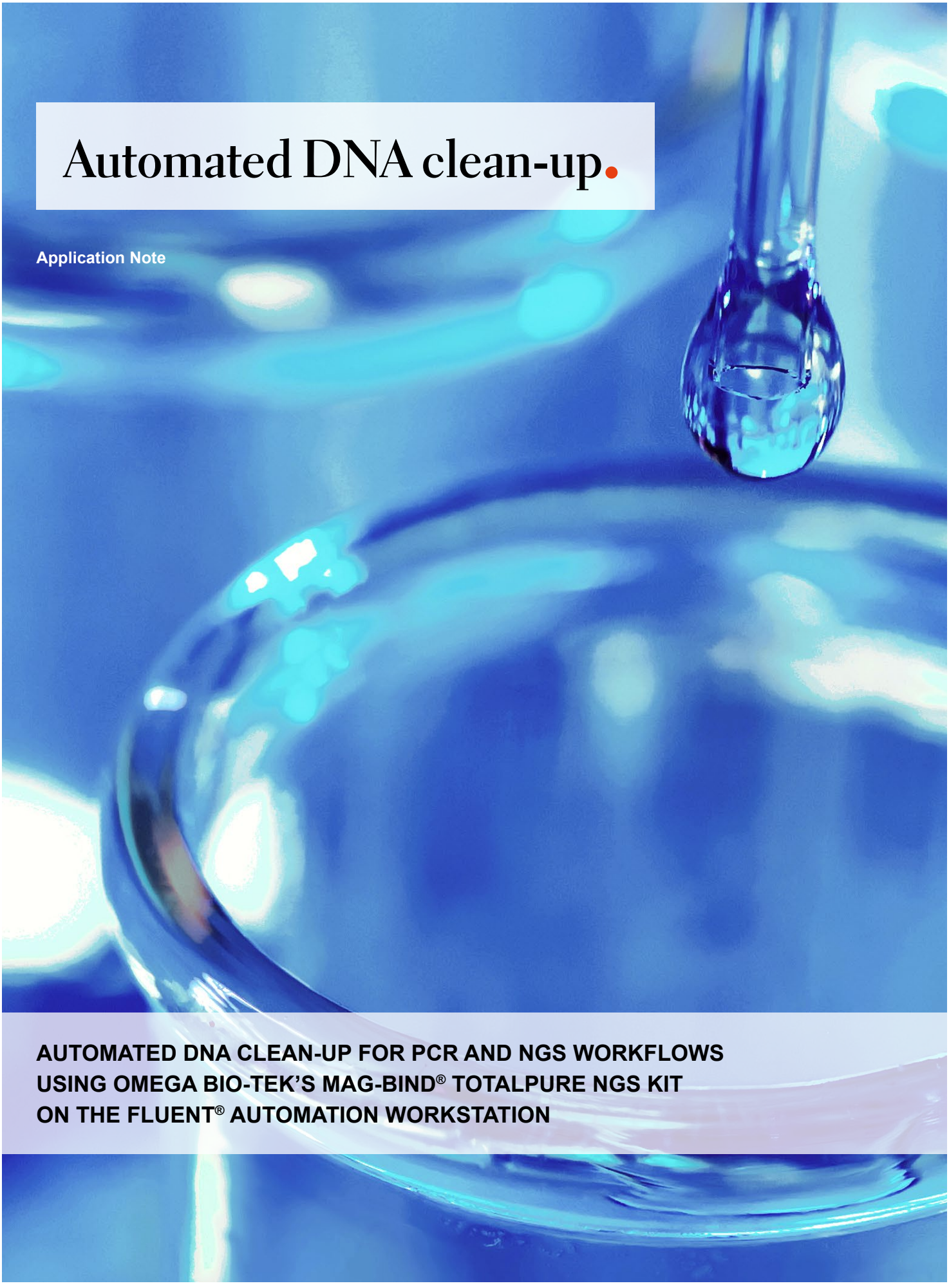
Automated DNA clean-up.

Application Note



AUTOMATED DNA CLEAN-UP FOR PCR AND NGS WORKFLOWS USING OMEGA BIO-TEK'S MAG-BIND® TOTALPURE NGS KIT ON THE FLUENT® AUTOMATION WORKSTATION

TECAN.

