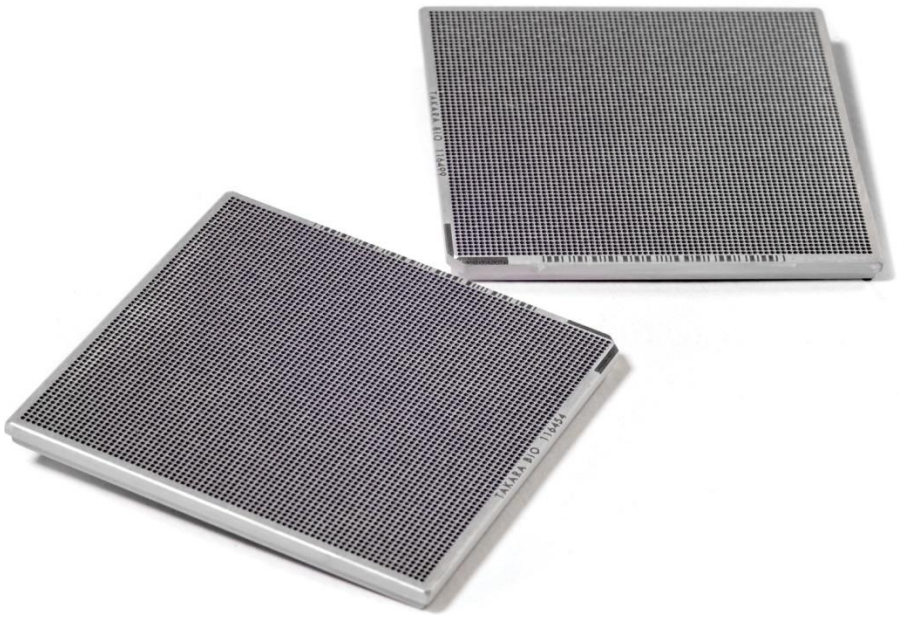
SARS-CoV-2 detection on the SmartChip™ Real-Time PCR System

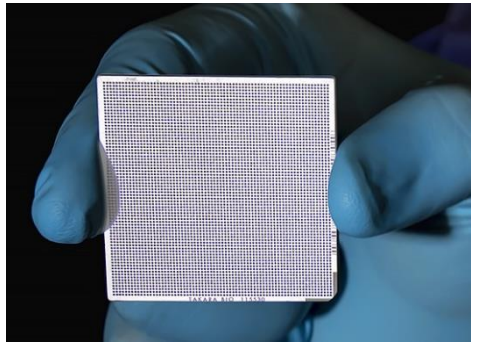
Outline

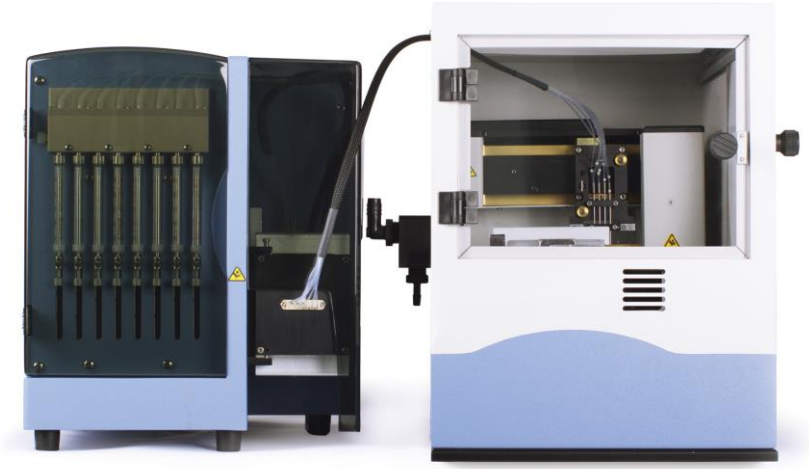


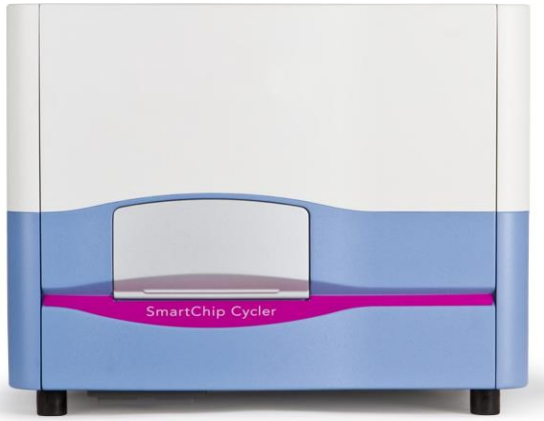
- SmartChip system introduction
- Experimental design and results
- Summary

