verifying the performance of miniaturized quantitative PCR (qPCR) reactions set-up with mosquito® HV and LV genomics and dragonfly® discovery

introduction

Quantitative PCR (qPCR) is one of the most widely used techniques for the detection, characterization and quantification of nucleic acids within a vast number of research applications. These reactions work in a very similar way to classic PCR, with a singularity, the use of fluorescent labelling. This enables the collection of data as the PCR progresses. A dsDNA binding dye is typically used, allowing the quantification of amplified DNA molecules.

The main limiting factors in the use of this technique are the scarceness of samples and the cost of reagents. Very often, the samples used as a template are unique and irreplaceable leaving no room for human error. In order to have confidence in qPCR results, reactions are usually run in duplicates or triplicates. Whilst necessary, increased replicate numbers not only deplete stocks of precious sample but increase reagent usage and therefore overall assay costs too.

To circumvent this problem a valued resource for qPCR assays is miniaturization, as it helps to reduce reaction costs and sample volume requirements. Nevertheless, very often, the quality of data from miniaturized qPCR reactions is questioned. In this application note, we have collected data on qPCR reactions at different levels of miniaturization, using mosquito® HV (High Volume) genomics, mosquito LV (Low Volume) genomics and dragonfly® discovery for reaction setup.

materials and methods

qPCR reactions were set up in 384-Well Skirted PCR Plates for Roche® Lightcycler® (STARLAB, E1042-9909). The reactions consisted of qPCR Control Kit (Jena Bioscience, PCR-354) and KAPA SYBR FAST qPCR Master Mix (2X) Universal (KAPA BIOSYSTEMS, KK4600), following the instructions of the manufacturers. qPCR-grade water was used as a negative control.

Reactions were set up with either:

- mosquito HV genomics
- mosquito LV genomics
- dragonfly discovery

In all cases, reagents were tested either by individual addition or as a master mix. The final concentration of reagents was as specified in table 1. Final reaction volumes were as specified in table 2.

Reactions were performed in a LightCycler® 480 Instrument II (Roche Molecular Systems, 05015243001) following the cycles as described in table 3. Analysis of samples was performed using LightCycler® 480 Software.

Table 1. Reagent concentrations in qPCR reactions.

Reagent	Desired concentration
qPCR Master Mix	1x
Premier mix	600 nM/ 100 µL
gDNA	2 ng/ 10 µL
dH_2O	required amount

key benefits

miniaturization of qPCR reactions >15x provides reproducible results.

- conserve valuable samples
- reduce reagent costs
- achieve great CVs

results and conclusions

The results of this study demonstrate that reproducible results can be obtained from qPCR assays where reaction volumes have been reduced 15-fold. For the mosquito HV genomics, this study demonstrated successful miniaturization down to 1 µL when using premixed reagents (Ct=25.68; CV=0.7) and 1.5 µL when reagents were dispensed individually (Ct=20.90; CV=0.2). For the mosquito LV genomics, successful results were achieved using just 0.6 µL of reagents, irrespective of whether reagents were premixed (Ct=25.44; CV=0.9), or dispensed individually (Ct=18.04; CV=1.7). Finally, qPCR assays set up using dragonfly discovery generated successful results when reagent volumes were miniaturized down to 0.6 µL for premixed reagents (Ct=25.92; CV=0.8) and 0.8 µL when dispensed individually (Ct=18.27; CV=1.5)

Table 2. Final reaction volumes dispensed with different instruments.

Instrument	Reagents dispensed individually	Reagents dispensed as master mix	Full volume reaction
mosquito HV genomics	1.5 µL	1 µL	20 µL
mosquito LV genomics	0.6 µL	0.6 µL	
dragonfly discovery	0.8 µL	0.6 µL	

sptlabtech

The low volume liquid handling instruments of SPT Labtech represent a perfect fit for qPCR reaction miniaturization in order to provide significant reagent, sample and cost savings. The mosquito genomics range enables highly accurate and precise multi-channel positive displacement pipetting from 500 nL to 5 µL for mosquito HV (High Volume) genomics and from 25 nL to 1.2 µL for mosquito LV (Low Volume) genomics. The dragonfly discovery offers highly accurate and precise multichannel dispensing with a broad dynamic range between 200 nL and 4 mL (figure 2).

