# Efficiency of DNA extraction from different carp tissue using the sodium chloride (NaCl) protocol

*Eficiência na extração de DNA de diferentes tecidos de carpas através do protocolo de cloreto de sódio (NaCl)*

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**ABSTRACT**

With advances of farming and research in the area of molecular genetics, studies that can help with obtaining good quality DNA are necessary. The objective was to evaluate the efficiency of DNA extraction in different types of tissues of three species of carp by using the protocol of DNA extraction with Sodium Chloride (NaCl). The samples were subjected to DNA extraction, and the integrity was visualized on 1.5% agarose gel. In a total of 72 samples used for DNA extraction, all of them were positive, confirmed by the presence of bands on agarose gel. The capacity of amplifying the extracted DNA was tested by amplification reactions using the RAPD (random amplification of polymorphic DNA) technique, confirming that the DNA was of good quality for use in subsequent studies with molecular markers.

**KEYWORDS:** carp, DNA extraction, RAPD.

**RESUMO**

Com o avanço da piscicultura e das pesquisas na área da genética molecular, existe a necessidade de estudos que possam aperfeiçoar a obtenção de DNA com boa qualidade. Diante disso, o objetivo desse trabalho foi avaliar a eficiência da extração de DNA em diferentes tipos de tecidos de três espécies de carpas, através do protocolo de extração de DNA com cloreto de sódio (NaCl). As amostras foram submetidas à extração de DNA e a integridade foi visualizada em gel de agarose 1,5%. Em um total de 72 amostras utilizadas na extração de DNA, todas foram positivas, confirmadas com a presença de banda no gel de agarose. A capacidade de amplificação do DNA extraído foi testada através de reações de amplificação utilizando a técnica RAPD (polimorfismos de DNA amplificados ao acaso), confirmando que o DNA apresenta boas condições para utilização em estudos posteriores com marcadores moleculares.

**PALAVRAS-CHAVE:** carpa, extração de DNA, RAPD.

## INTRODUCTION

Today, global aquaculture production is growing at a rapid pace. A significant proportion of aquaculture production corresponds to freshwater fish and one of the groups that produce more fish throughout the world is carp (FAO 2012). Different species of carp have been domesticated for centuries in Asia and their cultivation has expanded throughout the world in the early twentieth century. They are resilient fish and they adapt well to captivity (EL-ZAEEM et al. 2011). The farming technology for these species has evolved considerably, but there is a great demand for studies in this area yet (XU et al. 2011).

Due to the great commercial importance of freshwater fish, it is essential to develop researches on molecular biology. ODEGARD et al. (2011) report that the tools for genomic analysis are important elements when searching for genetic improvement of aquaculture species. To this sense, DNA extraction and the use of molecular markers is becoming increasingly common, and studies aimed at finding easier, cheaper, and faster methods for obtaining high-quality DNA are considered of great scientific importance (PARPINELLI & RIBEIRO 2009).

To obtain high-quality uncut DNA, it is necessary to make an efficient choice of samples and employ the most appropriate protocol for a particular procedure. Some studies report the use of different body parts for DNA extraction, such as blood, scales, eggs, buccal cells, muscles (LOPERA-BARRERO et al. 2008) fins, and gills (PARPINELLI & RIBEIRO 2009). Different choices of biological material for DNA extraction allow the use of what is currently available and, in some cases, prevent the sacrifice of animals with high zoo technical value.

There are several protocols that allow DNA extraction from different animal species and different cell types (CAWTHORN et al. 2011). Some methods

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are more cumbersome and some are easier. For DNA amplification using PCR (Polymerase Chain Reaction), for instance, the extraction of DNA using the Sodium Chloride (NaCl) protocol is a simple, easy, fast, and non-contaminant alternative which allows obtaining of high-quality DNA in sufficient quantities through samples of fish (LOPERA-BARRERO et al. 2008, BLANCK et al. 2009).

After DNA extraction, it is possible to perform the analysis of molecular markers, such as RAPD (Random Amplification of Polymorphic DNA), which consists of DNA amplification using short sequences of primers. This method is simple and very good especially for analysing and detecting polymorphisms and genetic variation, as well as for evaluating the genetic structure of populations (CORTINHAS et al. 2010). JAMSHIDI & KALBASSI (2011) reported that this type of molecular marker can be widely used in various studies related to the genomes of fishes.

Given the importance of obtaining DNA for genetic studies of fish through a fast and practical procedure, this study aimed to evaluate the efficiency of DNA extraction in different types of tissues of three species of carp, through the protocol of DNA extraction using NaCl, for further amplification test by RAPD makers.