The SmartChip Real-Time PCR System

High-throughput genotyping and gene expression analysis





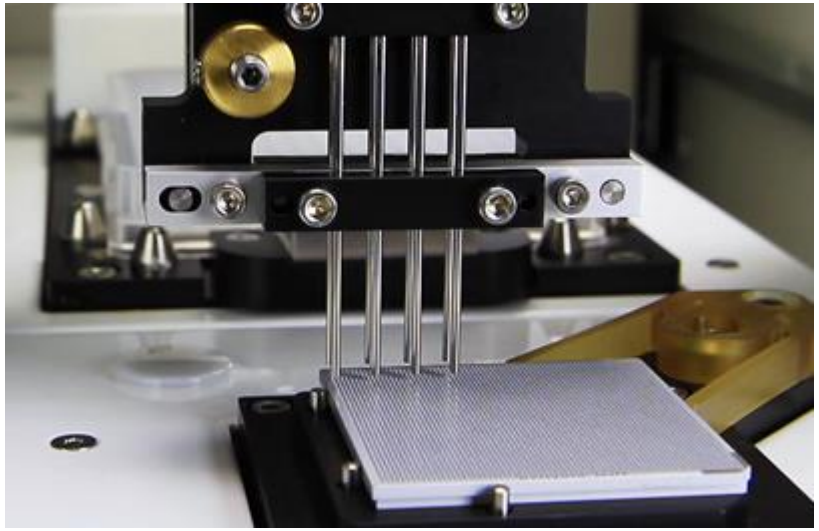


5,184 100-nl reactions/chip

Sample/assay dispense in <1 hour

Assay dispense → data in 2.25 hours

SmartChip technology enables maximum flexibility

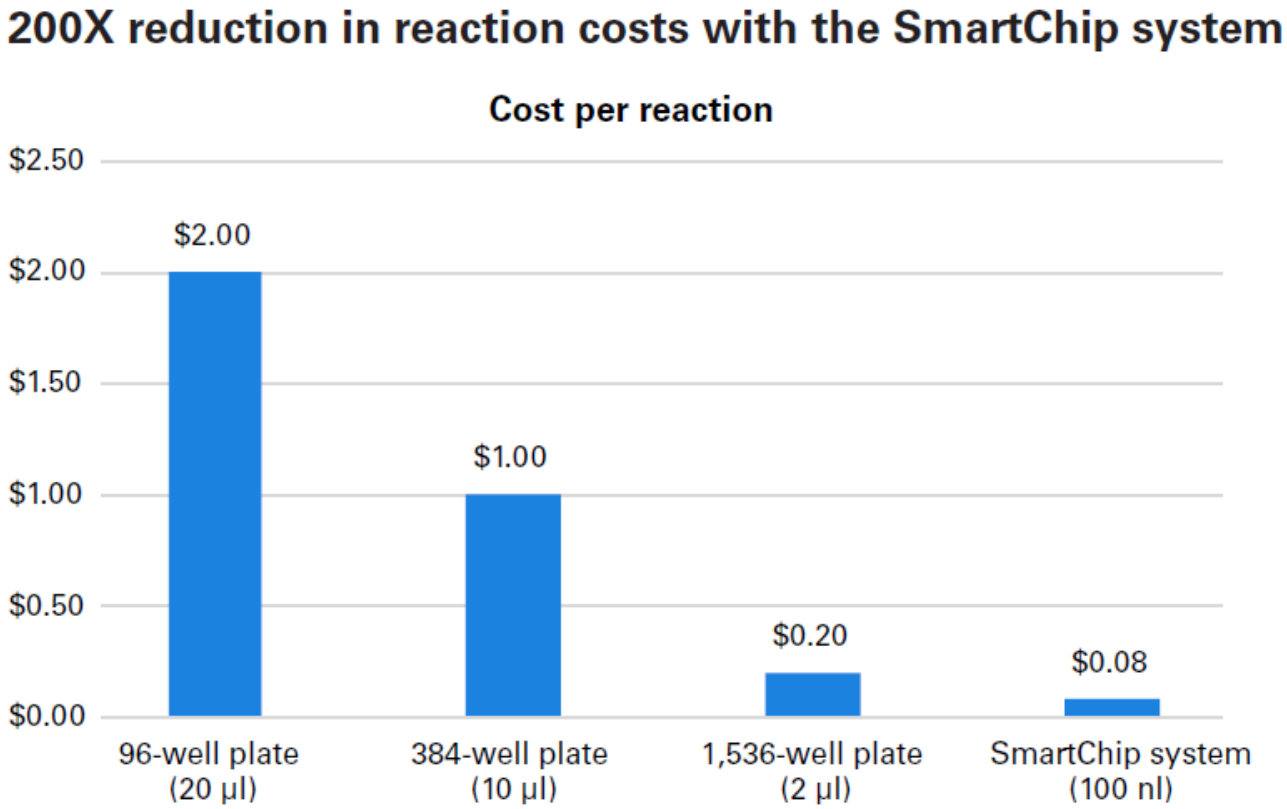


14 different combinations of samples and assays in blank chips—dispense the experiments you want without compromise

- Supports probe- and dye-based chemistries

Assays	Samples
12	384
24	216
36	144
48	108
54	96
72	72
80	64
96	54
120	42
144	36
216	24
248	20
296	16
384	12

SmartChip technology enables significant savings



