Reliable and cost-effective library preparation with INTEGRA's MAGFLO NGS beads for Illumina MiSeq amplicon sequencing

Abstract

This study shows the comparable performance of INTEGRA's and AMPure XP magnetic beads when used in size selection for next generation sequencing (NGS) library preparation, followed by Illumina MiSeq amplicon sequencing. Amplicons of 16S rRNA and internal transcribed spacer (ITS) were obtained from a microbial community standard containing genomic DNA from 8 bacterial species (3 Gram-negative and 5 Gram-positive) and 2 yeasts. We demonstrated no significant difference between the performance of INTEGRA's and AMPure XP magnetic beads during size selection steps in library preparation, or in consecutive sequencing results, with no sequencing bias in alpha diversity analysis of microbial composition.

Introduction

Size selection with magnetic beads is a gold standard technique used in NGS library preparation. It is used for either single size selection in PCR clean-up, or double size selection of fragments within a desired size range for sequencing. Reproducible and efficient size selection ensures consistent sequencing results.

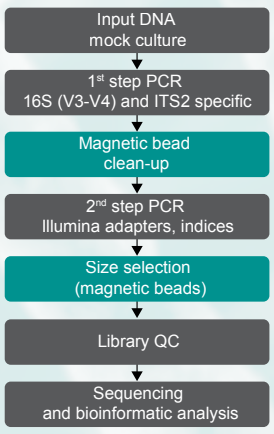

Figure 1: Amplicons for 16S and ITS2 were generated with the ZymoBIOMICS™ Microbial Community DNA Standard (D6305) with standard primers. Magnetic bead clean-up and size selection steps during the library preparation protocol were performed either with AMPure XP or MAGFLO NGS beads (indicated by color).

Species	Theoretical Composition (%)				
	Genomic DNA	16S Only[1]	16S & 18S[1]	Genome Copy[2]	Cell Number[3]
Pseudomonas aeruginosa	12	4.2	3.6	6.1	6.1
Escherichia coli	12	10.1	8.9	8.5	8.5
Salmonella enterica	12	10.4	9.1	8.7	8.8
Lactobacillus fermentum	12	18.4	16.1	21.6	21.9
Enterococcus faecalis	12	9.9	8.7	14.6	14.6
Staphylococcus aureus	12	15.5	13.6	15.2	15.3
Listeria monocytogenes	12	14.1	12.4	13.9	13.9
Bacillus subtilis	12	17.4	15.3	10.3	10.3
Saccharomyces cerevisiae	2	NA	9.3	0.57	0.29
Cryptococcus neoformans	2	NA	3.3	0.37	0.18

Table 1: The theoretical composition of 16S (or 16S & 18S) rRNA gene abundance was used as a reference, and was calculated from theoretical genomic DNA composition with the following formula: 16S/18S copy number = total genomic DNA (g) × unit conversion constant (bp/g) / genome size (bp) × 16S/18S copy number per genome. Use this as a reference when performing 16S targeted sequencing (Ref 3).

Methods

DNA libraries were prepared with 2 different magnetic beads: INTEGRA and AMPure XP (Beckman Coulter) were processed in parallel. Amplicon sequencing was performed on a MiSeq platform 2x250 bp v2 (Illumina) using Nextera protocol (Illumina), generating 60 k passed filter reads per sample on average. Fragment analysis was performed with Fragment Analyzer (Agilent). Experimental procedures and subsequent bioinformatics analyses were performed by Microsynth.

Results

Number of sequencing reads

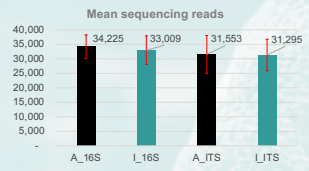

Figure 2: The mean number of sequencing reads obtained for both 16S (V3-V4) and ITS (ITS2) amplicons (n=3). (A: AMPure XP beads; I: INTEGRA's MAGFLO NGS beads)

