

accurate, miniaturized DNA quantification assays with positive-displacement liquid handlers

introduction

Quantification and subsequent normalization of nucleic acids are critical steps in many genomics applications. Conventional quantification workflows use high volumes of costly reagents and waste valuable DNA. Furthermore, highly viscous DNA samples pose a challenge for automated liquid handling.

Significant increases in throughput together with reduced reagent costs and sample saving can be achieved using the combination of a mosquito® pipetting robot and a dragonfly® discovery dispenser. Due to their unique positive-displacement technology, even highly viscous samples and reagents can be handled with speed and accuracy, down to nanolitre volumes.

instruments and pipetting technology

SPT Labtech's positive displacement pipetting technology enables accurate and precise liquid handling irrespective of viscosity, without any need for liquid classes.

mosquito® LV genomics delivers multi-channel pipetting in 8 or 16 channels from 25 nL - 1.2 µL. It can aspirate, dispense and mix, make plate copies and serial dilutions.

dragonfly® discovery is a non-contact dispenser with a dynamic range from 200 nL - 4 mL. dragonfly® discovery is available with three, six or ten independent dispense heads.

key benefits

- assay miniaturization offers a simple method to increase the number of technical replicates, whilst reducing sample and reagent input requirements
- validated with the Promega Quantifluor®, Quant-IT™ PicoGreen™ and Biotium AccuClear™ kits
- excellent assay linearity and reproducibility across the DNA concentration range (0.05 - 20ng/ml) tested

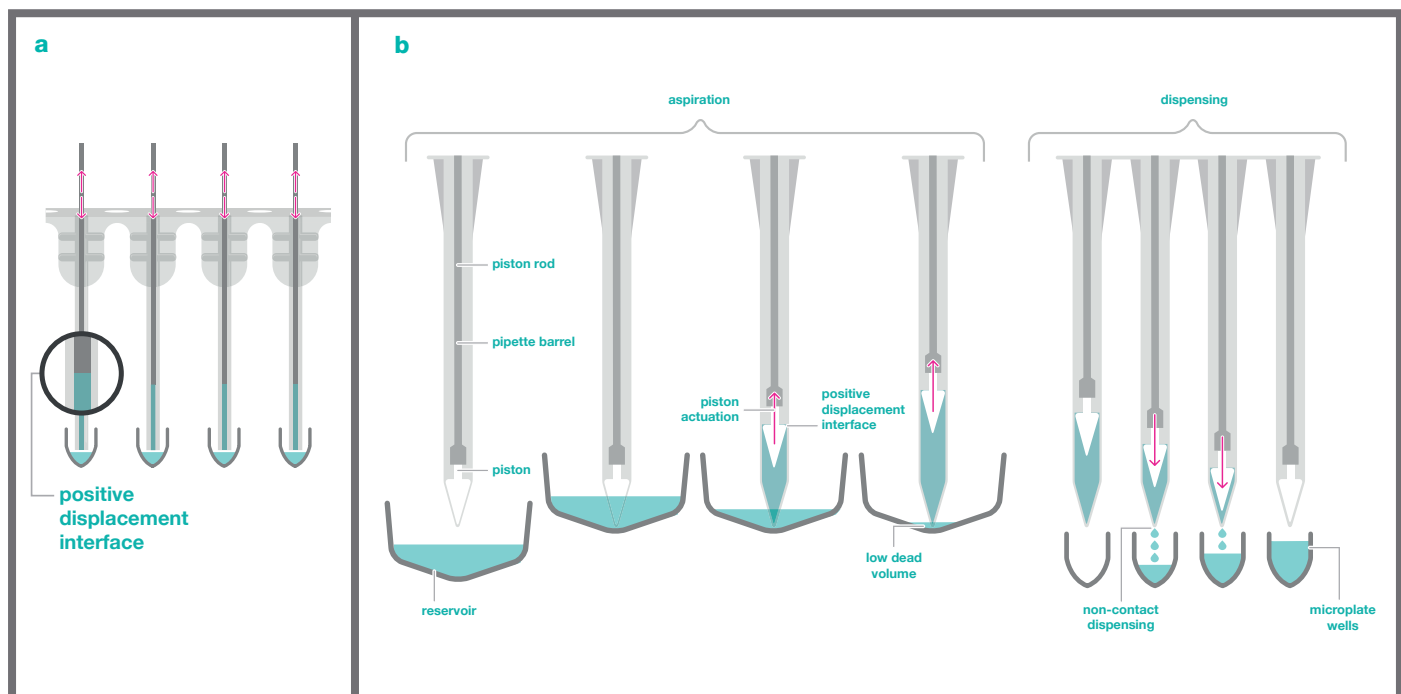


Fig 1. A) mosquito® tip design: disposable micropipettes with positive displacement pistons. B) dragonfly® discovery tip technology: schematic of an aspiration and dispense cycle with a single tip.

materials and methods

Sample quantification workflow (Fig. 2)

Step 1: A 384-well assay plate is pre-filled with the DNA quantification reagent using dragonfly® discovery.

Step 2: The samples are transferred in triplicates from a 96-well plate into the 384-well plate using mosquito® LV genomics.

Step 3: The 384-well plate scanned on a BMG ClarioStar® reader; results output in Excel, ready for normalization.

