Revolutionizing NGS-driven Laboratory Developed Tests: The impact of automated liquid handling on plate-based bead clean-up

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Abstract

Automated liquid handling technology is transforming the landscape of Laboratory Developed Tests (LDTs), especially those utilizing Next-Generation Sequencing (NGS), by enhancing accuracy, efficiency and scalability.

firefly® is a well-placed solution for labs developing LDTs due to its compact footprint, user-friendly software and exceptional versatility. Validated protocol templates that support NGS library preparation are readily available from the firefly® cloud and can be adapted to meet specific lab needs.

As an example, automated bead-based clean-ups designed for 96- and 384- NGS applications are available on firefly®. These remove DNA fragments of undesirable sizes, enzymes or primers from the amplified DNA after a PCR reaction. Automating this process facilitates higher throughput and scaling.

Methods

- firefly® protocols were developed and optimized to be compatible with a range of elution volumes and bead ratios.
- Protocol performance was tested using library DNA (~300bp). The same input DNA was plated out and quantified using the QuantiFluor dsDNA system (Promega) before and after running the protocol. This data was used to assess any reduction in DNA concentration and uniformity across the plate.
- The protocol's ability to successfully modulate the fragment size distribution was demonstrated using a 50bp DNA ladder and different bead ratios. Data was obtained using a Fragment Analyzer (Agilent, DNF-474 HS NGS Fragment Kit).
- Well-to-well contamination was assessed by running no-template-controls (NTCs) in wells adjacent to library DNA, then measured by qPCR using a LightCycler 480 System (Roche, KAPA Library Quantification kit).

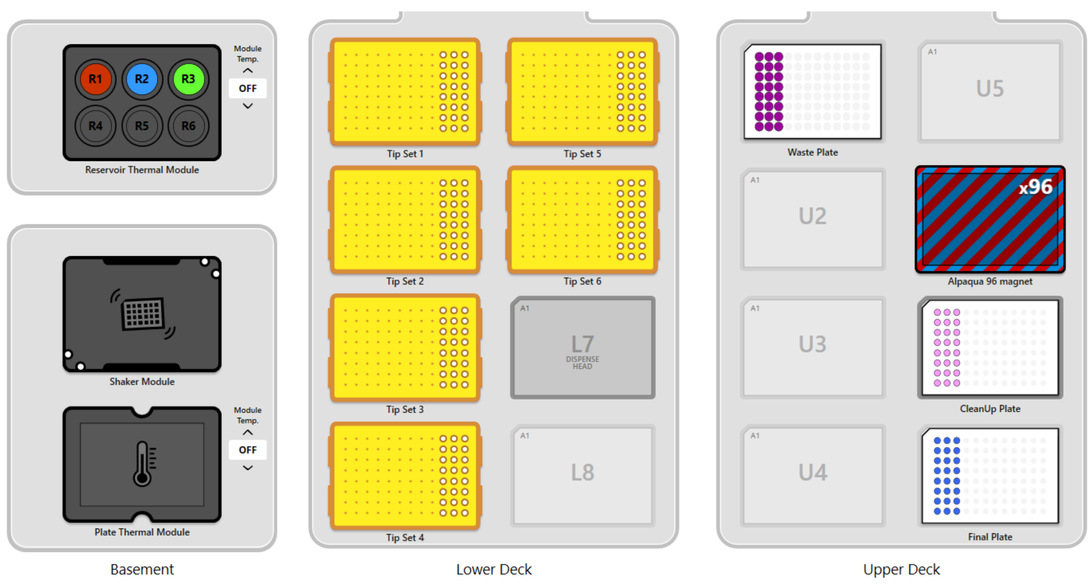

Figure 1. Example deck layout of a 3-column bead clean-up. R1 (reservoir 1) = Beads, R2 (reservoir 2) = Elution buffer and R3 (reservoir 3) = 80% Ethanol.

Protocol highlights

- Total protocol run time is 35 to 43 minutes (for 1 to 12 columns of samples respectively) with an additional 10-minute setup time.
- No user interactions are required once the protocol has started.
- Variables enable the user to tailor the bead ratio, final sample volume and columns to process.
- Tip mixing is used for the mix and resuspension steps.

Table 1. Variables and recommended limits for plate-based bead clean-up on firefly®.