For more information about this study, or the low volume liquid handlers used, contact us: discover@sptlabtech.com

Table 3. PCR conditions used for testing.

Stage	Temperature	Duration	Number of cylces
Initial denaturation	95°C	2 min	1x
Denaturation	95°C	10 sec	33x
Annealing	59°C	20 sec	33x
Elongation	72°C	30 sec	33x
Final elongation	72°C	2 min	1x

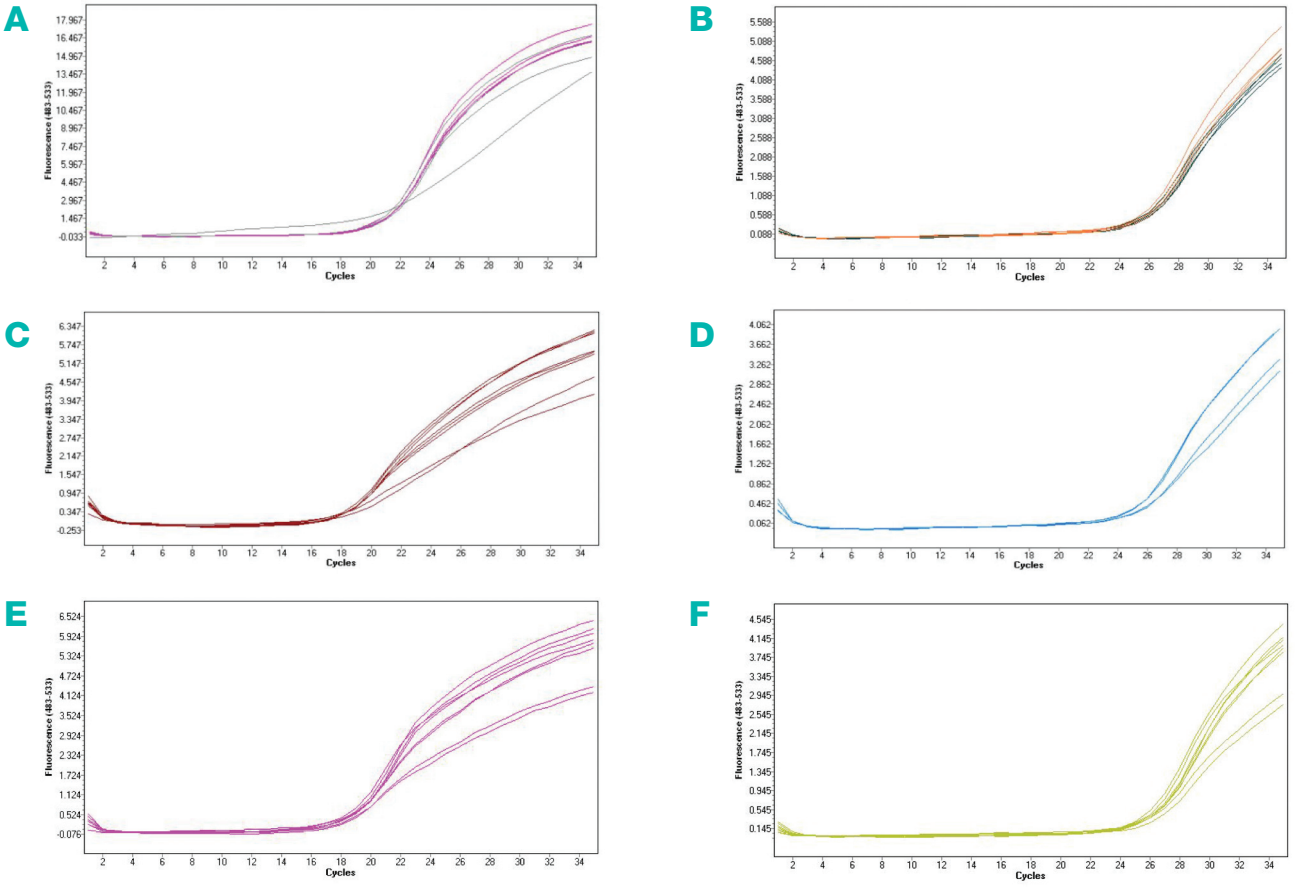

Figure 1. Amplification curves of reactions dispensed with a mosquito HV genomics (A. reagents