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Use of magnetic beads in Next-Generation Sequencing in the construction of low cost 16S rRNA gene genomic libraries

Uso de esferas magnéticas Mag-Bind Mag-Bind® TotalPure em sequenciamento de nova geração na construção de bibliotecas de genômicas de 16S rRNA gene com baixo custo

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ABSTRACT: This work aimed to test the new Mag-Bind® TotalPure magnetic beads in the construction of a genomic library of corn and sugarcane endophytes. The Next-Generation Sequencing (NGS) technique is very expensive and therefore, one of the most expensive reagents is the magnetic beads that purify the amplified DNA and separate by size depending on their proportion, as shown in the Illumina protocol. We observed that the use of these beads, which are five times cheaper and have the same capacity and quality as others on the market, are adequate for effective metagenomic and microbiome studies. We concluded that the use of omega brand beads presents excellent results in the construction of NGS genomic libraries.

KEYWORDS: Bioinformatic, metagenomic technique, NGS, 16S amplicon PCR.

RESUMO: *Este trabalho teve como objetivo testar novas esferas magnéticas da Mag-Bind® TotalPure na construção de uma biblioteca genômica de endofíticos de milho e cana-de-açúcar. A técnica de sequenciamento de nova geração (NGS) é muito cara e, para isso, um dos reagentes mais caros são as esferas magnéticas que servem para purificar o DNA amplificado e se separam por tamanho dependendo de sua proporção, conforme mostra o protocolo da Illumina. Observamos que a utilização desses esferas magnéticas, cinco vezes mais baratos e com a mesma capacidade e qualidade dos existentes no mercado, é importante para um estudo satisfatório de metagenômica e microbioma. Concluímos que o uso de esferas magnéticas da marca Ômega tem um excelente resultado na construção de uma biblioteca genômica para NGS.*

PALAVRAS-CHAVE: *Bioinformática, técnicas metagenômicas, NGS, 16S amplicon PCR.*

Metagenomics is the technique used to analyze the DNA of isolates from environmental samples including cultivable and non-cultivable microorganisms. Since less than 95% of prokaryotes are cultivable, the use of the next-generation sequencing (NGS) technique has shown interesting results for microorganisms (SCHOFIELD; SHERMAN, 2013; PALAZZOTTO; WEBER, 2018). Numerous studies have been carried out with metagenomics, with thousands of citations of works that apply the technique. However, it remains expensive and requires expensive reagents, which makes it difficult for the scientific community to use this technique of great importance for the microbiology and genetics of microorganisms, especially

for second and third world research groups. Seeking to reduce expenses with reagents and lower costs generally, this note aimed to evaluate a product that was described as similar to the most commonly used in the literature but with a cost five times lower. Cellular lysis of plant tissue (roots and culm – sugarcane and maize) was performed by maceration with an autoclaved mortar and liquid nitrogen. Total DNA was extracted separately from each sample using the Qiagen DNeasy kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. The resulting DNA samples were checked for integrity by electrophoresis on a 1% agarose gel and stored at -20°C. The total extracted DNA was in a three-band conformation, showing the different levels of DNA condensation.

The product used in this study was Mag-Bind® TotalPure NGS (SKU: M1378-00) (MANI et al., 2019), a magnetic bead that, according to the manufacturer's description, is comparable in quality to that used by its competitor. We found this product on the internet, and following communication with the technical sector of the company in the USA, they sent us the technical description. We found that it was viable as a substitute for cleaning (clean-up) and separation of the desired amplicon sizes. In this work, a 16S rDNA genomic library of endophytic bacteria from roots and culm of maize (*Zea Mays* L.) and sugarcane (*Saccharum officinarum* L.) were prepared. The methodology used was the protocol described in Illumina, Inc. (2013). The primers used in this work were the following: 16S Amplicon PCR Forward Primer: 5'CGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTAC GGGNGGCWGCAG 16S Amplicon Reverse Primer: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGA CTACHVGGGTATCTAATCC to amplify the V3 and V4 regions showing amplicon sizes from 450 to 630 bp. Omega BIO-TEK brand beads were replaced in the following steps adopting the same proportions as in the first clean-up step at a ratio of 0.8X the DNA volume. And in the second clean-up resuspending in 52.5 and 27.5 µL at the end of the process. Quantification was performed in a Qubit® 3.0 Fluorometer (Life Technologies). Three samples were randomly chosen from the sugarcane library for the test. They were drawn and the samples tested were: 24 (whose indexes used were N703/S511 and belonging to the treatment of sugarcane intercropped with peanuts with chemical nitrogen and stem bacteria applied); sample 29 (indexes N704/S507 and belonging to the treatment of sugarcane intercropped with beans without application of chemical nitrogen and stem bacteria); sample 32 (indexes N704/S511 and belonging to the treatment of sugarcane intercropped with beans and root bacteria). Raw sequences were processed using Mothur software v.1.44.3 (SCHLOSS et al., 2009). Forward and reverse sequences were combined in contigs and submitted to a quality filter, where sequences outside the range of 440-465 nucleotides, containing ambiguities or with more than 8 homopolymers were removed. They were then aligned with the SILVA 138.1 database (QUAST et al., 2012), after a virtual PCR with primers 341F-805R. After alignment, non-informative columns and sequences not aligned with the region were removed. The pre.cluster command was used, grouping sequences up to 2 nucleotides apart, and chimera.vsearch was used to remove chimeric sequences (ROGNES et al., 2016)