The SmartChip system utilizes 100-nl reactions, which offer significant reagent and cost savings over conventional plates. A typical experiment performed with the SmartChip system costs $0.08/reaction, compared to up to $2/reaction for a 96-well plate.

Higher sensitivity compared to other high-throughput platforms

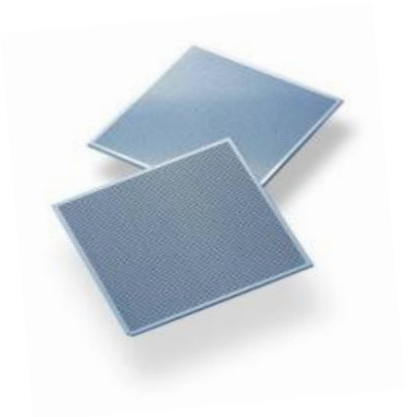
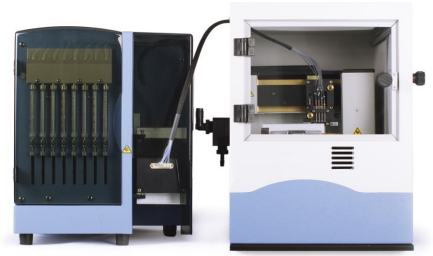
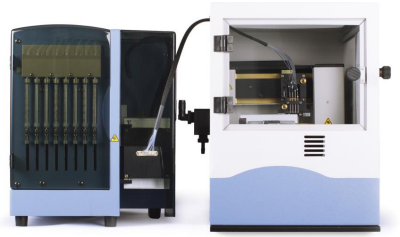
Reaction volume & platform	Input concentration or copies	Is preamplification required?
100 nl in SmartChip system	3–10 ng/µl or 10s of copies	Depends on sample quality
33 nl in 12K flex plate	50 ng/µl or 100s of copies	Yes
10 nl in IFC	60 ng/µl or 1,000s of copies	Yes

