Optimizing 384-well qPCR plate preparation while decreasing costs.

For research use only.
Not for use in diagnostic procedures.

Introduction

Quantitative Polymerase Chain Reaction (qPCR) is an important step in many laboratory experiments to accurately quantify nucleic acids in next generation sequencing (NGS) and other applications. Propagation of errors from inaccurate liquid transfers can greatly impact the quality of a qPCR assay. The FlexDrop™ iQ™ Non-Contact dispenser provides rapid, precise, and flexible dispensing from a 96-source position plate into 96, 384 or 1536 well plates with a dead volume of less than 1 μL. This automation compatible dispenser greatly reduces carryover and cross-contamination while conserving reagents.

The Centre for Cancer Biology (SA Pathology and the University of South Australia) compared multiple ways of preparing qPCR plates using:

- Manual Preparation

- Revvity® JANUS® G3 Varispan 4-tip Workstation Single Dispense

- Revvity JANUS G3 Varispan 4-tip Workstation Multiple Dispense

- Qiagen® QIAgility® Instrument

- Revvity FlexDrop iQ Non-Contact Dispenser (500 μL version)

Where the JANUS G3 workstation single dispense is a single aspirate per a single dispense while a multiple dispense is a single aspirate to multiple dispenses.

FlexDrop iQ Non-Contact dispenser

Materials

- Qiagen® QuantiTect® Reverse Transcription Kit (cat # 205311)

- Qiagen® QuantiTect® SYBR Green PCR Kit (cat # 204143)

- GAPDH in house primers

- Cal-51 RNA, generated from the human epithelial breast carcinoma cell line CAL-51 using TRIzol™ Reagent (Thermo Fisher Scientific)

Methods

qPCR reactions with two levels of cDNA input and differing final volumes (5 µL and 10 µL) were tested across several known automated platforms alongside the FlexDrop iQ Non-Contact dispenser. To start, cDNA was generated using 1ug of RNA and QuantiTect® Reverse Transcription kit. The cDNA was diluted at 1:80 ratio then further diluted to a 1:20000 ratio to aim for both a high and med-low cycle quantification (Cq) values. Following cDNA dilution, a bulk qPCR master mix was prepared manually, using the QuantiTect® SYBR Green PCR kit and GAPDH primers. The diluted cDNA and master mix were placed into the automated liquid handler and 8 µL or 3 µL of master mix was aliquoted into a 384 well plate followed by 2 µL of cDNA for a total of 10 µL or 5 µL volume per well. Normally a 2 µL minimum volume is used when dispensing cDNA to increase accuracy. However, to thoroughly test the FlexDrop iQ Non-Contact dispenser's low volume capabilities, an extra variation was only added to the

FlexDrop iQ Non-Contact dispenser, having it deliver 1 µL of cDNA and 4 µL of master mix. 16 replicate wells were dispensed for each condition. The plate was then sealed, vortexed, centrifuged and run in a Thermo Fisher® Scientific QuantStudio™ 6 Pro Real-Time PCR instrument. Cycling conditions were as follows:

- 95 °C for 15 minutes

- 50 cycles of PCR at 95 °C for 15 seconds, 60 °C for 20 seconds, then lastly 72 °C for 20 seconds

- 95 °C for 15 seconds

- 72 °C for 15 seconds

- Melt Curve from 72 °C to 95 °C

Results

The results from the FlexDrop iQ Non-contact dispenser were compared with the QIAgility® Instrument, JANUS (multiple and single dispense) G3 workstations, and a manual setup. The average cycle quantification (Cq), standard deviation and percent CV (%CV) were reported for each variable between 16 replicates as shown in table 1. The %CV between all instruments are comparable, fall below 5%. The setup time and cost (for consumables and master mix), for a full 384 well plate, is also compared between the instruments in figure 2 and 3. The FlexDrop iQ Non-Contact dispenser has the lowest setup time and cost between all instruments.

