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Using the G.PURE to conduct automated, non-contact bead-based DNA purification

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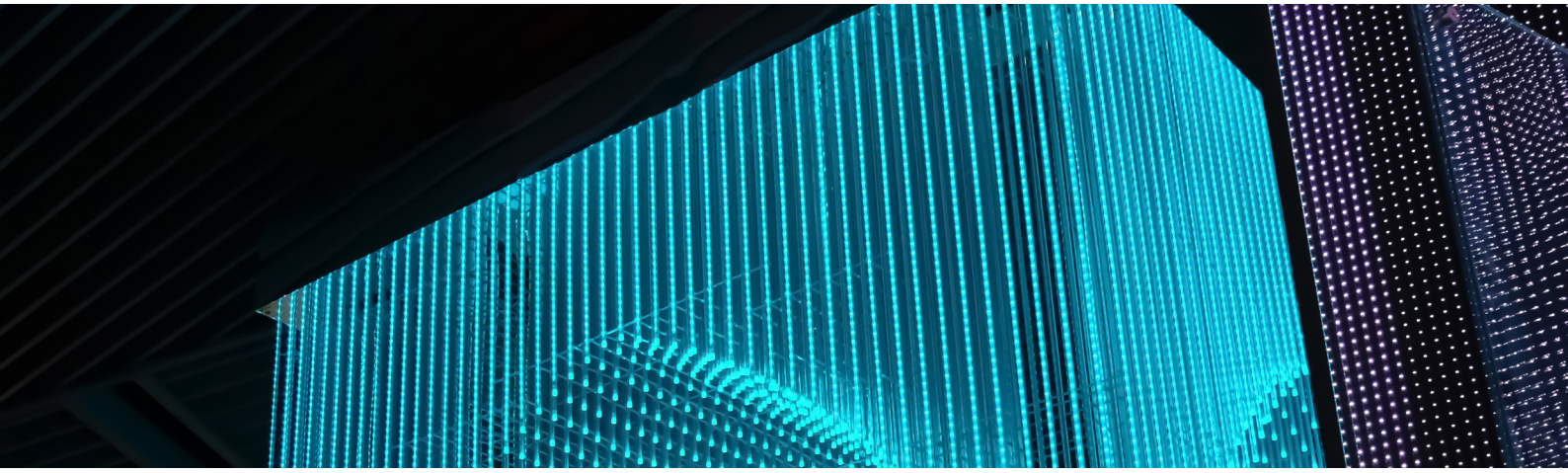

Introduction

DNA purification of PCR products is a critical step in the NGS library preparation workflow. It rids the sample of unwanted contaminants such as buffers, lower molecular weight fragments and nucleotides, resulting in a pure sample which is essential for downstream processes.

The most common method of purifying low concentration DNA samples is to perform a cleanup step using paramagnetic solid-phase reversible immobilization (SPRI) beads. In this situation, a specific ratio of polyethylene glycol (PEG) and salt to sample solution is used to preferentially bind only one thing to the beads — the DNA fragments intended for downstream processes. Through adjusting the ratio of the bead suspension to sample volume, we can readily adapt the cutoff size.

The process of NGS library prep is becoming increasingly automated through the use of liquid handlers such as the I.DOT. However, magnetic bead cleanup is still often performed manually, creating a time-consuming bottleneck in the automation workflow. Manual bead cleanup relies heavily on the quality of the pipetting, and inconsistencies can lead to variation between samples. Furthermore, each additional wash step creates an opportunity for bead loss or over-drying of the sample, as processing time can vary. Additionally, the multiple steps involved often require the use of many boxes of pipette tips.

In contrast, automated magnetic bead cleanup has the potential to streamline the NGS library prep workflow, ensuring consistent results.

The G.PURE is a plate washer and liquid dispenser designed specifically for cellular assays and bead-based DNA purification. It quickly and gently removes liquids from PCR plates using centrifugal forces, while magnetic plate carriers maintain the magnetic beads in the plates. The non-contact dispenser quickly and uniformly adds reagents, such as ethanol, for the wash steps. These features enable automated bead-based DNA purification, even at the reduced volumes that are common in miniaturized NGS library preparation workflows. Non-contact washing saves several hundreds of pipette tips per plate.

In this experiment, beginning with unpurified cDNA post SMART-seq2 library prep, we compare sample purification between the G.PURE and manual bead cleanup at both standard and miniaturized volumes. Through comparison of the bioanalyzer traces, we demonstrate that the G.PURE provides equivalent or better results in DNA purification when compared with manual bead cleanup, while taking only a fraction of the time.

The G.PURE provides standalone operation, along with seamless integration into automated high-throughput workflows.

