Automated DNA Cleanup for PCR and NGS Workflows: Mag-Bind® TotalPure NGS on Tecan Fluent® 780 Workstation

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Introduction

Next-generation sequencing technologies are increasingly utilized in a variety of fields from basic biological research to pharmacogenomics to clinical medicine. Library preparation is the first crucial step of a typical NGS workflow and it involves several DNA cleanup steps. To fully exploit the potential of NGS technologies, rapid advancements are needed in terms of throughput processing and turnaround time. To meet this need, Omega Bio-tek has developed a fully automated protocol employing Mag-Bind TotalPure NGS beads (M1378) on a Tecan Fluent 780 Workstation to perform the required cleanup steps during library preparation. This application note demonstrates the ability of Mag-Bind TotalPure NGS beads to selectively bind different fragment lengths by altering the ratio of volume of magnetic beads used to the volume of input DNA. The performance of this high throughput solution at different bead-to-sample ratios was evaluated based on DNA recovery and quality. Our results indicate that this automated workflow can efficiently cleanup ninety-six samples with a sample input of DNA > 100 bp and up to 100 µL volume in less than 35 minutes.

Materials and Methods

A 25 µL volume of a 50 bp ladder was diluted 20 times and cleaned up using Mag-Bind TotalPure NGS beads at bead-to-sample volume ratios of 0.6X, 0.8X, 1.0X and 1.2X. Four 25 µL aliquots of the 50 bp ladder (1:20 dilution) were transferred to a 96-well plate in quadruplicate and moved to the Tecan Fluent workstation for DNA cleanup at the four different ratios mentioned above. The Tecan instrument was programmed to perform various liquid handling and magnetic bead-based tasks as demanded by the Mag-Bind TotalPure NGS beads protocol. A Tecan Fluent 780 was configured to accommodate automation of the Mag-Bind TotalPure NGS protocol, including automatic dispensing beads as per cleanup ratio, binding, washing and finally elution of the DNA in 30 µL of 10 mM Tris-HCl (pH 8.5). The cleanup workflow was fully automated starting with the sample aliquot in the 96-well plate to final eluted product. An aliquot of unprocessed 50 bp ladder (1:20 dilution) was included as a control for post cleanup analysis and to shed light on the fragment sizes recovered at the four different bead-to-sample ratios tested.

The fragment sizes of DNA eluted after cleanup ratios of 0.6X, 0.8X, 1.0X and 1.2X were analyzed on Agilent's TapeStation® 2200 and compared to the unprocessed 50 bp ladder (1:20 dilution control) using a High Sensitivity D1000 ScreenTape®. TapeStation 2200 analysis software was used to estimate the percentage of DNA recovered as well as fragment sizes removed after cleanup at four different ratios. The results were also compared to an unaffiliated, third-party internal report[1] published by the Genomics & Cell Characterization Core Facility of University of Oregon to validate the automation cleanup methodology and setup.

TapeStation Analysis of DNA Post Cleanup at Different Bead-to-Sample Ratios

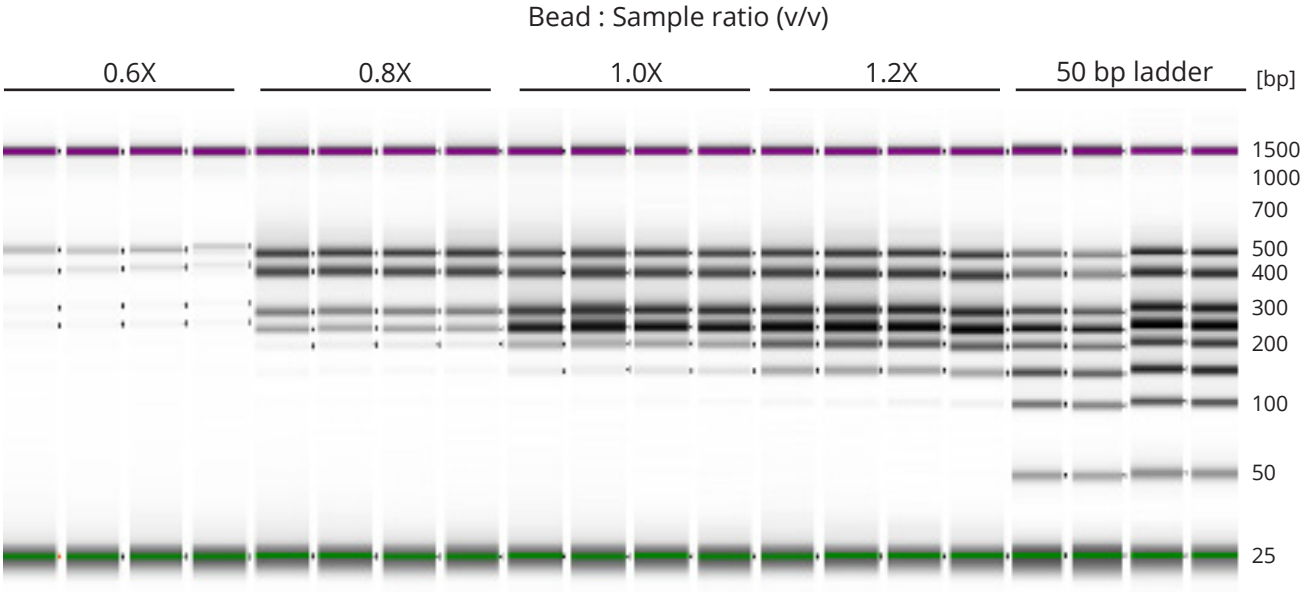


Figure 1. TapeStation analysis was performed on 25 µL of 20X diluted 50 bp ladder following cleanup with Mag-Bind TotalPure NGS beads on Tecan Fluent 780 workstation and an unprocessed 50 bp ladder as a control. The fragment sizes of DNA eluted after different cleanup ratios are as shown above.