SARS-CoV-2 detection experimental design

- Goal:
  - Demonstrate limit of detection and relative confidence intervals for varying copy numbers in 200-nl reactions
- Samples:
  - Serial dilutions of synthetic control targets (ATCC) were spiked into 250 pg of human universal reference RNA
    - 10,000; 1,000; 100; 10; 1; 0.1; 0.01; or 0 control copies/rxn
- Primers:
  - Viral Nucleocapsid 1 (N1) & Nucleocapsid 2 (N2) and human RNase P primers from IDT (US CDC protocol)
- Reagents:
  - Takara Bio SMARTScribe™ Reverse Transcriptase and SmartChip Probe qPCR Master Mix

Copies/rxn	Copies/µl
10,000	50,000
1,000	5,000
100	500
10	50
1	5
0.1	0.5
0.01	0.05
0	0

SmartChip system workflow for COVID-19 detection

1	2	3	4	5
Blank MyDesign chips	Dispense samples into chip	Blot and spin	Dispense assays into chip	Thermal cycle and collect data
				
	~55 minutes		~15 minutes	~2 hours

Sample and assay plate setup for one chip

	Samples per chip	Assays per chip
Proof of concept	• 8 different concentrations + 1 NTC • 8 sample replicates on each chip	• 5 replicates of N1 assay • 5 replicates of N2 assay • 2 replicates of RNase P assay
Scale-up in submitted EUA	• 382 samples • 1 positive and 1 negative control	

Take away:

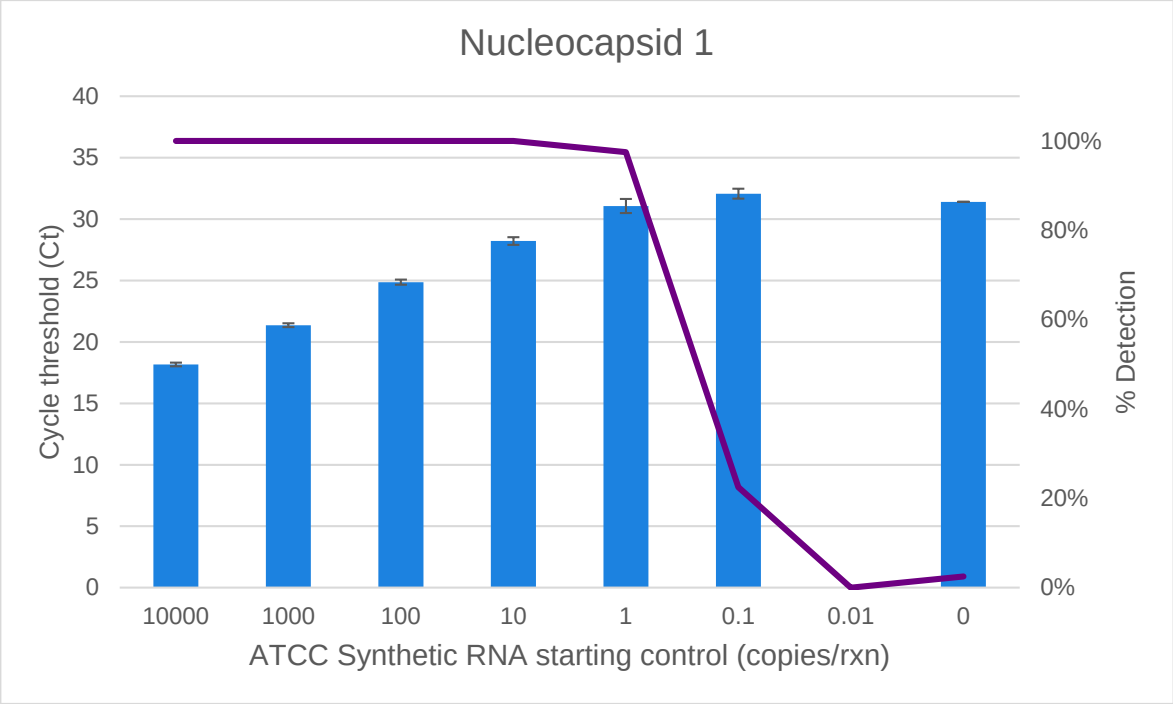
- Using this setup, run up to 382 samples across the 3 US CDC assays in quintuplicate
- Total time: <4 hours, with <30 minutes hands-on time
- Total cost/sample: <$3