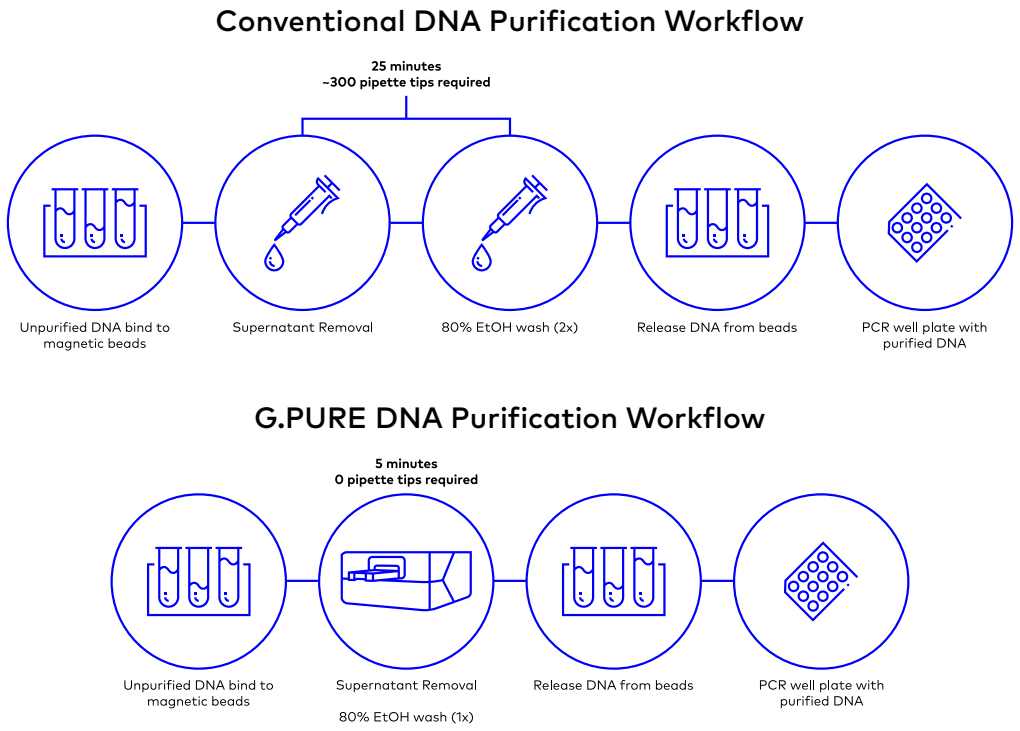

Figure 1. *The G.PURE provides a fast and automated workflow for bead-based DNA purification. Standard manual bead cleanup workflows are time-consuming and require many pipettes due to the several removals, wash and dispense cycles used to purify the DNA. The G.PURE performs the critical wash steps 5x faster than conventional purification while requiring no pipetting steps.*

Materials and methods

cDNA bead-based cleanup procedure

The bead-based DNA purification protocol used here was adapted from the SMARTSeq v4 Ultra Low Input RNA Kit for Sequencing user manual (Takara Bio). An unpurified cDNA sample post library prep was used for all comparison tests. Briefly, 25 µL of cDNA sample was mixed with 25 µL of AMPure XP beads (A63881) in a 96 PCR well plate (4titude, 4TI-0960). The samples were incubated at room temperature for 8 minutes. Samples were placed on the G.PURE magnetic carrier for 5 minutes, until the liquid was clear.

For manual prep, the supernatant was removed with a pipette, and the samples were washed twice with 80% EtOH for 30s each.

In the case of the samples processed by the G.PURE, the 1x and 2x wash steps of EtOH were processed automatically on the device.

The samples were removed from the magnet, and 17 µL of fresh water was added to the samples and mixed. The samples were incubated for 2 minutes. Samples were again placed on the magnet, and 15 µL of the clear supernatant was removed and kept for analysis. The miniaturized samples were similarly processed by the G.PURE, with a starting amount of 5 µL of cDNA and an equal volume of beads. The final water volume used for DNA elution was 3.4 µL.

cDNA purification analysis
1 µL of each purified sample was analyzed via the Agilent 2100 Bioanalyzer. The resulting bioanalyzer traces were compared in order to determine the consistency of the manual and G.PURE bead cleanup.

Results and discussion

G.PURE automates and reduces the bead-based DNA purification process
Conventional or manual bead-based DNA purification is a time-consuming workflow, given that it involves several wash steps (~25 minutes) (***Figure 1***). Given that each well contains a distinct library, a new pipette tip must be used for each processing step in order to prevent cross-contamination. The number of pipette tips required grows considerably when processing many plates per day, which is not uncommon in some automated workflows. Additionally, pipetting errors can lead to variability between samples, which is especially critical when working with and comparing single cell derived samples.

The G.PURE reduces the critical wash steps to just 5 minutes. Since both reagent dispensing and removal are conducted through non-contact methods, no pipette tips are needed to perform the wash steps.

This also brings more uniformity to the cleanup, compared to manual bead cleanup. Other automated bead cleanup methods utilize pipetting robots, but these still involve the use of pipette tips and other consumables. Additionally, the processing time is not always improved with this method, since reagent dispensing and removal is normally performed sequentially, as it would be in manual prep.