dispensed individually; B. reagents pre-mixed), with a mosquito LV genomics (C. reagents dispensed individually; D. reagents pre-mixed) and with dragonfly discovery (E. reagents dispensed individually; F. reagents pre-mixed).

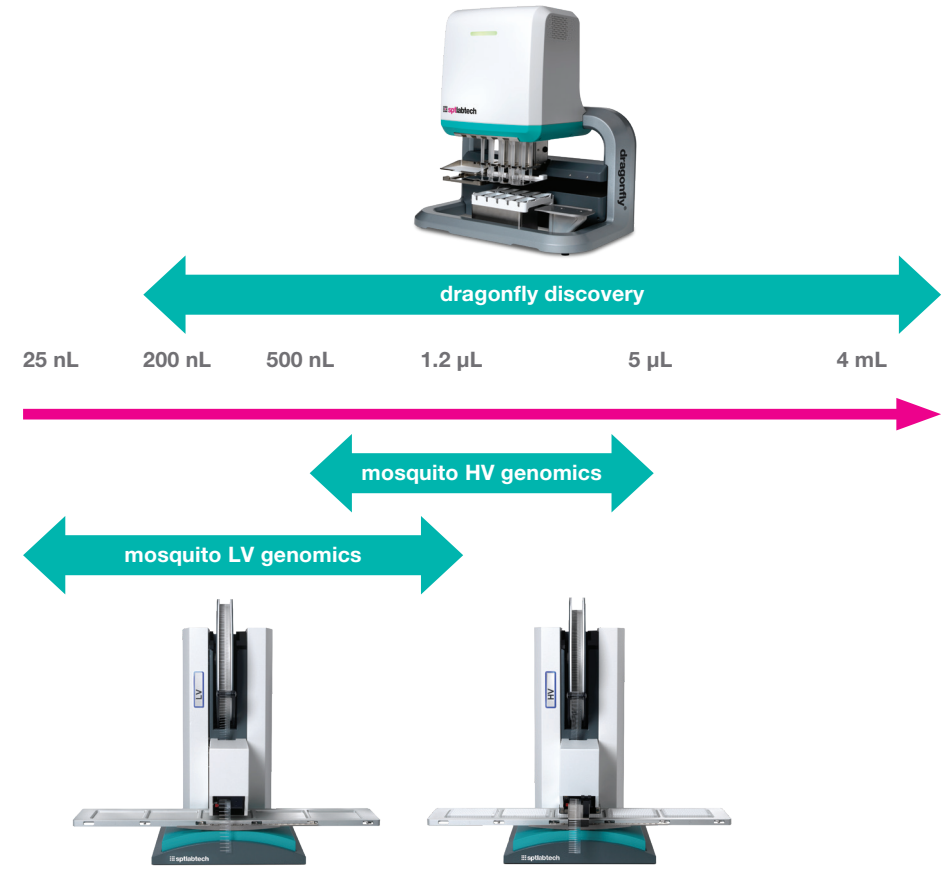

Figure 2. Volume ranges of SPT Labtech low volume liquid handling instruments.

designed for discovery

For more information contact us at discover@sptlabtech.com

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