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Table 1: The average Cq values, standard deviation, and percent CV with 16 replicates for each instrument and variable.

|	Sample	Average C_q	STDev	%CV
QIAgility®	10 µL High cDNA Input	18.91	0.38	2.00%
	5 µL High cDNA Input	18.35	0.44	2.41%
	10 µL Medium cDNA Input	26.84	0.49	1.82%
	5 µL Medium cDNA Input	26.85	0.60	2.25%
JANUS multi-dispense	10 µL High cDNA Input	18.68	0.29	1.55%
	5 µL High cDNA Input	18.14	0.81	4.49%
	10 µL Medium cDNA Input	26.99	0.50	1.87%
	5 µL Medium cDNA Input	26.18	0.37	1.40%
JANUS single dispense	10 µL High cDNA Input	18.74	0.37	2.00%
	5 µL High cDNA Input	18.22	0.28	1.54%
	10 µL Medium cDNA Input	27.00	0.35	1.30%
	5 µL Medium cDNA Input	26.34	0.52	1.97%
Manual	10 µL High cDNA Input	18.83	0.43	2.28%
	5 µL High cDNA Input	18.14	0.74	4.06%
	10 µL Medium cDNA Input	27.55	0.36	1.32%
	5 µL Medium cDNA Input	26.41	0.46	1.76%
FlexDrop iQ non-contact dispenser	10 µL High cDNA Input	17.52	0.44	2.51%
	5 µL High cDNA Input (3+2)	17.56	0.45	2.57%
	5 µL High cDNA Input (4+1)	17.82	0.79	4.43%
	10 µL Medium cDNA Input	26.11	0.30	1.14%
	5 µL Medium cDNA Input (3+2)	26.07	0.44	1.67%
	5 µL Medium cDNA Input (4+1)	26.59	0.27	1.03%
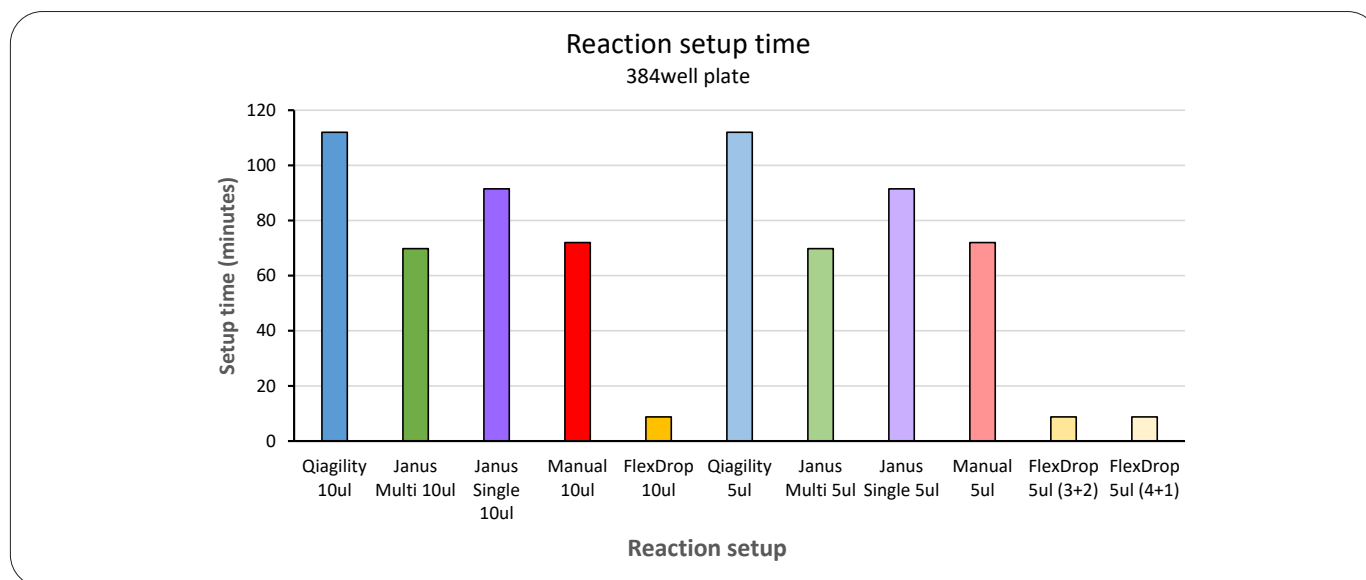