## MATERIAL AND METHODS

### Fish origin

The collection of the biological material used for the extraction of DNA was carried out from fish reared in the Department of Fish Farming, at the Centre of Agroveterinary Sciences of the Santa Catarina State University – CAV/UDESC, in the city of Lages, SC. Then, this material was taken to the Genetic Analysis Laboratory of UDESC for later genomic material extraction and RAPD marker analysis.

### Biological material samples

The samples were collected from three freshwater fish species: Bighead carp (*Anstichtys nobilis*), Grass carp (*Ctenopharyngodon idella*), and Common carp (*Cyprinus carpio*). Three animals of each species were used for the analysis, totalling nine animals. The fishes were represented by the following nomenclature to facilitate data control: BC I, BC II, and BC III (Bighead carp), GC I, GC II, and GC III (Grass carp), and CC I, CC II, and CC III (Common carp). The samples were collected from the eight body parts of each animal: gill (G), heart (H), liver (L), spleen (SP), swim bladder (SB), dorsal muscle (DM), scales (SC), and caudal fin (CF), totalling 72 samples for the DNA extractions. The fish were weighed and identified to create a database of the material collected. Samples of 600 mg of tissue were placed into micro tubes with 1.5 mL of absolute ethanol and stored at a temperature of –20 °C, and after 24 hours were subjected to DNA extraction.

### Sodium chloride (NaCl) protocol

The absolute ethanol used in the preservation was removed and the samples used for DNA extraction were weighed (approximately 250-350 mg each) and placed into micro-tubes where 550 µL buffer were added (50 mM of TrisHCl, pH 8.0, 50 mM of EDTA and 100 mM of NaCl), plus 5.5 µL of 10% CTAB (cetyltrimethylammonium bromide) and 7 µL of proteinase K (200 µg $mL^{-1}$), and were then incubated for 12 hours in a water bath at 50 ºC. Subsequently, 600 µL of NaCl 5M were added to the samples and the phases were mixed by inversion and centrifuged for 10 minutes at 125 g. The supernatant was transferred to new micro tubes and then 700 µL of cold absolute ethanol were added, homogenizing samples with swirling until precipitation of the DNA occurred. Then, the samples were incubated in a –20 °C freezer for two hours. Once removed from the freezer, they were centrifuged for 10 minutes at 125 g and the ethanol was discarded carefully not to lose the "pellet". An aliquot of 600 µL of 70% ethanol was added and centrifuged for more 10 minutes at 125 g and then the ethanol were discarded again. After these procedures, the samples remained on the bench to dry at room temperature for about five minutes. After this interval, 45 µL of TE buffer were added to each tube (10 mM of Tris, pH 8.0 and 1 mM of EDTA) and treated with 0.6 µL of RNAse (2 mg $mL^{-1}$). The DNA samples were kept in a water bath at 37 ºC for 40 minutes, and then stored at –20 ºC till they were checked on agarose gel and submitted for amplification tests. The time needed for carrying out this protocol is 24 hours.

The integrity of the genomic DNA extracted was analyzed on 1.5 % agarose gel and visualized with an ultraviolet transilluminator. Aliquots of 5 µL of DNA, 4 µL of bromophenol blue (0.25% of bromophenol blue, 40% of sucrose and ultrapure water), and 1.5 µL of Gel Red (1 µL of gel red and 250 µL of ultrapure water) were used. Electrophoresis was performed in a

horizontal unit at 80 V for 60 minutes, using TBE 1X buffer (10.8 g of Tris HCl 1M pH 7.4, 5.5 g of boric acid 1M, 4 ml of EDTA 0.5 M pH 8.0 and $H_2O$). The image was captured using Vilber Lourmat (Figure 1).

**RAPD amplification**

The integrity, quality, and quantity of the DNA extracted from the samples were tested by amplification reactions using the RAPD technique. The amplification reaction had a final volume of 12 µL, with 1.9 µL of water, 4 µL of dNTPs (1.25 mM), 2 µL of 10X buffer, 2 µL of primer (10 µM), 1 µL of $MgCl_2$ (50mM), 0.1 µL of Taq DNA Polymerase (5U $\mu L^{-1}$) and 1 µL of DNA (1:10 dilution). The sequences of primers used in the reactions are shown in Table 1.

