Adoption of versatile pipetting platform for highly accurate and precise qPCR assay setup

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Introduction

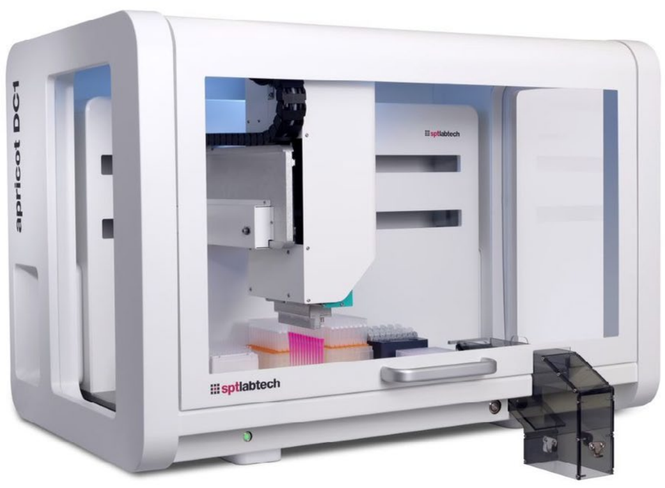
qPCR is a highly sensitive method that allows users to accurately quantify the DNA or cDNA content of samples. The technique has been applied to range of scientific and diagnostic use cases, for example gene expression analysis, microbe and pathogen detection, as well as NGS sequencing library quantification.

qPCR assays have a very large assay windows that can span 6-8 orders of magnitude [Life Technologies, Real Time PCR Handbook, *https://www.gene-quantification.de/real-time-pcr-handbook-life-technologies-update-flr.pdf* accessed 15 Dec 2022]. The cDNA concentration of individual samples is determined by reference to a standard curve of assay threshold cycle (C_t) against log [concentration] for a positive control. Standard curves are typically generated by 5-10 fold serial dilutions of the positive control supplied with a kit, and the serial dilution steps are extremely sensitive to any pipetting inaccuracies. Even if each individual dilution step carries an error of just 2-3%, this can compound to much larger errors for the most dilute samples and potentially invalidate the standard curve. Accurate qPCR assays therefore rely critically on precise and accurate pipettes or liquid handlers, as well as appropriate pipetting technique, to achieve high quality assay metrics.

In this poster, we showcase the liquid handling performance of the apricot DC1 and its suitability for setting up qPCR assays. We present a fully automated workflow starting from the individual reagent tubes supplied with a qPCR kit to the fully assembled qPCR plate comprising a standard curve and samples that is ready for analysis.

Materials and methods

Liquid handing



Apricot DC1 (above)

All liquid handling steps were performed on an apricot DC1 automated liquid handler. Apricot DC1 is compact, benchtop liquid handler that features:

- a unique 4-in-1 head and dual dispense cores to enable 1/8/12-channel pipetting from 0.5 to 1000 µL
- versatile portrait and landscape orientation pipetting across up to 9 and 7 deck positions, respectively
- optional process modules such as heaters, coolers, shakers and motorized magnets
- a broad range of compatible labware that includes: 24/96/384 well plates (standard SBS, deep well blocks and PCR plates), 1.5/2.0/5.0 mL microcentrifuge tubes, PCR strips, and reagent reservoirs

Dispense specification

Dispensing precision	High volume core: <3% CV at 10 µL Low volume core: <3% CV at 1 µL
Dispensing accuracy	High volume core: +/-2% CV at 10 µL Low volume core: +/-2% CV at 1 µL
Volume	Low volume core (1/8/12 channels): 1 - 125 µL High volume core (1/8/12 channels): 10 - 1000 µL
Resolution	High volume core: 1 µL Low volume core: 0.1 µL

Serial dilution performance by qPCR analysis

qPCR assays were performed using the TaqMan™ Gene Expression Master Mix (Applied Biosystems, 4369016) and positive control virus at 10^8 ng/mL. apricot DC1 was used to perform serial dilutions of the positive control in a 96 well U-bottom plate with 3 replicates for each concentration: First, using 8 channels and a 125 µL tip, 90 µL of dilution buffer were transferred from a column-based reservoir into columns 2-8 of the serial dilution plate. Then column 1 of the same plate was filled with 20 µL of the positive control. A column-wise 1:10 serial dilution was performed by aspirating 10 µL of positive control from column 1 and dispensing into the dilution buffer in column 2. Then 70 µL of the diluted sample was mixed (aspirated and dispensed back into the same well) 10 times. Using a fresh set of tips for each dilution step, the serial dilution was continued across columns 3-8. A dragonfly discovery dispenser was used to fill columns 1-8 of a qPCR plate (Applied Biosystems, 4306737) with 9 µL of the qPCR Master Mix. The plate was transferred to the apricot DC1 deck, and 1 µL of the diluted standard from the serial dilution plate was copied into the assay plate. The pipetting scheme is summarised in Figure 1.

The plate was sealed with optically clear adhesive film (Applied Biosystems, 4311971) and read on a QuantStudio 6 Flex (Applied Biosystems, 4485697) qPCR machine using the program: 95°C for 10 minutes; 40 cycles of 95°C for 15 seconds. The qPCR readout (assay threshold cycle, C_t) was plotted against the log [concentration of positive control] and fitted to a linear regression model. From this, the slope and R^2 of the linear fit were determined.