Figure 2: Reaction setup time compared between the QIAgility® instrument, JANUS G3 workstation multi-dispense, JANUS G3 workstation single dispense, manual, and FlexDrop iQ Non-Contact dispenser.
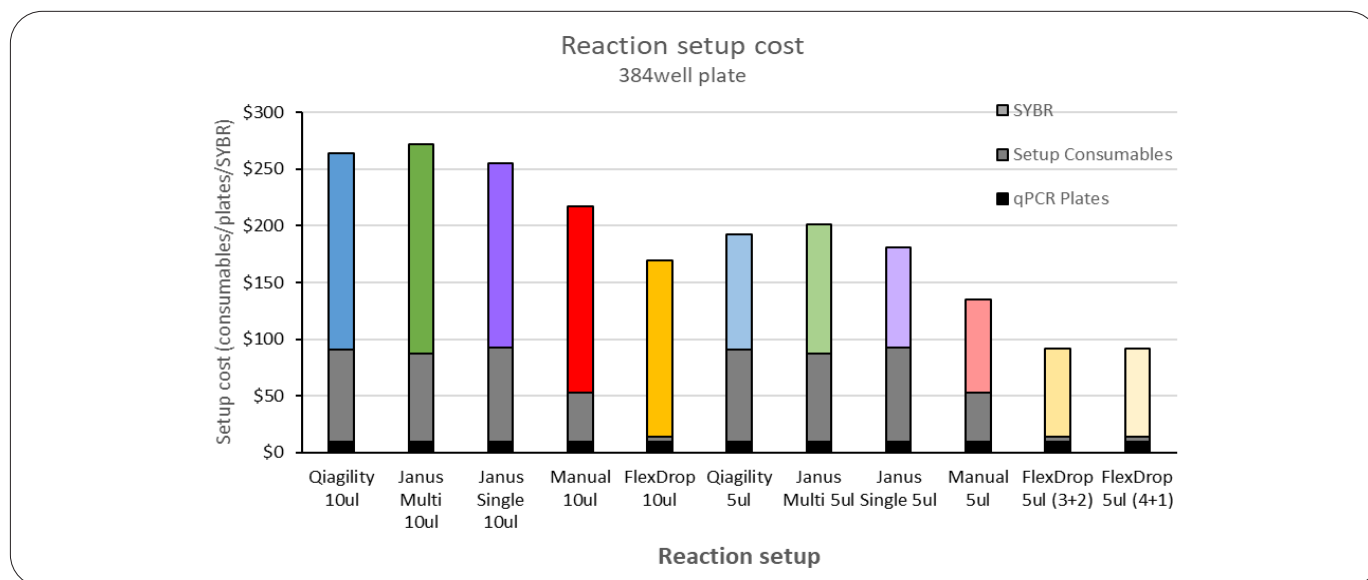
Figure 3: Reaction setup cost compared between QIAgility® instrument, JANUS G3 workstation multi-dispense, JANUS G3 workstation single dispense, manual, and FlexDrop iQ Non-Contact dispenser.

Conclusion

This study compared multiple methods to prepare 384 well qPCR plates. Manual preparation, the JANUS G3 workstation (single and multiple dispense), the Qiagen® QIAgility® instrument and the FlexDrop iQ Non-Contact dispenser were tested. Plates made using the FlexDrop iQ Non-Contact dispenser were comparable to the preparation from both liquid handling instruments while the reaction setup time and cost was lower than all other preparation methods. This occurred for both the standard FlexDrop iQ Non-Contact dispenser setup and the additional variation of adding 1 µL of cDNA and 4 µL of master mix. The FlexDrop iQ Non-Contact dispenser is an easy to use, rapid, reliable method for qPCR plate preparation. from a 96-source position plate into 96, 384 or 1536 well plates with a dead volume of <1 µL. This automation compatible dispenser greatly reduces carryover and cross-contamination while conserving reagents.

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