The number of sequences per sample, after the quality control steps, was obtained for comparison. The first interesting result was that the amount of DNA between the samples was very similar compared with the samples used with the competitor's beads. In Figure 1 we show the samples in agarose gel visualized under ultraviolet light. In this figure we observe the presence of dimers in the four samples, however, the amount of DNA in the samples using Omega Beads showed a greater amount of

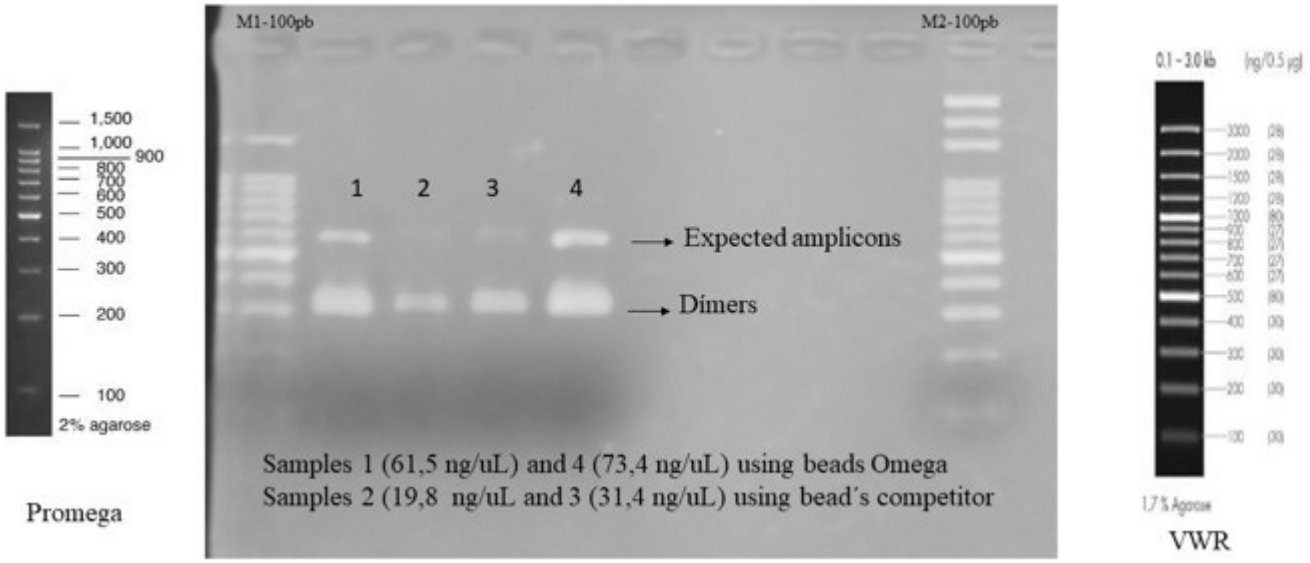

Figure 1. 1.5% agarose gel stained with ethidium bromide. Legend: samples 1 and 4 using Mag-Bind® TotalPure beads and samples 2 and 4 concurrent beads. 100 bp Molecular Markers from Promega and VWR.

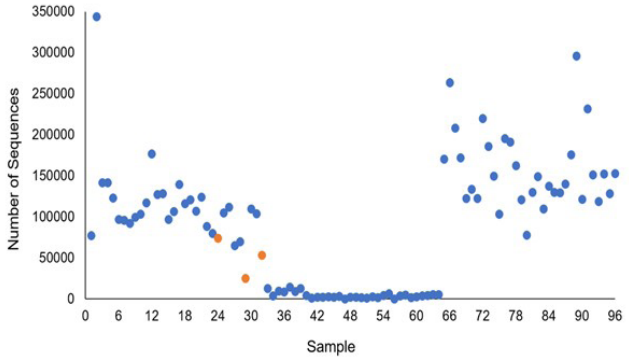

Figure 2. Number of sequences per sample after quality filter steps in the Mothur software. Samples in which Mag-Bind® TotalPure NGS Beads were used are colored orange.

DNA in ng/µL. Due to the presence of dimers, we performed a third cleaning using only 0.6X the proportion of beads to DNA volume as supported by Illumina. The dimers disappeared and the amount of DNA in the samples was as follows: sample 24: 29 ng/µL, 29: 25.1 ng/µL, and 32: 30.8 ng/µL. This showed that there was not as much loss after the third cleaning with a smaller portion of 0.8X and eliminating the beads. These concentrations are sufficient to perform a sequencing run in MiSeq.

After sequencing, we send the results to be analyzed by researchers in bioinformatics at LABEM. Figure 2 shows that the orange circles are the samples tested in this study and we observe that, in relation to the number of sequences, they obtained good coverage compared to the other samples treated with competitor's beads. It is noteworthy that these sequences were submitted to different types of filters that are part of the bioinformatics analysis, such as expected size, ambiguities, homopolymers and alignment with the 16S rDNA region. Therefore, only high quality sequences are presented in this step, that are able to further bioinformatic analysis. We conclude that Omega BIO-TEK beads present excellent performance for cleaning DNA when DNA libraries of endophytic bacteria were assembled.

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