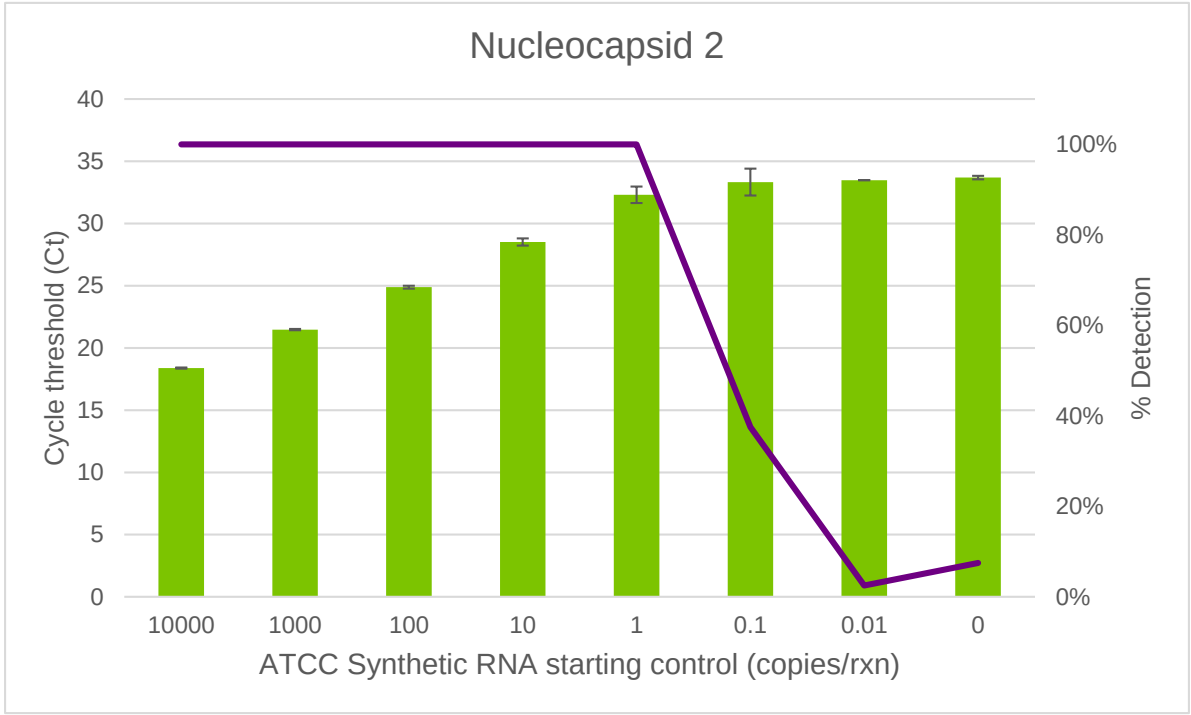
Benefits of replicates from the SmartChip system

The system enables up to 5 replicates for each assay with your samples. This gives you:

- Confidence in the data you generate by having multiple data points per sample
- Assurance that there are no false negatives that could arise due to pipetting errors during reaction set-up
- Flexibility to add more assays (by reducing replicates) to your experiment without lowering your throughput
- Increased likelihood of detecting rare or low-copy targets by having 5 chances vs.1

