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Short Communication

Protocol for extraction of genomic DNA from swine solid tissues

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Abstract

Molecular diagnostics are performed by using DNA from different body tissues. However, it is necessary to obtain genomic DNA of good quality. Due to the impossibility of collecting blood from slaughtered animals, DNA extraction from solid tissues is necessary. The objective of this study was to describe a protocol of DNA extraction from swine skin, adipose, brain, liver, kidney and muscle tissues. We obtained high molecular weight DNA of good quality, shown by agarose gel and amplification of two DNA fragments, 605bp and 891pb, by PCR. Spectrophotometric analysis of DNA concentration showed variation among the DNA from different tissues, with the liver and adipose tissues presenting the greatest and the smallest concentration, respectively. The described protocol has proven to be advantageous due to its simplicity, quickness, affordable reagents and absence of phenol, resulting in a high molecular weight DNA of good quality from several tissues.

Key words: DNA extraction, swine tissues.

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Introduction

Professionals that control swine meat and carcass quality work basically with slaughtered animals, and it is not possible to collect blood for DNA extraction when genetic exams are needed. For this reason, DNA must be extracted from solid tissues.

Many genomic DNA extraction protocols have been described for prokaryotes and eukaryotes, from cell sample to specific tissues (Sambrook *et al*., 1989). However, there are not many studies using DNA from swine solid tissues.

Like reagents, good quality DNA is essential to achieve good results in experiments (Hoy, 1994), especially in the Polymerase Chain Reaction (PCR), in which excess of cell debris and proteins may inhibit the amplification process (Saiki, 1990).

Due to the importance of having good quality DNA extracted from solid tissues, this paper describes a fast and simple protocol for genomic DNA extraction from swine skin, adipose, brain, liver, kidney and muscle tissues.

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Material and Methods

Three samples from skin, adipose, brain, liver, kidney and muscle tissues were stored at -20 °C after slaughter, from which DNA was extracted.

A sample consisted of a 200 mg biopsy. Specimens were ground separately with mortar and pestle in liquid nitrogen. The frozen powder was transferred to a 2 mL eppendorf tube and 800 µL of extraction solution (50 mM Tris-HCL, pH8.0; 25 mM EDTA and 400 mM NaCl), 100 µL 10% SDS, and 20 µL Proteinase K (10 µg/µL) were added. The extract was homogenized and incubated at 65 °C for 3 h. After incubation, proteins and cellular debris were precipitated by adding a 300 µL 6 M NaCl, kept at 4 °C for 15 min. Centrifugation was done at 25,000 g for 20 min. 500 µL of the supernatant were transferred to a new eppendorf, with 500 µL 8 M guanidine hydrochloride (pH 8.0), and 0.49 M ammonium acetate solution, and kept in mild agitation for 90 min. Nucleic acids were precipitated by adding 800 µL of cold 100% isopropyl alcohol, followed by centrifugation at 8,000 g for 5 min. Pellets were washed with 400 µL of 70% isopropyl alcohol. After drying, pellets were resuspended in 150 µL TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA and 50 µg/µL RNAse). DNA samples were stored at 4 °C.

DNA quality and concentrations were evaluated by spectrophotometer and by ethidium bromide-stained

agarose gel under Ultraviolet light. Photographs were documented through a Videodocumantetion system, the VDS ImageMaster (Pharmacia Biotech).

Fragments chosen to probe DNA integrity are in different regions of the porcine Growth Hormone gene. A 605pb fragment was amplified under the following conditions: 100 ng of genomic DNA, 1U Taq DNA Polymerase, 0.5 µM Primer, 400 µM dNTP, Taq buffer 1X and 3 mM $MgCl_2$. Another fragment, 891bp, was amplified in the following conditions: 100 ng genomic DNA, 1U Taq DNA Polymerase, 0.2 µM Primer, 400µM dNTP, Taq buffer 1X and 1.5 mM $MgCl_2$. Both in a 20 µL final volume.