The G.PURE removes liquids in a single spin, dramatically reducing the overall time required. Since the reagent dispensing is non-contact, the G.PURE also saves time as it removes the need for pipette tip exchange. In this way, a higher number of plates can be processed in a single day using the G.PURE, creating a significant reduction in the cost of consumables.

G.PURE performs uniform library cleanup and is compatible with miniaturization
To assess the performance of both the G.PURE and manual bead-based cleanup, unpurified cDNA generated through a SMART-seq2 protocol was purified. After bead cleanup, the expected result should span between 100-10,000 bp, with the peak around 2,000 bp. Ideally, after cleanup, unwanted smaller fragments would also be removed from the sample. For the purposes of this experiment, we performed the DNA purification in 96 well plates, as specified above.

After bead accumulation on the magnet, the G.PURE spun the plate at 22 g to remove the supernatant. After the dispensation of 180 µL of the 80% EtOH by the G.PURE, it was spun again at 28 g to remove the remaining EtOH, then proceeded with DNA elution. Figure 2 shows the resulting bioanalyzer traces for the manual vs. G.PURE processed samples. The G.PURE samples have fewer small molecular weight fragments in the traces when compared to the manually prepped sample, demonstrating the thoroughness of the washing step.

Additionally, the manually prepped sample that was washed twice produced a similar result to the G.PURE sample that was only washed once. This demonstrates that the second wash step, as recommended in the manual cleanup protocol, is not required with the G.PURE opening the door for additional time savings.

Bead-based cleanup was also performed in a miniaturized sample with the G.PURE. Here, only 5 µL of cDNA was purified. The subsequent volumes were also reduced by one-fifth. When comparing bioanalyzer traces, the bioanalyzer trace of the miniaturized sample was comparable to the standard volume samples, along with having a minimal number of smaller fragments. These results demonstrate that even in 96 well plates, the G.PURE is compatible with miniaturized volumes, something which is difficult to achieve with manual bead cleanup methods.

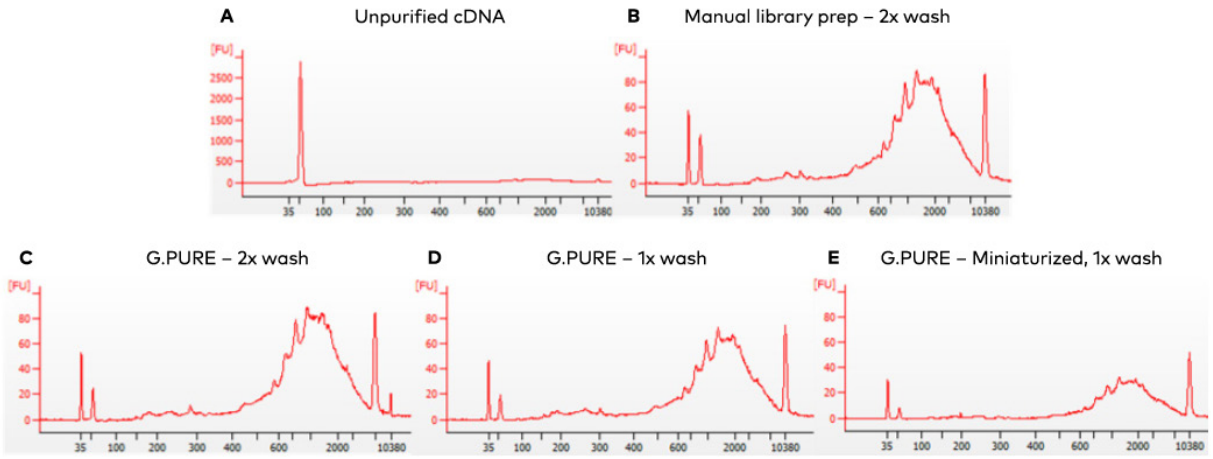

Figure 2. *The G.PURE provides equivalent or better performance as compared to manual bead cleanup methods with faster processing time. Prior to bead cleanup, the bioanalyzer trace of the unpurified cDNA has a dominant peak with smaller fragments (A). The same sample underwent DNA bead cleanup by manual prep, which resulted in a trace with the expected size distribution and most of the smaller fragments removed (B). The sample processed by the G.PURE with one wash step (C) and two wash steps (D) also resulted in ideal traces with minimal small fragments present. The G.PURE also produced similar results in a 1/5th miniaturized sample as with standard volumes (E).*

Conclusion

- The G.PURE reduces both the time and cost required to perform bead-based cleanup, when compared to conventional manual methods.
- Since the residual volume of the G.PURE is <0.1 µL/well, only one wash cycle is necessary to obtain a purified sample and is comparable to two manual wash steps, further reducing processing time.
- The G.PURE is compatible with miniaturized workflows with starting volumes of at least 5 µL in 96 well plates, performing similarly in the case of full reaction volumes.

References

1. Takara Bio. SMART-seq v4 Ultra Low Input RNA Kit for Sequencing User Manual. 2019; takarabio.com.

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