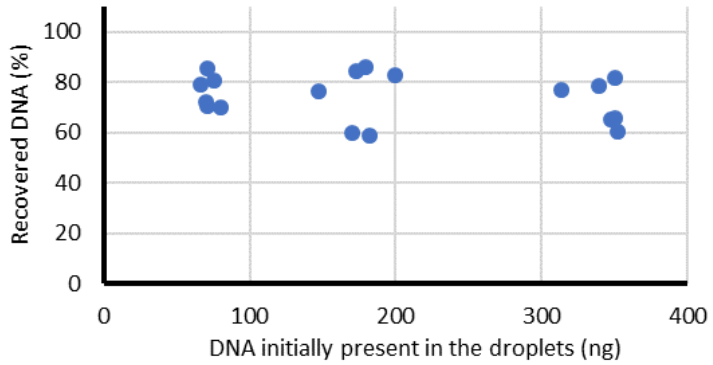
Remove the Sapphire chips from the Geode and disconnect the blue caps Pipette out the emulsion.

03



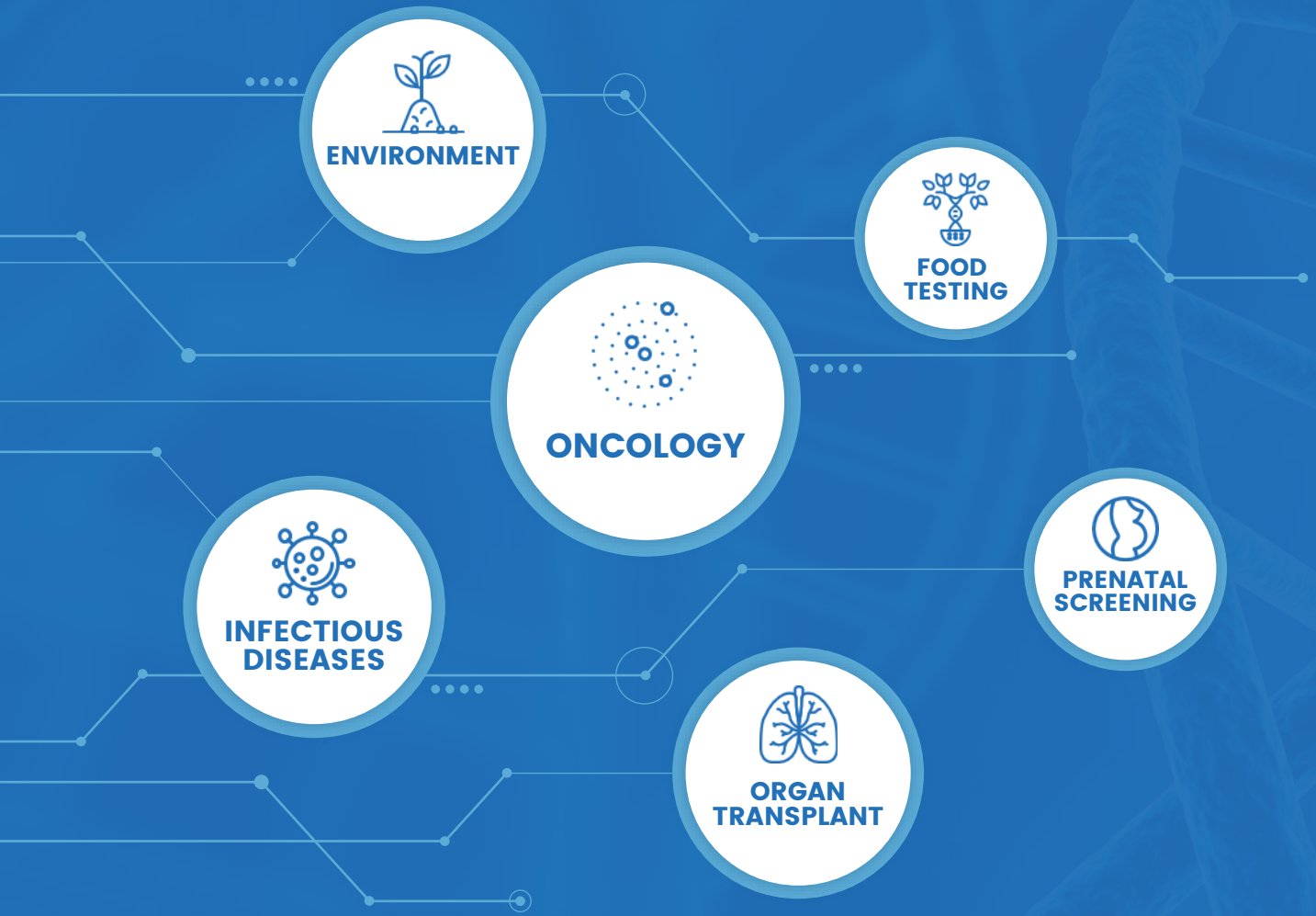
Break the emulsion and extract DNA with chloroform