Plate layout

Step1 (DC1): Sample dilution in 96-well U-bottom plate

conc.	1	2	3	4	5	6	7	8	9	10	11	12
A (STD1)	1E8	1E7	1E6	1E5	1E4	1E3	1E2	1E1				
B (STD2)	1E8	1E7	1E6	1E5	1E4	1E3	1E2	1E1				
C (STD3)	1E8	1E7	1E6	1E5	1E4	1E3	1E2	1E1				
D												
E												
F												
G												
H												

Step2 (DFD): PCR master mix addition in 96-well PCR plate

vol.	1	2	3	4	5	6	7	8	9	10	11	12
A (STD1)	9µL	9µL	9µL	9µL	9µL	9µL	9µL	9µL				
B (STD2)	9µL	9µL	9µL	9µL	9µL	9µL	9µL	9µL				
C (STD3)	9µL	9µL	9µL	9µL	9µL	9µL	9µL	9µL				
D												
E												
F												
G												
H												

Step3 (DC1): 1 µL of sample transfer from 96-well U-bottom plate to 96-well PCR plate

well vol.	1	2	3	4	5	6	7	8	9	10	11	12
A	10µL	90µL	90µL	90µL	90µL	90µL	90µL	90µL				
B	10µL	90µL	90µL	90µL	90µL	90µL	90µL	90µL				
C	10µL	90µL	90µL	90µL	90µL	90µL	90µL	90µL				
D												
E												
F												
G												
H												

1µL

final vol.	1	2	3	4	5	6	7	8	9	10	11	12
A (STD1)	10µL	10µL	10µL	10µL	10µL	10µL	10µL	10µL				
B (STD2)	10µL	10µL	10µL	10µL	10µL	10µL	10µL	10µL				
C (STD3)	10µL	10µL	10µL	10µL	10µL	10µL	10µL	10µL				
D												
E												
F												
G												
H												

Figure 1 serial dilution, qPCR MasterMix distribution, and sample transfer liquid handling steps

Results

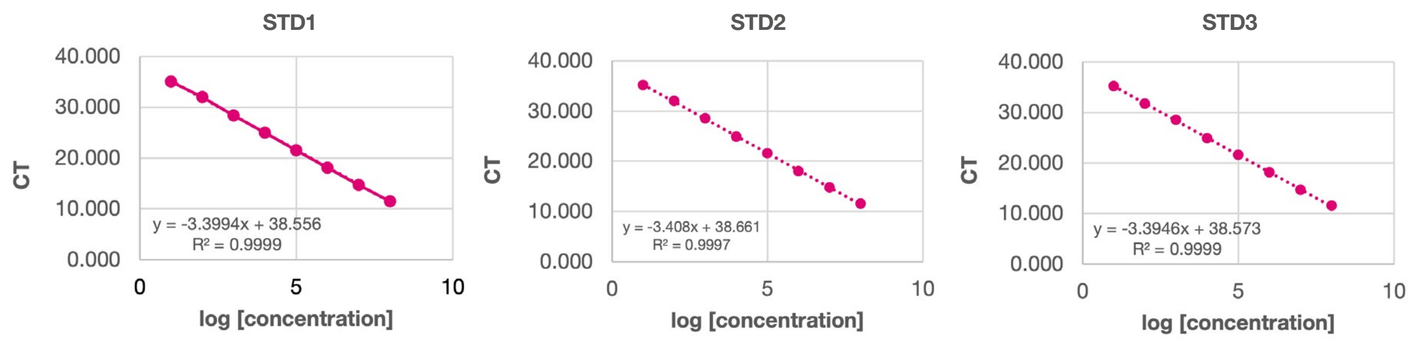
qPCR assay analysis

	Concentration (ng/mL)	Log [concentration]	C_T value
STD1	10^8	8	11.444
	10^7	7	14.723
	10^6	6	18.063
	10^5	5	21.538
	10^4	4	24.964
	10^3	3	28.364
	10^2	2	31.945
	10^1	1	35.031

	Concentration (ng/mL)	Log [concentration]	C_T value
STD2	10^8	8	11.583
	10^7	7	14.758
	10^6	6	18.045
	10^5	5	21.580
	10^4	4	24.903
	10^3	3	28.514
	10^2	2	32.051
	10^1	1	35.165

	Concentration (ng/mL)	Log [concentration]	C_T value
STD3	10^8	8	11.540
	10^7	7	14.716
	10^6	6	18.155
	10^5	5	21.590
	10^4	4	24.925
	10^3	3	28.532
	10^2	2	31.696
	10^1	1	35.223

	Concentration (ng/mL)	C_T mean	C_T SD
Mean STD 1-3	10^8	11.522	0.071
	10^7	14.733	0.022
	10^6	18.088	0.059
	10^5	21.569	0.028
	10^4	24.931	0.031
	10^3	28.470	0.092
	10^2	31.897	0.182
	10^1	35.140	0.099



Conclusions

We have shown that the apricot DC1 with its unique 4-in-1 pipetting head is an ideal tool to perform accurate and precise serial dilutions, which are routinely used for the generation of qPCR standard curves. The automatic tip exchange function of the instrument allows users to utilize fresh tip for each dilution step, thereby eliminating the risk of unintended sample carryover from a previous workstep.

The multi-position deck is ideally suited to automating multistep protocols, and although not shown in this poster, could be utilized to make up the qPCR MasterMix from individual components prior to assay setup. Similarly, 8/12 tip functionality could be utilized to distribute the MasterMix prior to sample addition. Overall apricot DC1 provides a versatile, easy-to-use and economical solution for automating qPCR workflows.