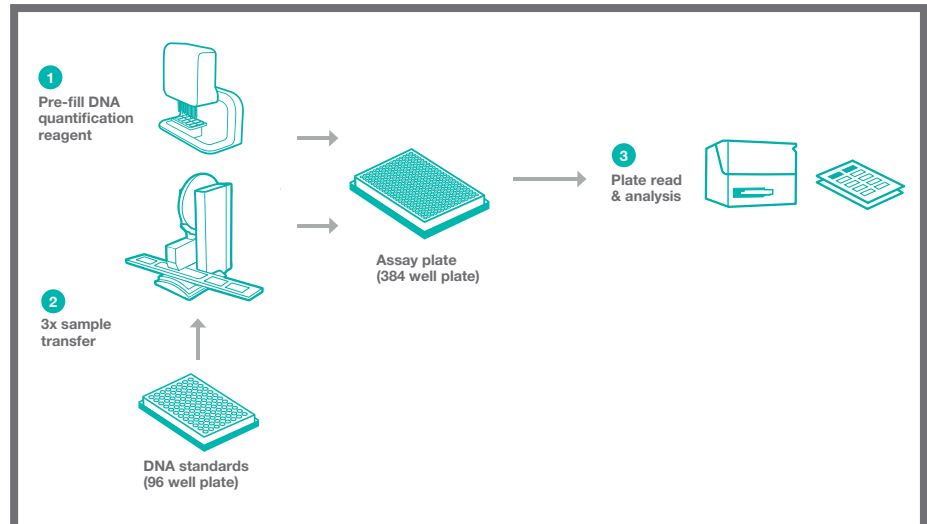


Fig 2. Workflow schematic for automated, dye-based DNA quantification process.

validation of miniaturized DNA quantification

We adapted common dye-based DNA quantification methods: Promega's QuantiFluor®, Quant-iT™ PicoGreen™ and Biotium's AccuClear™. Miniaturized high-throughput protocols were established for each kit using only 600 nL of DNA sample in triplicate and significantly reduced the amounts of fluorescent dye (see Table).

samples and reagent input

The table shows the amounts of DNA sample and fluorescent dye required for each quantification kit with the manual and the automated, miniaturized protocols, respectively.

	Final volumes in triplicates					
	Manual volume		Automated volume		Savings	
	DNA	Dye	DNA	Dye	DNA	Dye
Promega QuantiFluor®	3-60 µL	200 µL	600 µL	10 µL	Up to 100x	20x
Quant-iT™ PicoGreen™	<1000 µL	1000 µL	600 µL	10 µL	Up to 1500x	100x
Biotium AccuClear™	30 µL	200 µL	600 µL	50 µL	50x	4x

results

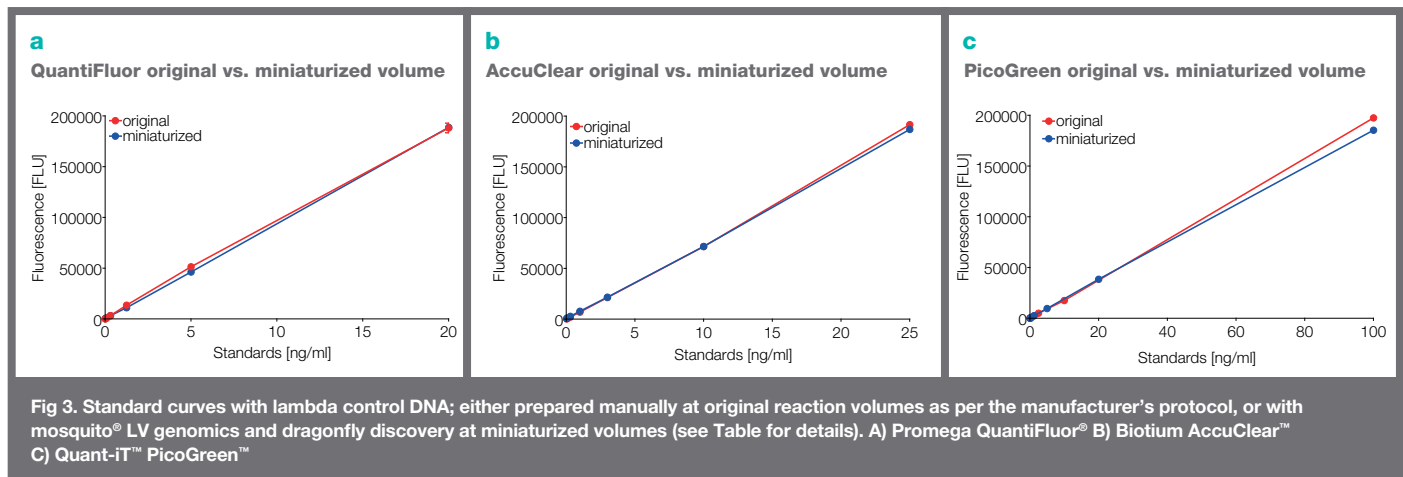


Fig 3. Standard curves with lambda control DNA; either prepared manually at original reaction volumes as per the manufacturer's protocol, or with mosquito® LV genomics and dragonfly® discovery at miniaturized volumes (see Table for details). A) Promega QuantiFluor® B) Biotium AccuClear™ C) Quant-iT™ PicoGreen™

conclusion

We show the utility of mosquito® LV genomics and dragonfly® discovery to automate and accurately miniaturize DNA quantification with dye-based kits. This approach allows for DNA quantification set-up in high throughput. It delivers highly reliable results, while reducing reagent cost and preserving valuable samples.

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for discovery



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