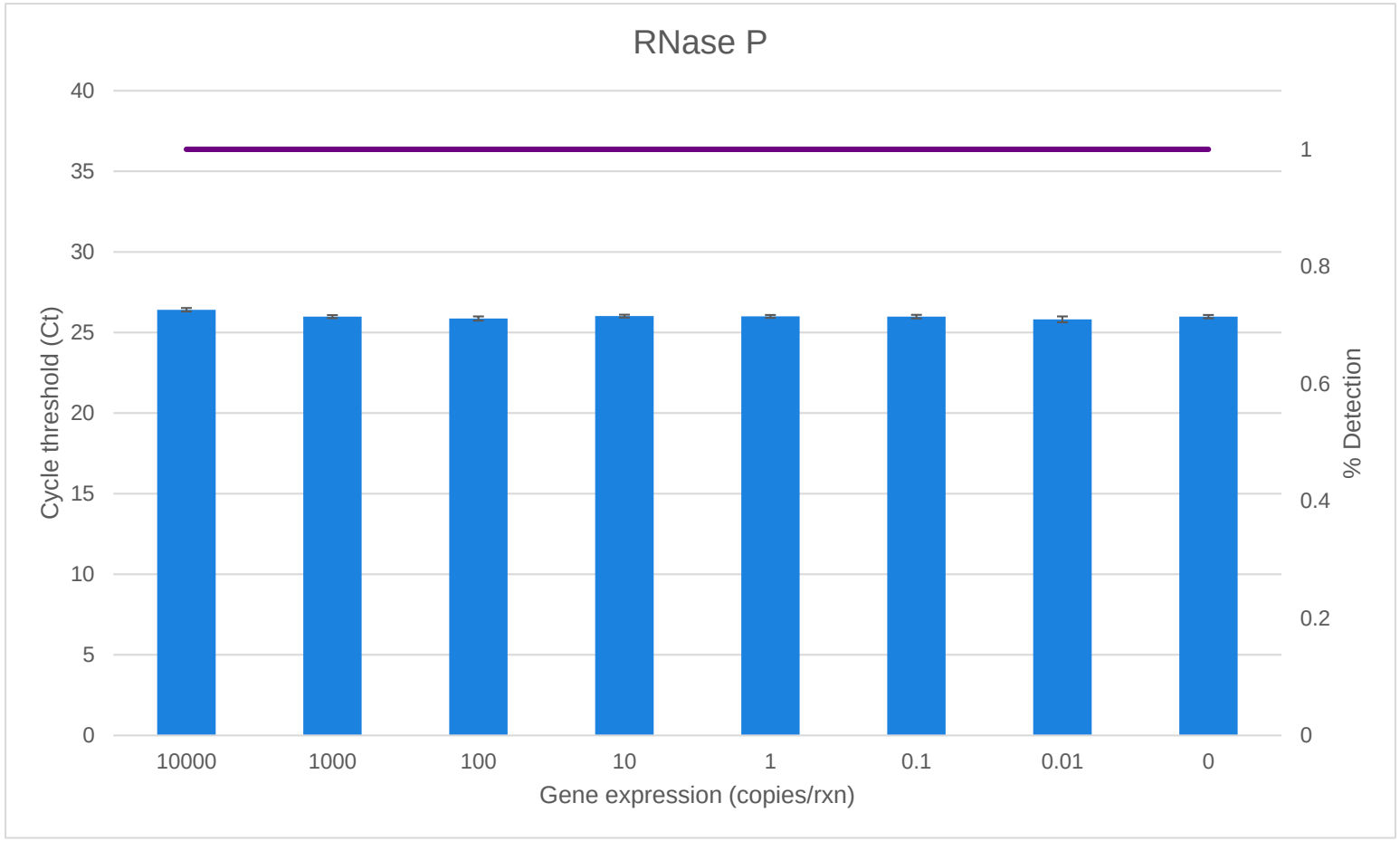
Proof-of-concept results: nucleocapsid assays





- N1 and N2 assays, wells with ≥10 copies/rxn (25 copies/µl): ~100% amplified
- N1 and N2 assays, wells with 1 copy/rxn (5 copies/µl): 98–100% amplified
- N1 and N2 assays, wells with 0.1 copy/rxn (0.5 copies/µl): 23–38% amplified

Proof-of-concept results: RNAse P assay and NTCs



- RNAse P assay works well across a wide range of control input concentrations with 100% amplification
- No amplification was observed in any of the NTC wells

Summary

- The SmartChip system is a rapid, flexible, and cost-effective way to run a large number of samples
- Using our layout, the average cost per sample is <$3
- Our limit of detection is ~5 copies/µl starting sample or ~1 copy per reaction with a ±1 Ct confidence interval of 95%
- We have successfully collaborated with bioSyntagma, Inc. and their partners, who are now seeking EUA from the FDA: https://www.takarabio.com/about/announcements/takara-bio-usa-inc-and-biosyntagma-inc-develop-method-for-large-scale-automated-covid-19-testing

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