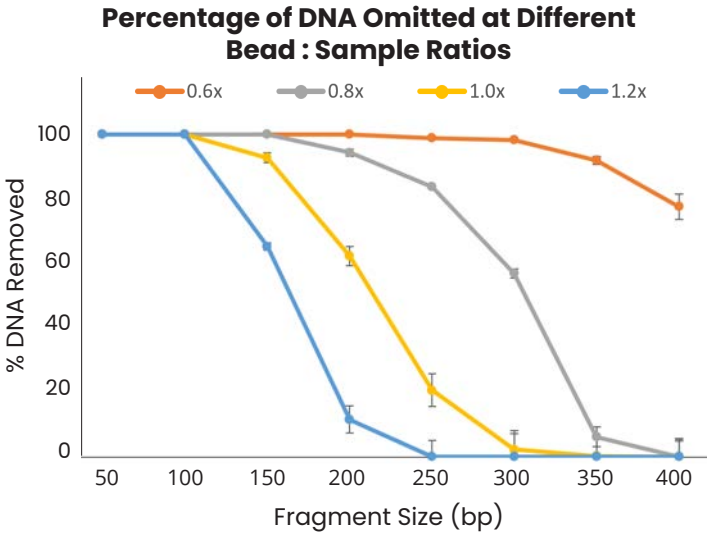
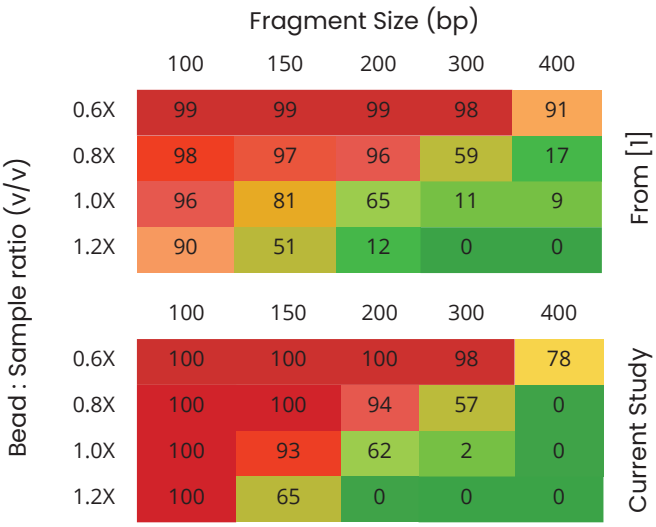


Figure 2. Average percentage of DNA removed (n=4) at various fragment sizes at different volume ratios of Mag-Bind TotalPure NGS beads to input sample volume. Note: Negative percentages are reported as 0%.

Table 1. Comparison of average percentage of DNA removed (n=4) at various bead : sample ratios following automated protocol to that of the data reported in [1]. Note: Negative percentages are reported as 0%.



Results and Discussion

TapeStation analysis of the DNA after cleanup at various ratios of Mag-Bind TotalPure NGS beads to sample volume is as shown in Figure 1. The results show that by altering bead-to-sample ratio, it is possible to selectively bind DNA fragments. The average percentage of DNA removed at 0.6X, 0.8X, 1.0X and 1.2X bead : sample ratios (v/v) was calculated based on the control (unprocessed 50 bp ladder) at different fragment sizes (Figure 2). 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 omitted at all the different ratios tested. The results demonstrate higher bead volumes are capable of binding smaller fragment sizes compared to lower bead volumes. For instance, a 1.2X ratio of beads to sample recovered fragments over 150 bp whereas only fragment sizes 300 bp and over were recovered when using a 0.8X ratio. Table 1 shows that the average percentage of DNA removed following the automated protocol on Tecan Fluent 780 is in consensus with the results published in [1]. The results not only match but show similar trends in terms of DNA binding capability at different bead-to-sample volumes. These results validate the instrument setup and automated cleanup protocol.

Conclusions

Omega Bio-tek's Mag-Bind TotalPure NGS (M1378) bead protocol integrated onto the Tecan Fluent workstation offers an automated, high throughput DNA cleanup solution for PCR and NGS applications. Using this workflow, up to ninety-six samples up to 100 µL in volume can be processed in less than 35 minutes. The proposed deck configuration with this workflow can easily be adapted to different Fluent configurations according to liquid handling arm availability and size. The DNA cleanups at different bead-to-sample ratios were carried out on the same plate elucidating the ease and flexibility of the automated workflow. The proposed workflow using the magnetic beads is not only user-friendly but also eliminates the need for time consuming electrophoresis based cleanups. The high throughout promise and the tunability of an automated bead cleanup system to select desired DNA fragment sizes offers significant potential in NGS applications.

Note:
The third-party report[1] put together by the Genomics & Cell Characterization Core Facility of 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 appropriate cleanup ratios(s) needed for the selection of DNA fragments of choice.

References

1. Evaluation of Omega Mag-Bind® TotalPure NGS Beads for DNA Size Selection. Genomics & Cell Characterization Core Facility, University of Oregon Technical Note - http://gc3fstorage.uoregon.edu/IMAGES/Evaluation_of_Omega_Mag-Bind_TotalPure_NGS_Beads_MWeitzman_April2018.pdf.

Product Information

Product No.	Description
M1378-00	Mag-Bind® TotalPure NGS beads (5 mL)
M1378-01	Mag-Bind® TotalPure NGS beads (50 mL)
M1378-02	Mag-Bind® TotalPure NGS beads (500 mL)

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