	Variable	Minimum	Maximum
Sample volume per well (uL)*	✓	n/a	n/a
Bead volume per well (uL)	✓	0.2uL	179.8uL
Total sample + bead volume per well (uL)		10uL	180uL
Elution buffer volume (uL)		15uL†	60uL‡
Final library volume (uL)	✓	10uL	100uL
Number of columns to run	✓	1	12
Starting column	✓	1	12

* The protocol starts with the CleanUp plate containing a constant known volume of sample in each well which MUST be specified as is used to dictate the mix volume. The bead : sample ratio is modified by specifying the bead volume to add to the sample.
† Minimum volume required to resuspend the bead pellet and the minimum library volume is set 5 µL higher than this.
‡ Set to 60 µL to align with the ethanol wash volume.

Results

- Uniformity of concentration was seen across the plates (Figure 2).
- Comparison of the library concentration before and after the clean-up showed a 5% reduction in DNA concentration after the 96-well plate clean-up and an 11% reduction in library concentration when using the 384-well plate clean-up (Figure 3).
- Fragment size analysis showed that fragment sizes can be successfully modulated on firefly® by changing the bead ratio of the firefly® run (Figure 4) and no detectable well-to-well contamination was measured by qPCR in both 96 and 384-well formats (Figures 5 and 6).

Variation in DNA concentration after clean up

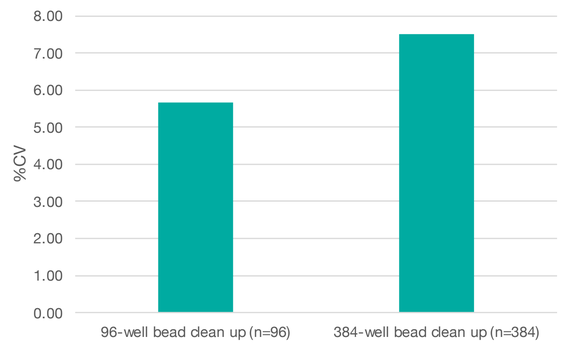

Figure 2: Variation in DNA concentration after performing bead clean-up protocols on firefly®

DNA concentration before and after a bead clean-up on firefly®

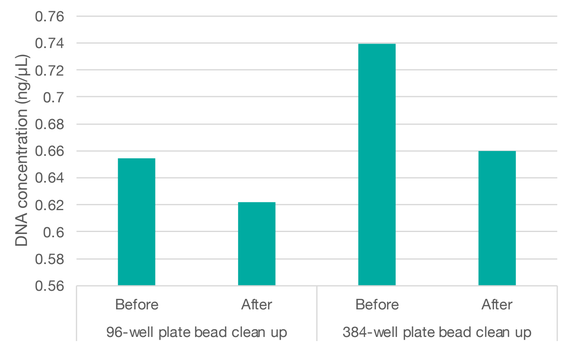

Figure 3: DNA concentration before and after bead clean-up on firefly®

The effect of varying bead ratio

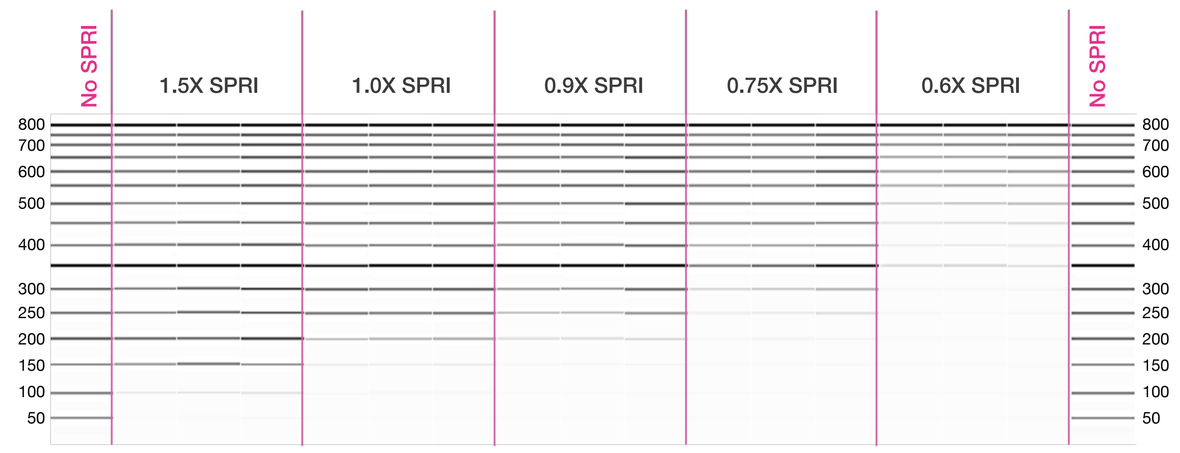

Figure 4: Fragment Analyzer gel images showing the abundance of remaining DNA fragments after running the 96-bead clean-up protocol on firefly® using a 50bp DNA ladder