Quality of sequencing reads

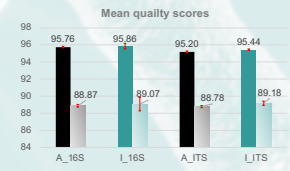

Figure 3: The mean sequencing quality scores for reads with Q20 (filled bars) was around 95 %, and with Q30 (shaded bars) around 88 %, for both amplicons (n=3). Q values for individual samples were calculated based on mean quality score values from forward and reverse reads. (A: AMPure XP beads; I: INTEGRA's MAGFLO NGS beads)

Sequencing libraries

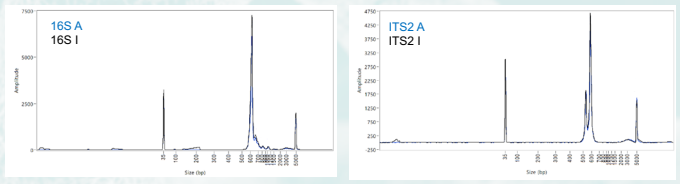

Figure 4: Overlapping DNA fragment profiles obtained prior to sequencing for 16S (left) and ITS2 amplicon (right, 2 visible PCR products accommodate for genetic variants captured with used primers). (A: AMPure XP beads; I: INTEGRA's MAGFLO NGS beads)

Theoretical vs detected microbial species

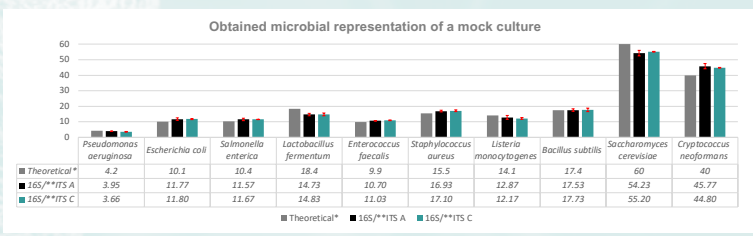

Figure 5: Species aggregation analysis of obtained sequences showed good correlation with theoretical microbial composition of a reference samples. * Theoretical composition is explained in Table 1; ** For ITS, the theoretical composition was based on the copy genome ratio of 0.57/0.37=1.5, therefore 60 and 40 % was used, as only 2 species out of 10 were the target for the ITS specific primers.

Alpha diversity of amplicon

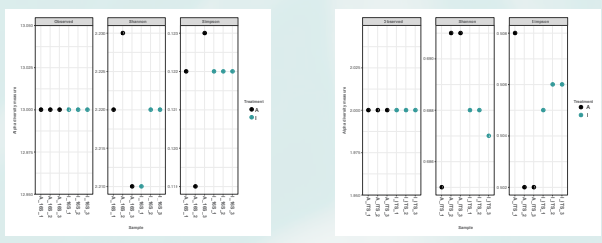

Figure 6: *Alpha* diversity scattered plot, with Shannon and Simpson analysis (16S on the left, ITS2 on the right). Observed *alpha* diversity of microbial species detected in samples was comparable between INTEGRA's MAGFLO NGS (I) and AMPure XP (A) beads, showing no statistical difference or bias in library preparation or sequencing.

Discussion

Regardless of the brand of magnetic beads used, sequencing reads with good quality scores ensured reproducible results across triplicates (**Figures 2 & 3**). We showed that bead clean-up efficiencies and final DNA fragment lengths were similar for both bead types (**Figure 4**). Detected microbial diversity correlated with the theoretical reference sample composition for all species (**Figure 5**), and *alpha* diversity analysis demonstrated that there was no sequencing bias (**Figure 6**).

Conclusion

The library quality control data and bioinformatics analysis demonstrated the interchangeability of INTEGRA's MAGFLO NGS and AMPure XP magnetic beads in a complex NGS amplicon sequencing workflow, particularly in the context of sequencing 16S rRNA and ITS2 phylogenetic markers, ensuring robust microbial composition analysis. The lower reagent price of MAGFLO NGS magnetic beads reduced the processing costs for library preparation, establishing it as the cost-effective alternative to AMPure XP beads without compromising quality.

References

16S primers, Klindworth *et al*. 2013
ITS primers, White *et al*. 1990
https://files.zymoresearch.com/protocols/_d6305_d6306_zymobiomics_microbial_community_dna_standard.pdf

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