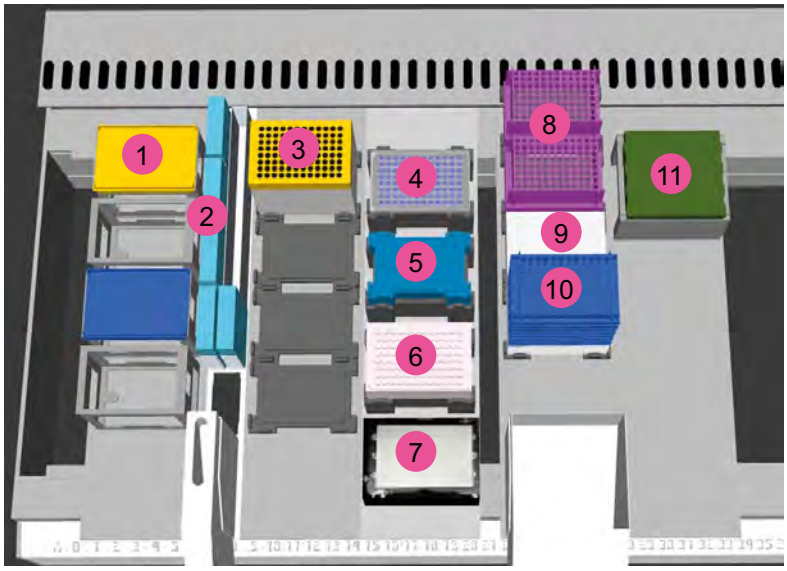
INTRODUCTION

Next generation sequencing (NGS) technologies are increasingly being used in a variety of fields, from basic biological research to pharmacogenomics and clinical medicine. Library preparation is the first crucial step of a typical NGS workflow, and involves several DNA clean-up stages. To fully exploit the potential of NGS technologies, rapid advancements are needed in throughput and turnaround time. To meet this need, a fully automated protocol has been developed that uses Mag-Bind TotalPure NGS beads (Omega Bio-tek®) to perform the required library preparation clean-up steps on a Fluent 780 workstation. This application note demonstrates the ability of Mag-Bind TotalPure NGS beads to selectively bind DNA of different fragment lengths by altering the ratio of magnetic beads to input DNA. The performance of this high throughput solution at different bead-to-sample ratios was evaluated based on DNA recovery and quality. The results indicate that the automated workflow can efficiently purify 96 samples, with an input DNA >100 bp and up to 100 µl volume, in less than 35 minutes.

MATERIALS AND METHODS

Four 25 µl aliquots of 20x diluted 50 bp ladder were transferred to a 96-well plate in quadruplicate, and purified using Mag-Bind TotalPure NGS beads on a Fluent 780 automation workstation at bead-to-sample ratios of 0.6x, 0.8x, 1.0x and 1.2x. The Fluent workstation was configured with an Air Flexible Channel Arm™ (Air FCA), a MultiChannel Arm™ (MCA), a Robotic Gripper Arm™ (RGA), a BioShake® D30-T elm (QInstruments®) for heating and shaking, and a Magnum FLX® Enhanced Universal Magnet Plate (Alpaqua®). This worktable configuration allows for full automation of the Mag-Bind TotalPure NGS protocol, including automatic dispensing of beads as per the clean-up ratio, binding, washing and, finally, elution of the DNA in 30 µl of 10 mM Tris-HCl (pH 8.5). An aliquot of unprocessed 50 bp ladder (1:20 dilution) was included as a control for post clean-up analysis, and to evaluate the fragment sizes recovered at the four different bead-to-sample ratios.

The fragment sizes of the eluted DNA were determined using a TapeStation® 2200 (Agilent®), and compared to the unprocessed 50 bp ladder control using a High Sensitivity D1000 ScreenTape® (Agilent). TapeStation 2200 analysis software was used to estimate the percentage of DNA recovered, as well as the fragment sizes removed after clean-up at the four different ratios. The results were also compared to an unaffiliated, third-party internal report published by the Genomics and Cell Characterization Core Facility of the University of Oregon,[1] to validate the automation clean-up methodology and set-up.



