VALIDATION OF THE BONE TISSUE EXTRACTION PROCESS WITH THE USE OF THE COMMERCIAL BONE DNA EXTRACTION KIT, CUSTOM

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Bone is a tissue composed mainly of collagen, a protein that provides a soft structure, and minerals that add strength and harden the structure. About 70% of bone consists of the inorganic mineral hydroxyapatite, which includes calcium phosphate, calcium carbonate, calcium fluoride, calcium hydroxide, and citrate. Areas of extensive mineralization within bone represent physical barriers to extraction reagents and therefore prevent the release of DNA molecules. In addition to the composition of bone samples, time, environmental conditions, soil, and other chemical factors degrade DNA, which makes the challenge of obtaining an interpretable genetic profile greater. In this study, the Promega Bone DNA Extraction Kit Custom was validated with different types of bone samples, as well as with various extraction conditions.

Three different bone samples were selected, each one was pulverized 32 times (each sample had a previous cleaning with soap, 2% chlorine and ethanol), with the help of a Mill. Amounts of 100, 200, 400 and 800mg of bone powder were placed in a microtube. Each quantity was digested with volumes of 400 and 800ul of digestion buffer (Bone DNA Extraction Kit Custom) and digested for different times (2.5, 5, 8 and 24 hours), resulting in 32 different processes for each sample. They were purified by DNAIQ System (Promega Co.), eluting at 20ul. The genomics were quantified by PowerQuant System, and according to the quantification, amplification was decided by PowerPlex Fusion 6c or PowerPlex Y23, the samples were typed in a 3500 equipment. Once the best extraction conditions were observed, 10 different bone remains were processed, each with three variables, to observe the reproducibility of the process. These samples were only quantified to verify the success of the extraction and were not amplified.

It was observed that, according to the amount of bone powder, double the volume of digestion buffer should be placed, in addition that the most successful results are obtained at a longer digestion time. Although we could also observe that, although it is the same bone rest, each extraction will have a different yield, in the same way, the type of sample influences the amount of DNA obtained. It was also observed that an important step is the prior cleaning and / or decontamination of the bone tissue. Therefore, this new technique can be used as an alternative to other processes that currently exist.