The amplification was performed in four steps in a thermal cycler: an initial step at a temperature of 94 °C for 2 minutes; the second step of 3 cycles: denaturing at 94 ºC for 1 minute, annealing at 35 °C for 1 minute and elongation at 72 ºC for 2 minutes; the third step of 40 cycles at three different temperatures: denaturing at 94 ºC for 1 minute, annealing at 40 °C for 1 minute, and elongation at 72 ºC for 2 minutes; and a final step at 72 ºC for 5 minutes.

The amplification patterns were visualized on 1.5% agarose gel with an ultraviolet transilluminator. Aliquots of 10 µL of PCR product, 8 µL of bromophenol blue (BSB), and 1.5 µL of Gel Red were used. Electrophoresis was performed in a horizontal unit at 80 V for 60 minutes, using TBE 1X buffer. Images were captured using Vilber Lourmat (Figure 2a and 2b).

**RESULTS AND DISCUSSION**

The use of the protocol for DNA extraction with NaCl proved to be effective for obtaining DNA from different body parts of the three analyzed species (Table 2). From a total of 72 samples used for DNA extraction, all of them were positive. There are no studies using the different parts for DNA extraction in carps with NaCl protocol, so this study validates the use of the methodology of extraction for these species and enables its use in further studies. The Figure 1 shows the electrophoresis of some of the positive samples.

Table 1 - Sequence corresponding to primers used in RAPD amplification reaction.

| Primer | Sequence (5' – 3') |
|---|---|
| Tube C-08 | TGGACCGGTG |
| Tube E-04 | GTGACATGCC |
| Tube G-03 | GAGCCCTCCA |
| Tube H-18 | GAATCGGCCA |
| Tube O-13 | GTCAGAGTCC |
| Tube Z-04 | AGGCTGTGCT |
| Tube AA-03 | TTAGCGCCCC |

The body weight of fish used for the extractions is presented in Table 2. Differences between the weights did not influence the efficiency of DNA extraction. According to BUCKINGHAM et al. (2003), development of the vertebrate cell is a process that involves various events, and the numbers of

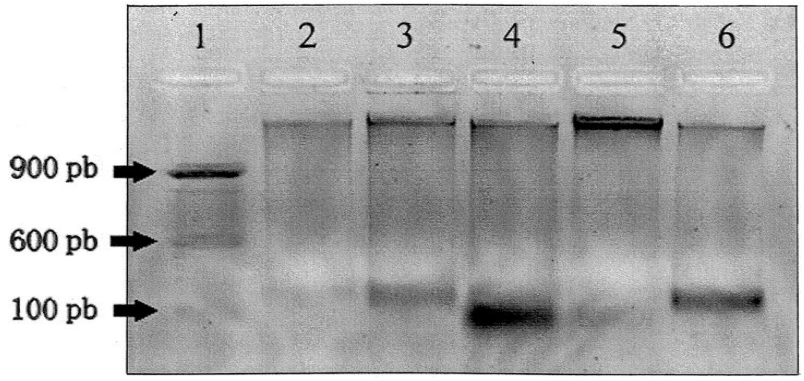


Figure 1 - 1 % agarose gel containing samples of the DNA extraction using the NaCl protocol. Lane 1 corresponds to the molecular marker consisting of a 100 bp ladder (base pairs), lane 2 is the extraction from the dorsal muscle of common carp, lanes 3, 4 and 5 correspond to extractions from the heart of common carp, grass carp and bighead carp and lane 6 is the DNA extracted from the spleen of common carp.

Table 2 - Result of DNA extraction from different tissues of the four analyzed species.

| Species | Body weight (g) | Extractions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | G | H | L | SP | SB | DM | SC | CF |
| BC I | 80 | + | + | + | + | + | + | + | + |
| BC II | 60 | + | + | + | + | + | + | + | + |
| BC III | 70 | + | + | + | + | + | + | + | + |
| GC I | 250 | + | + | + | + | + | + | + | + |
| GC II | 200 | + | + | + | + | + | + | + | + |
| GC III | 75 | + | + | + | + | + | + | + | + |
| CC I | 125 | + | + | + | + | + | + | + | + |
| CC II | 55 | + | + | + | + | + | + | + | + |
| CC III | 215 | + | + | + | + | + | + | + | + |

BC I, BC II and BC III = Bighead carp; GC I, GC II and GC III = Grass carp; CC I, CC II and CC III = Common carp; G = gill; H = heart; L = liver; SP = spleen; SB = swim bladder; DM = dorsal muscle; SC = scales; CF = caudal fin; + = presence of genomic DNA.

muscle fibers are determined during embryogenesis and thus muscle growth on embryo occurs by the process of hyperplasia, or by cell proliferation. After that, birth growth is controlled hypertrophy, which is a consequence of increased cell volume. Thus, the amount of cells present in the samples is near standardization, it is possible to perform extractions in fish of all weights and ages.