1. 1,000 µl hanging DiTis
2. 100 ml reagent troughs
3. 1,000 µl SLAS, filtered DiTis
4. 96-well standard microplate
5. Magnum FLX plate
6. Nunc 96-well DeepWell plate
7. BioShake D30-T elm
8. 500 µl MCA filtered DiTis
9. Liquid waste trough
10. 200 µl MCA filtered DiTis, nested 8 stack
11. Extended Volume Adapter

Figure 1: Fluent deck layout for extraction of 96 blood samples with 250 µl input volumes. DiTi = disposable tips.

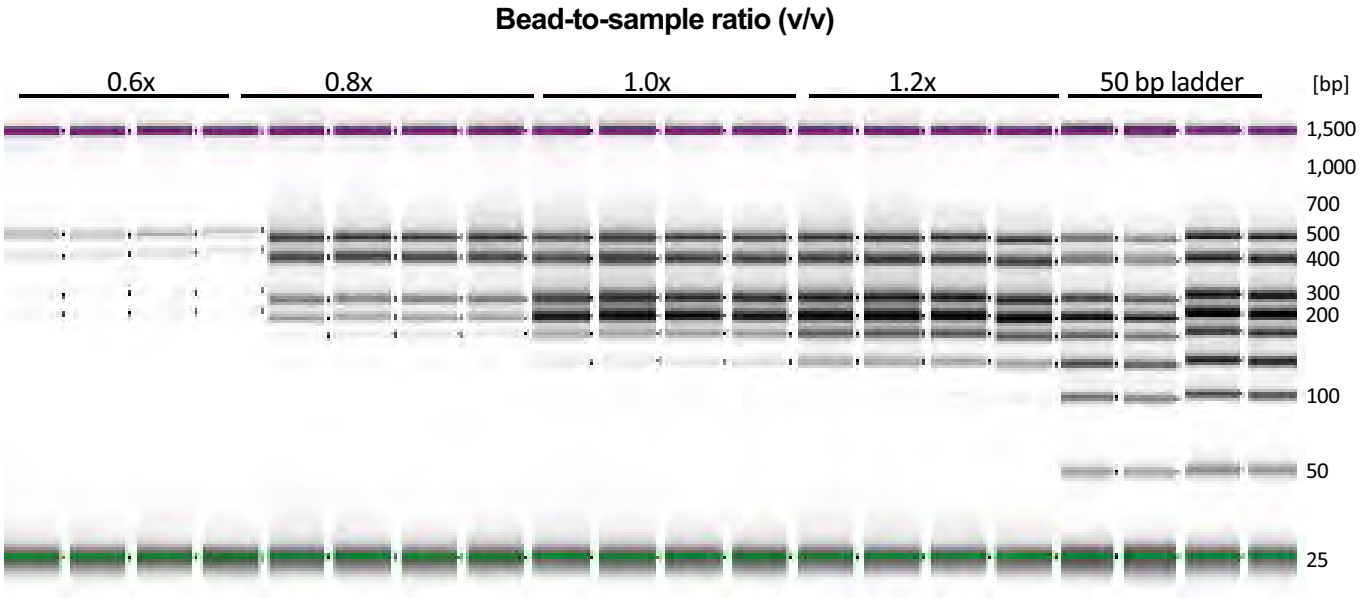


Figure 2: Fragment sizes of eluted DNA after clean-up with different bead-to-sample (v/v) ratios. TapeStation analysis was performed on 25 µl of 20x diluted 50 bp ladder following clean-up with Mag-Bind TotalPure NGS beads on a Fluent 780 automation workstation, using unprocessed 50 bp ladder as a control.

Eight 250 µl aliquots from the same lot of human whole blood were transferred to a 96-well deep-well plate, then placed on the Fluent workstation for automated gDNA extraction and purification using the Mag-Bind Blood & Tissue DNA HDQ 96 Kit. DNA was eluted in 100 µl of 10 mM Tris-HCl (pH 8.5). All consumables and carriers were placed onto the Fluent workdeck (Figure 1). The workflow was fully automated, from preparation and extraction of the sample aliquots contained in the 96-well plate, to elution of the final product. Aliquots of the same lot of human whole blood were manually extracted in parallel, and the results of the two workflows compared to validate the automated methodology and instrument set-up.

