Comparative analysis of gene expression patterns in RNA sequencing using INTEGRA's MAGFLO NGS and AMPure XP magnetic beads

Abstract

This is a comprehensive analysis of the interchangeability of AMPure XP and INTEGRA's MAGFLO NGS beads in nucleic acid clean-up, specifically in the context of RNA sequencing (RNA-Seq) of human reference RNA samples. Samples were prepared using an Illumina TruSeq RNA library preparation kit, and sequenced on an Illumina NovaSeq platform.
The magnetic bead clean-up steps were conducted in triplicate for both AMPure XP and MAGFLO NGS beads. Analysis demonstrates no significant difference in gene expression profiles between samples processed with AMPure XP and INTEGRA's MAGFLO NGS beads, underscoring their efficacy and interchangeable use in nucleic acid purification workflows.

Introduction

RNA-Seq is an indispensable tool in modern biology, using next generation sequencing (NGS) to analyze the complexity of gene expression dynamics and molecular pathways across diverse experimental conditions. The selection of appropriate bead-based purification/size selection methods is critical for obtaining high quality RNA-Seq data, as it directly impacts downstream analysis. Therefore, we conducted a comparative analysis to evaluate the performance of AMPure XP beads and MAGFLO NGS beads in terms of their effect on gene expression profiling.

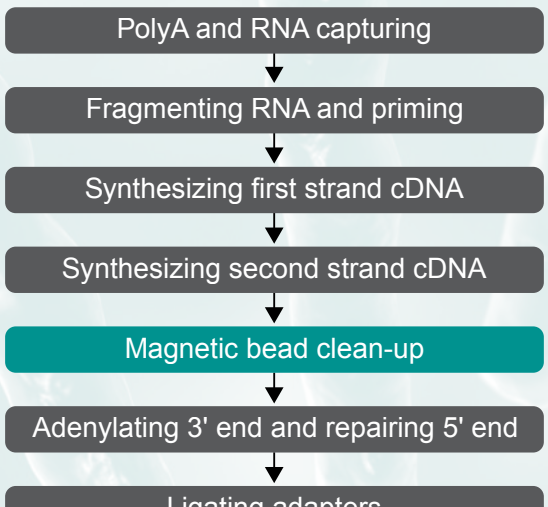

Figure 1: Illumina TruSeq RNA library preparation workflow.

Methods

RNA samples were processed using both AMPure XP beads and MAGFLO NGS beads throughout the Illumina TruSeq RNA library preparation workflow (**Figure 1**), followed by sequencing on Illumina NovaSeq (2 x 100 bp). Subsequently, sequencing quality assessment was performed using the FastQC tool. Differential gene expression analysis was visualized using Volcano plot (**Figure 4**), which depicts significance versus fold-change values, allowing for the identification of statistically significant changes in gene expression between the 2 conditions. Furthermore, heatmaps representing the top 30 up- and down-regulated genes provided insights into the overall expression patterns by the different bead-based purification methods (**Figure 5**). Experimental procedures and subsequent bioinformatics analyses were performed by Microsynth.

Results

Bead purification

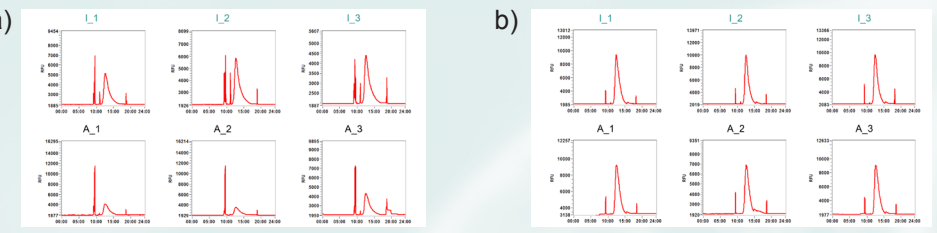

Figure 2: Fragment analyzer electropherograms showing the bead-based size selection results at the 'Enriching ligated fragment' step in the RNA-Seq workflow (a) before clean-up and (b) after clean-up. For both bead types, clean-ups were efficient in removing the small fragment peak. (I: MAGFLO NGS beads; A: AMPure XP beads)

Read mapping

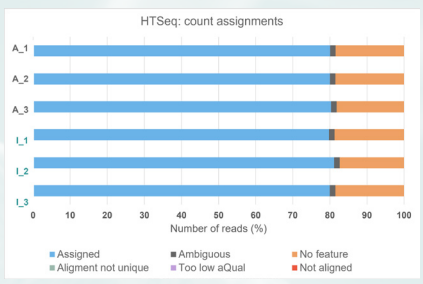

Figure 3: Around 80 % of counts could be mapped to genomic features whether samples were purified with AMPure XP (A) or INTEGRA's MAGFLO NGS (I) beads.

Differential gene expression

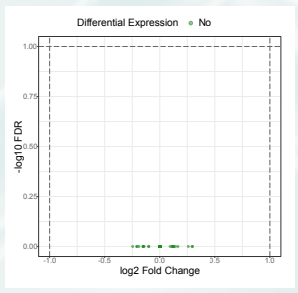

Figure 4: A volcano plot showing no significant differences in gene expression between samples processed using AMPure XP or MAGFLO NGS beads for bead clean-up steps. (Dark green: MAGFLO NGS beads; light green: AMPure XP beads)

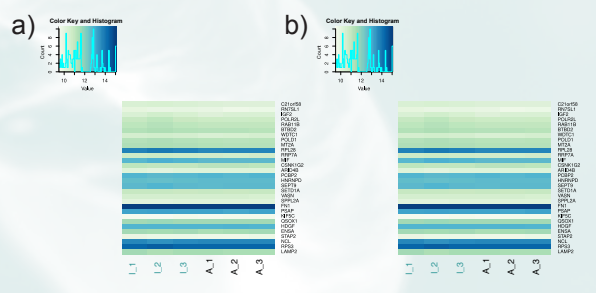

Figure 5: Heatmaps to visualize the differential expression of the top (a) 30 up- and (b) down-regulated genes. No significant differences were detected in the differential gene expression between the 2 conditions across the three replicates. (I: INTEGRA's MAGFLO NGS beads; A: AMPure XP beads)

Discussion

The results of this study provide valuable insights into the interchangeability of AMPure XP and INTEGRA's MAGFLO NGS beads in nucleic acid purification workflows for RNA-Seq sequencing. The similarity in gene expression profiles between samples processed with these 2 bead-based purification methods highlights their comparable efficacy and reliability in generating high quality RNA-Seq data. The electropherogram analyses confirm the adequacy of bead clean-up results for both AMPure XP and MAGFLO NGS beads, further supporting their effectiveness in removing unwanted components. Moreover, the comparable read mapping rates observed in samples purified with AMPure XP and MAGFLO NGS beads indicate similar efficiencies in capturing and sequencing RNA transcripts. The differential gene expression analysis, as depicted by Volcano plots and heatmaps, underscores the consistency of gene expression patterns between samples processed with the 2 different bead types.

Conclusion

The cost-effectiveness of MAGFLO NGS beads, relative to AMPure XP beads, offers researchers a practical alternative for nucleic acid purification without compromising data quality. Our findings provide valuable guidance for researchers seeking to maximize efficiency and affordability of RNA-Seq experiments.

References

Human reference RNA – Agilent (Cat No. 750500)
Illumina TruSeq RNA library – RS-122-2001
Reference human genome for bioinformatic analysis – UCSC hg38

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