98% of the droplets and 70% of the total DNA present in the initial droplet crystal is recovered.



dPCR

POTENTIAL APPLICATIONS



+3300

publications for digital PCR in 2018*
Oncology is driving the field

TYPE OF ASSAYS:

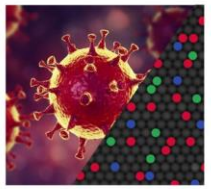
- ✓ Absolute quantification (DNA/RNA)
- ✓ Copy number variation
- ✓ Rare event detection
- ✓ Gene expression

*Source: Google Scholar

APPLICATION & TECHNICAL NOTES

STILLA

A 3-color
Crystal Digital PCR™ kit
for detection of COVID-19



Development of one-step RT-dPCR models for COVID-19 detection

The 2019-2020 outbreak of COVID-19 caused by the SARS-CoV-2 virus first reported in Wuhan, Hubei, China has been declared a pandemic by the World Health Organization. To facilitate the action of health authorities, the development of robust laboratory tests is of primary importance. Using the numerous publicly accessible SARS-CoV-2 and SARS-related sequences, several PCR-based assays specific for SARS-CoV-2 have been designed (Chan et al., 2020). The Naica compatible 3-color Crystal Digital PCR™ kit (Figure 1), developed by ApexBio (Hsinchu Science-based Industrial Park) includes primers and FAM- and HEX-labeled probes specific to two distinct regions (**ORF1ab** and Nucleocapside (N) genes, respectively) of the SARS-CoV-2 positive strand RNA genome. The 3rd channel of the Naica™ system has been used as an endogenous PCR reference detecting a human housekeeping gene with a Cy5-labeled probe. This single assay design permits the simultaneous detection of two independent SARS-CoV-2 sequences reported as conserved while concurrently monitoring PCR effectiveness using the third channel of detection (Figure 2).

apexbio

Kit components

- dPCR master mix1
- dPCR master mix2
- Primers and probes mix
- SARS-CoV-2 positive control
- SARS-CoV-2 negative control

Figure 1: The RUO ApexBio-developed ready-to-use kit contains all reagents required to perform a one-step RT 3-color Crystal Digital PCR™ on the Naica™ System.

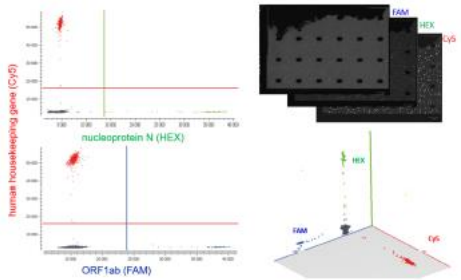


Figure 2: Crystal Miner-generated 2D (left) and 3D dot plots (right) and crystal droplet images obtained on positive controls containing human RNA and synthetic target sequences of the RUO RT-dPCR SARS-CoV-2 detection kit.