RESULTS AND DATA ANALYSIS

TapeStation analysis of DNA after clean-up at various bead-to-sample ratios is shown in Figure 2. The results show that, by altering the bead-to-sample ratio, it is possible to selectively bind DNA fragment sizes. The average percentage of DNA removed at ratios of 0.6x, 0.8x, 1.0x and 1.2x (v/v) was calculated based on the unprocessed 50 bp ladder control at different fragment sizes (Figure 3).

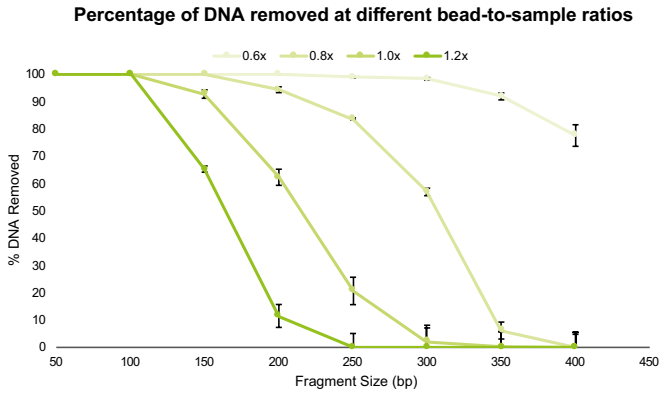


Figure 3: Average percentage of DNA removed (n = 4) at various fragment sizes using different volume ratios of Mag-Bind TotalPure NGS beads to input sample. Note: negative percentages are reported as 0 %.

The calculations were based on the concentrations of the different DNA fragments of the 50 bp ladder, as estimated by the TapeStation 2200 analysis software. The 50 bp and 100 bp fragments of the 50 bp ladder were completely removed at all the different ratios tested. The results demonstrate that higher bead volumes are capable of binding smaller fragment sizes compared to lower volumes. For example, a bead-to-sample ratio of 1.2x recovered fragments over 150 bp, whereas only fragment sizes of 300 bp and over were recovered using a 0.8x ratio. Table 1 shows that the average percentage of DNA removed using the automated protocol on a Fluent 780 workstation is in agreement with published results.[1]

Bead-to-sample ratio (v/v)

	100	150	200	300	400
0.6x	99	99	99	68	91
0.8x	98	97	96	59	17
1.0x	96	81	65	11	9
1.2x	90	51	12	0	0

Published data[1]

	100	150	200	300	400
0.6x	100	100	100	98	78
0.8x	100	100	94	57	0
1.0x	100	93	62	2	0
1.2x	100	65	0	0	0

Current study

Table 1: Comparison of average percentage of DNA removed (n = 4) at various bead-to-sample ratios using the automated protocol with that of published data.

[1] Note: negative percentages are reported as 0 %.

The results also show similar trends in terms of DNA binding capability at different bead-to-sample volumes. These results validate the instrument set-up and automated clean-up protocol.

SUMMARY

Integration of the Mag-Bind TotalPure NGS bead protocol with the Fluent workstation demonstrates a fully automated, high throughput DNA clean-up example for PCR and NGS applications. Using this workflow, full or partial 96-well plates containing up to 100 µl samples can be processed in less than 35 minutes. The Fluent deck configuration can easily be adapted according to the size and availability of other liquid handling arms. DNA clean-up at different bead-to-sample ratios was carried out in the same plate, demonstrating the ease and flexibility of the automated workflow. The proposed magnetic bead-based workflow is not only user-friendly, but also eliminates the need for time-consuming electrophoresis-based clean-up. The high throughput capabilities, together with the ability of the automated bead clean-up system to select the desired DNA fragment sizes, has significant potential in NGS applications.

NOTE

The third-party report published by the Genomics and Cell Characterization Core Facility of the University of Oregon evaluated and validated the performance of Mag-Bind TotalPure NGS beads at different bead-to-sample ratios (0.3x to 3x). This report serves as a guideline for the appropriate clean-up ratio(s) needed for the isolation of the DNA fragments of choice.

REFERENCES

1) Technical Note: Evaluation of Omega Mag-Bind® TotalPure NGS Beads for DNA Size Selection. Genomics and Cell Characterization Core Facility, University of Oregon. **https://www.omegabiotek.com/product/ngs-workflow-pcr-clean-up-mag-bind-total-pure-ngs/?mc_phishing_protection_id=55255-cbpvb4a80btra8187us0#protocols**

ACKNOWLEDGEMENTS

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