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Novel kit for efficient extraction of long-segment genomic DNA by nanodisk adsorption

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Abstract

The acquisition of complete DNA is usually the first and most critical step in many basic molecular biology applications. Isolation of complete total DNA from tissue samples is related to different physical and biochemical properties of tissue. Long fragments of DNA are easily degraded and fragmented, which poses a challenge for extracting complete long fragments of DNA. By comparing several different extraction methods and verifying the concentration and length of extracted DNA, it is proved that the kit we used has high efficacy. We provide tissue kit DNA extraction for long fragments and obtain long fragments of high purity DNA from almost any type of tissue, especially muscle and blood tissues.

Keywords: genomic DNA, long fragments, kit.

1. Introduction

The acquisition of complete DNA is usually the first and most critical step in many basic molecular biology applications. Isolation of complete total DNA from tissue samples is related to different physical and biochemical properties of tissue. Long fragments of DNA are easily degraded and fragmented, which poses a challenge for extracting complete long fragments of DNA.

2. Result and Discussion

The total genomic DNA was extracted using a modified phenol-chloroform-isoamyl alcohol (PCI) protocol [1]. Sari Stark reported that the V1–V3 region of the 16S rRNA gene PCR products were cleaned using the Agencourt AMPure XP magnetic beads purification system (Beckman Coulter) and quantified using the Qubit dsDNA HS Assay Kit (Invitrogen). After quality filtering, 152,348 reads were obtained from all of the samples, with an average of 4,009 reads per sample (min = 1892; max = 9885) [2]. It can be observed that nucleic acid extraction for short fragments is more efficient in the extraction of long fragments by using AMPure XP Magnetic Beads combined with nucleic acids,

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which is then separated from impurities, ethanol washing and transfer elution products. The problem is mainly due to the fact that the magnetic beads will break the DNA fragment.

Recent updates on Magnetic Nano-Particles (MNPs) based separation of nucleic acids have received more attention because of their easy manipulation, simplicity, ease of automation and cost-effectiveness. It has been indicated that DNA molecules absorb on solid surfaces via hydrogen-bonding [3]. We obtained pH 8 and 1.5 M NaCl as an optimal condition for desorption of DNA from MNPs [4]. The result further showed that, 0.2 mg nano-particle and 10 min at 55 °C are the optimal conditions for DNA desorption from nano-particles [4].

Cigene long fragment DNA extraction nano kit is based on a four-step process. The first step in the purification procedure lyses the cells and the nuclei. For isolation of DNA from white blood cells, this step involves lysis of the red blood cells in the Cell Lysis Solution, which is followed by lysis of the white blood cells and their nuclei in the Nuclei Lysis Solution. An RNase digestion step may be included at this time, and it is optional for some applications. Most notably, after this step, the long-segment DNA was adsorbed using the nanodisk in the cigene kit. The cellular proteins are then removed by a salt precipitation step, which precipitates the proteins but leaves the high molecular weight genomic DNA in solution. Finally, the genomic DNA is concentrated and desalted by isopropanol precipitation. DNA purified with this system is suitable for a variety of applications. Our products provide high quality genomic DNA for many basic downstream molecular biology experiments with excellent repeatability.

As research on bacteria progresses, we are learning more about the important role of bacteria in human health. Research on the microbiome is changing the way we look at disease and providing new treatment ideas for disease [5]. Not all bacterial cells are identical, and Gram-positive samples may be particularly difficult to dissolve. The actual cartridge that we need to develop is also tested by the extraction test of Gram-positive bacteria, which is currently well solved. Therefore the scope of the kit is relatively wide.

DNA extraction is from agarose and polyacrylamide gels, which are for desalting and concentrating DNA from solutions. The agarose is remelted using the reagent for breaking the cross-linking, which is washed with ethanol, and the recovered DNA fragment is obtained by adsorption on the nanodisk.

Table 1. DNA sequencing results extracted using Cigene long fragment DNA extraction nano kit

Total number of sequences	Total length of sequences	Shortest sequence length	Longest sequence length	N50	combined length
983,117	4,303,384,992 bp	37 bp	147,940 bp	7,535	2,151,694,272 bp

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