Results and Discussion

The protocol was efficient in extracting genomic DNA from all solid tissues. Analysis of whole genomic DNA in agarose gel (Figure 1) and amplified fragments (605 and 891bp) by PCR (Figures 2a and b) demonstrated that the extracted DNA had high molecular weight, one of the most important aspects for successful amplifications of larger fragments (Borges, 1997).

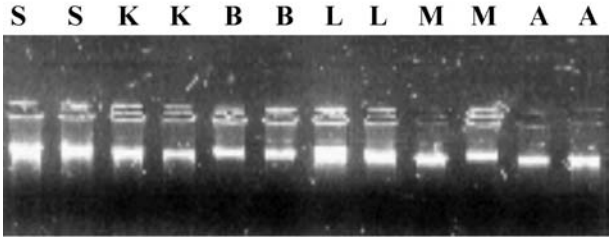

Figure 1 - Genomic DNA sample submitted to electrophoresis in 0.8% agarose gel. (S) Skin, (K) Kidney, (B) Brain, (L) Liver, (M) Muscle and (A) Adipose tissues.

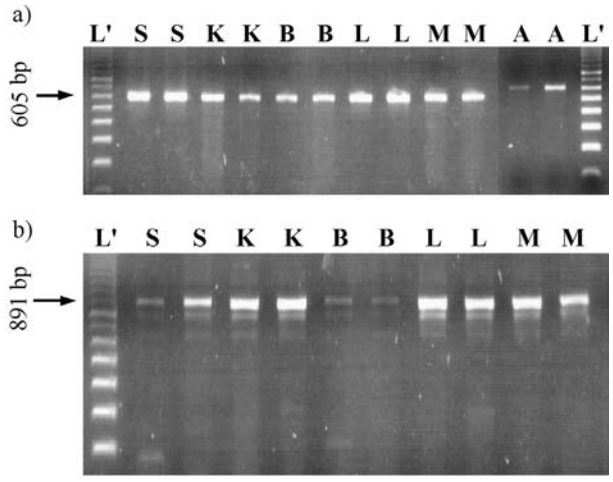

Figure 2 - a: 605bp fragment amplified by PCR visualized in 1.5% agarose gel under UV light. (L') 100bp DNA Ladder, (S) Skin, (K) Kidney, (B) Brain, (L) Liver, (M) Muscle and (A) Adipose tissues. b: 891bp fragment amplified by PCR visualized in 1.5% agarose gel under UV light. (L') 100pb DNA Ladder, (S) Skin, (K) Kidney, (B) Brain, (L) Liver, (M) Muscle and (A) Adipose tissues.

Table I - DNA concentration and OD ratio (A260/A280) of DNA extracted from different tissues.

Tissues	DNA concentration ($\bar{x}$) ηg/µL	OD260/OD280 ($\bar{x}$)
Liver	1950	2.05
Kidney	840	2.23
Brain	486.6	1.93
Muscle	483.3	1.80
Skin	160	2.24
Adipose	33.3	*

*Spectrophotometric readings did not detect proteins.

Spectrophotometer measurements indicated differences in DNA concentration and purity, according to the tissue origin (Table I). Higher concentrations were obtained from the liver, kidney and brain tissues, similar to those obtained by Atmadja *et al*. (1995) for human tissues. The OD260/OD280 ratio values satisfied those suggested by Sambrook *et al*. (1989), ≥ 1.8, which were higher than those obtained by Akane *et al*. (1993) and Atmadja *et al*. (1995).

The variability in DNA quality and purity can be explained by tissue specific structural complexity. Liver, kidney and brain tissues are composed of delicate membrane cells with few fibrous cells. On the other hand, the skin consists of stratified tissue with keratin and other fibrous cells. The muscle tissue is constituted by many proteins within the cell. Adipose tissue consists mainly of lipids, which increase cell volume and decrease cell number. In addition, lipid is water insoluble, which hinders extraction, lysing a smaller number of nuclei (Junqueira and Carneiro, 1995).

This protocol proved to be advantageous because of its simplicity, quickness and affordable reagents, besides the high molecular weight DNA and purity achieved in a variety of tissues. Furthermore, there is no phenol in DNA purification, known as a strong PCR inhibitor (Saiki, 1990).

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