Amplification curves

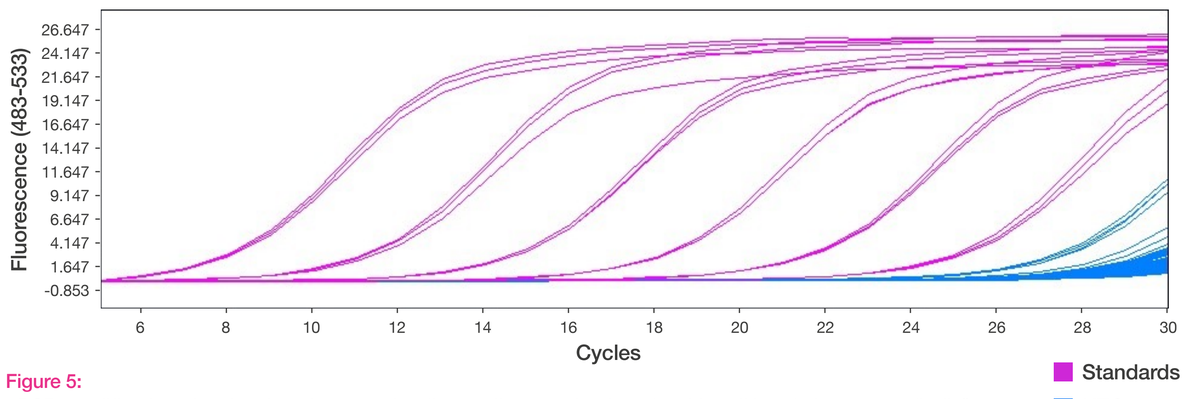

Figure 5:
qPCR amplification plot showing absence of well-to-well contamination after 96-well bead clean-up on firefly®.

Amplification curves

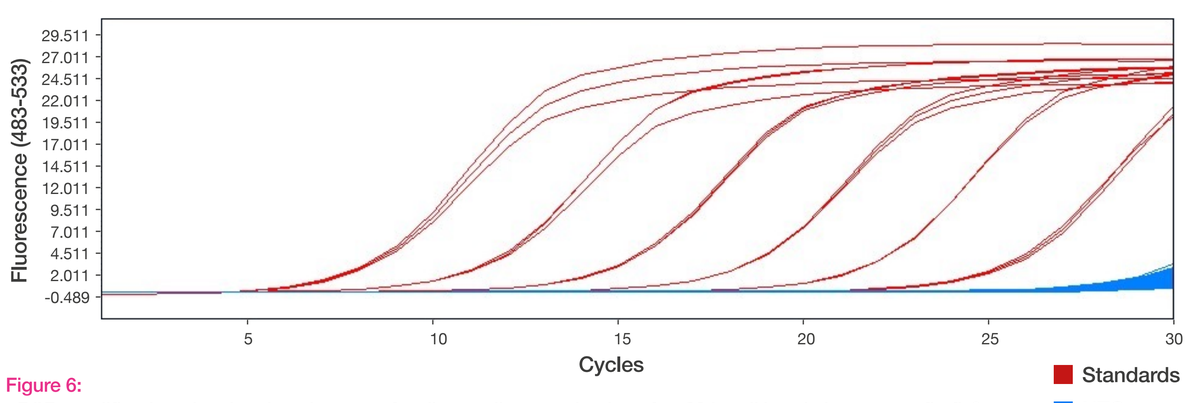

Figure 6:
qPCR amplification plot showing absence of well-to-well contamination after 384-well bead clean-up on firefly®.

Conclusion

This poster demonstrates that automated bead clean-ups on firefly® show uniformity across both 96- and 384-well plates, with no detectable well-to-well contamination, to deliver the consistent and robust results required for an LDT.

Bead-based cleanups on firefly® offer a fast, 100% walkaway solution. By running 96-samples at once on firefly®, we see approximately a 6-fold increase in throughput than when performed manually by a single user.

Ready-to-implement protocols can be accessed directly from the firefly® cloud to accelerate protocol setup, and can be used in isolation or as a building block for a custom protocol.