Chan JF, Yip CC, To KK, Tang TH, Wong SC, Leung KH, Fung AY, Ng AC, Zou Z, Tsoi HW, Choi GK, Tam AR, Cheng VC, Chen KH, Tsang OT, Yuen KY. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-polymerase chain reaction assay validated in vitro and with clinical specimens. J Clin Microbiol. 2020 Mar 4.

Sensitive and specific detection of COVID-19

An experimental model containing synthetic sequences targeted by the SARS-CoV-2 detection kit was serially diluted and seven dilution points were assessed in triplicate. A total of 1ng of human RNA was added to each replicate. The results indicated a robust and specific detection of SARS-CoV-2 sequences down to 0.6 copies per µl of positive control (5 copies per 25µl reaction) of the ORF1ab gene and down to 0.9 copies per µl of positive control (7 copies per 25µl reaction) of the Nucleocapside (N) gene in all tested samples. Further dilutions showed an extremely sensitive but stochastic detection **down** to 0.25 copies per µl of positive control (2 copies per 25µl reaction) for both genes (Figure 3). In parallel, a total of 15 controls containing only human RNA were tested as negative controls and no false positives were observed.

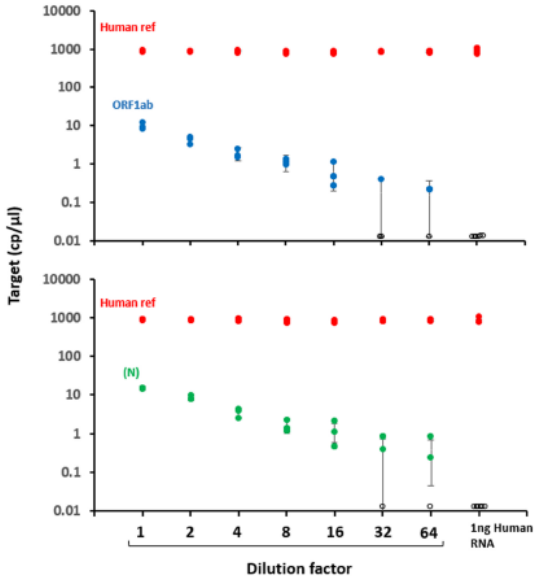
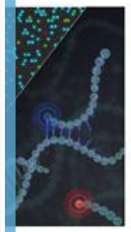


Figure 3: Sensitivity of the 3-color RUO RT Crystal Digital PCR™ kit for SARS-CoV-2 detection. Serial dilutions of SARS-CoV-2 synthetic targets were assayed in triplicate in a background of 1ng of human RNA. A total of 8µl of positive controls was added to each 25µl reaction. The vertical bars represent the theoretical 95% Poisson confidence intervals for the pool of 3 replicates. The empty circles represent replicates where SARS-CoV-2 sequences were not detected.

