Electronic Supplementary Material (ESI) for Analyst.

SUPPLEMENTARY INFORMATION

Reverse Transcription-Free Digital-Quantitative-PCR for microRNA Analysis

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Table S1. Nucleic acid sequences used in base-stacking PCR.

Reagent	Sequence (5'-3')
DNA Guide	Biotin-GGCTAAGACAGATGCTCTTTGCCAACAGGCCACAGAA TTCCTACACTCAAAGTCGTACTGAACTATACAACCTACTACCT CATCGCACT
let-7a miRNA	UGAGGUAGUAGGUUGUAUAGUU
Forward Primer	TACGAGAGATGCGA
Reverse Primer	GGCTAAGACAGATGCTC

Table S2. Reagents used in the PCR master mixes and the microfluidic device loading buffer.

Reagent	Components	Concentration
Microfluidic dqPCR master mix	KAPA SYBR Fast mix	1x
	SYBR Green	5x
	$MgCl_2$	2 mM
	Primers	6 µM
	Kapa 2G Polymerase	0.15 U/µL
Bulk commercial qPCR master mix	KAPA SYBR Fast mix	1x
	Rox Reference Dye	100 nM
	DNA Guide	200 nM
	Primers	200 nM
Loading buffer	Tris HCl, pH 8	20 mM
	KCl	20 mM
	$MgCl_2$	2.5 mM
	Tween 20	0.1%
	BSA in 1x TBS	1%

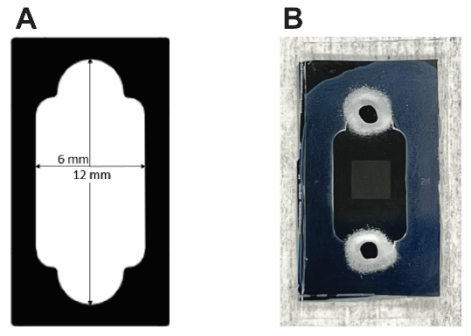

Figure S1. (A) Illustration of a glass fluidic dome feature. **(B)** Image of an assembled silicon-glass microwell array device. The total volume of the device is 1.5 µL.

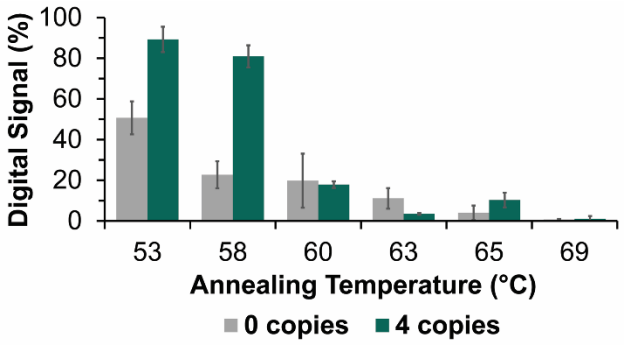

Figure S2. PCR annealing temperature was evaluated by measuring the digital assay signal using 300,000 guides/bead. Negative controls decreased in signal uniformly with increasing temperature, whereas positive controls experienced a sharp decrease at 60 °C.

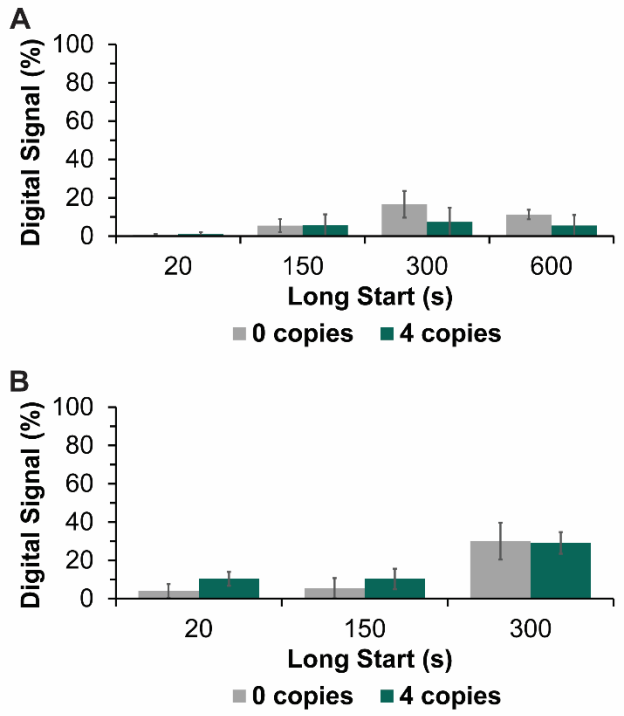

Figure S3. The Cycle 1 annealing time was evaluated to determine its effect on the digital signal of a BS-PCR assay. Long-start programs using annealing temperatures of **(A)** 69 °C and **(B)** 65 °C produced similar results between both positive and negative controls at each condition. Annealing for Cycles 2-35 was done for 20 s at the same annealing temperature as Cycle 1.

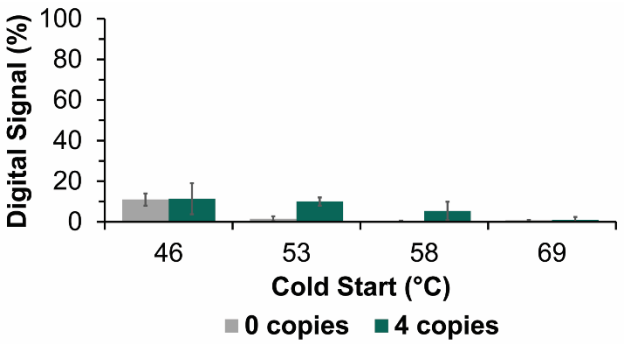

Figure S4. The Cycle 1 annealing temperature was evaluated to determine its effect on the digital signal of a BS-PCR assay. Cold-start programs exhibited a gradual decrease in digital signal from positive controls as temperature increased, while negative controls decreased sharply between 46 °C and 53 °C. An annealing temperature of 69 °C was used for Cycles 2-35.

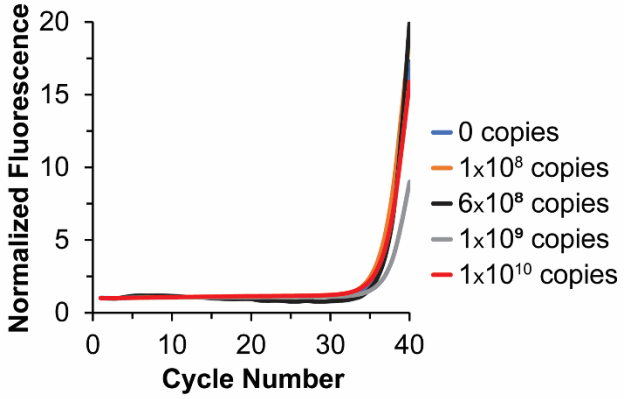

Figure S5. Averaged qPCR curves obtained from a commercial qPCR instrument. The copy numbers of let-7a did not affect the measured C_q values.