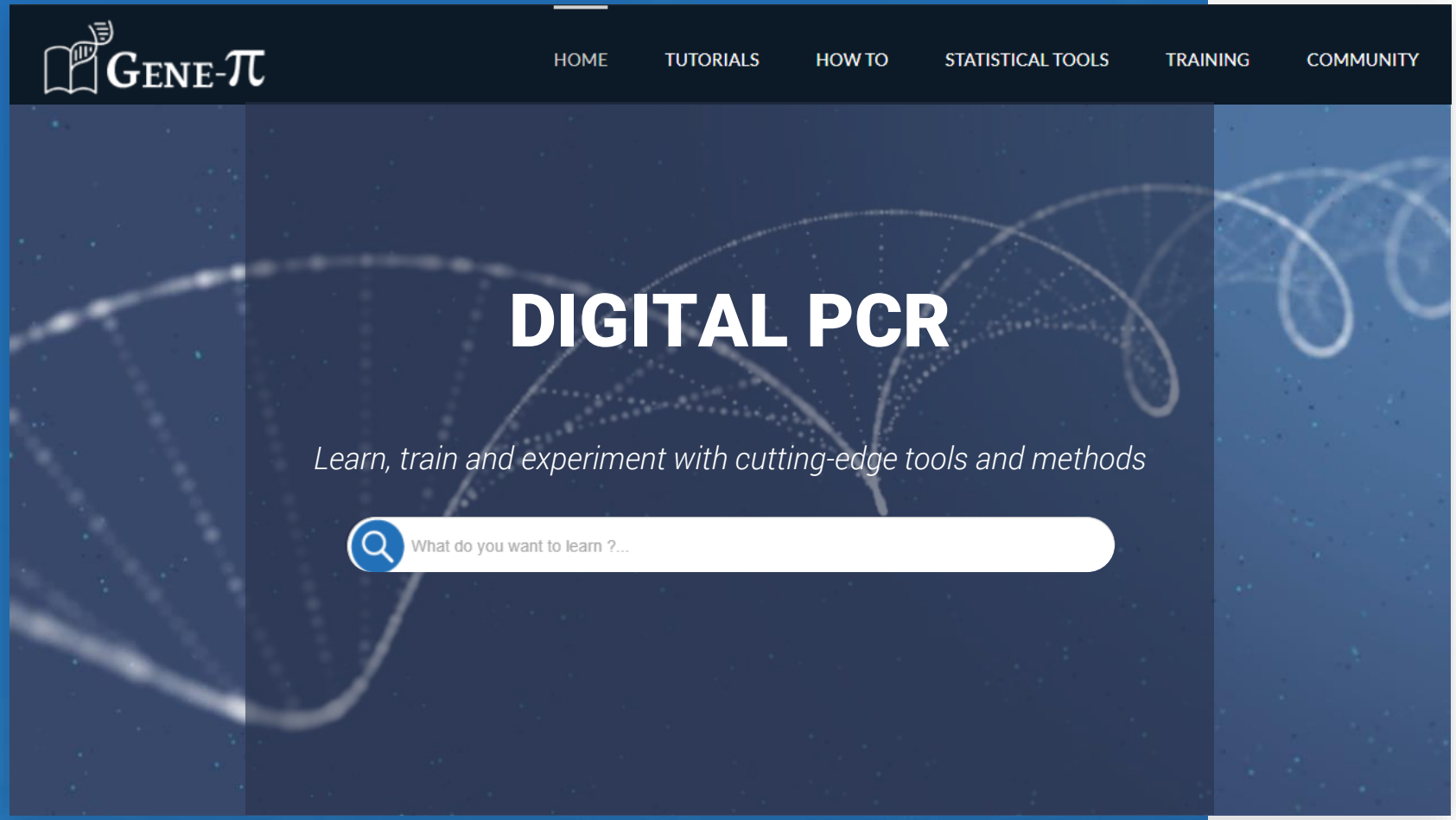
LA

ify Drop-Off
R assays
al™ Miner



STILLA

LEARNING CENTER:
www.gene-pi.com



LAUNCH IN MARCH 2019:

- **3 tutorials**
  - Rare Mutation Detection
  - CNV
  - Drop-off Assay
- **1 video**
- **14 how to's**
- **3 memos**
- **3 online statistical tools**
  - Poisson Law
  - CNV
  - Limit of Blank/Limit of Detection

Stilla Technologies – 1 Mail du Professeur Georges Mathé, 94800 Villejuif – www.stillatechnologies.com

Demo Lab & Training Center

STILLA

THANK YOU FOR YOUR ATTENTION!

ANY QUESTIONS?

For more information on product and workflow, visit our website at

www.stillatechnologies.com

Stilla Technologies – 1 Mail du Professeur Georges Mathé,
94800 Villejuif – www.stillatechnologies.com