The extraction method with NaCl provides a convenient and fast procedure (PARPINELLI & RIBEIRO 2009). Samples being positive in DNA extraction correspond to published reports found in the literature in which the methodology was tested in some species of fish. LOPERA-BARRERO et al. (2008) used caudal fin tissue from five different species for the extraction of DNA, reaching positive result not only in the extractions, but also in the amount of DNA obtained. BLANCK et al. (2009) successfully performed extractions from caudal fin samples of Nile tilapia using the NaCl protocol.

It is essential to enable the extraction of different kinds of tissues, as it is not always possible to obtain a sample from a specific organ. The positive results obtained for scales and fins are of great importance as they allow the analysis of DNA without sacrificing the animals, which usually have a considerable economic value as they are breeding fish with high genetic potential.

PCR amplification parameters are very important for extracted DNA (CAWTHORN et al. 2011). Thus, RAPD amplification was performed using a set of 7 primers (Table 1). With amplification it was possible to show that the DNA was in good condition for use in further studies with molecular markers. Amplification reactions were carried out with DNA samples from the extractions, and the electrophoresis results are shown in Figure 2.

Some studies of fish genome were conducted using RAPD markers. LOPERA-BARRERO et al. (2008) performed successful DNA extractions with NaCl and RAPD amplifications working with *Brycon orbignyanus, Piaractus mesopotamicus, Oreochromis niloticus, Leporinus elongates,* and *Prochilodus lineatus*, finding good amplification conditions, which is in accordance with the present study.

The identification of genetic markers RAPD makes it possible to determine associations with genetic improvement, to monitor genetic changes along the selection processes and to identify strains. LI et al. (2010) conducted a study evaluating these parameters, including the use of these markers to enhance the understanding of its performance in Nile tilapia.

Another point to consider is using it to assess the genetic characterization of populations. CORTINHAS et al. (2010) characterized populations of pejerrey, *Atherinella brasiliensis*, from three different geographical regions, using the analysis of markers RAPDs. With this information, it is clear that this type of marker is widely used in genomic studies of fish.

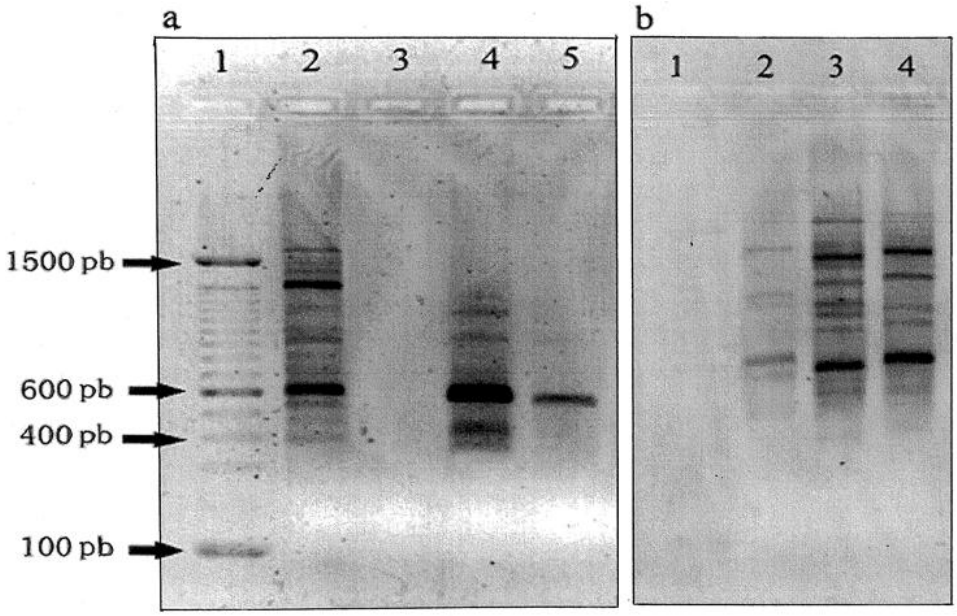


Figure 2 - RAPD amplified fragments visualized on 1.5 % agarose gel. a) lane 1 corresponds to the molecular marker consisting of a 100 bp ladder, lane 2 is the amplification of DNA extracted from the spleen of grass carp, lane 3 is the negative control, lane 4 dorsal muscle of a bighead carp and 5 dorsal muscle of another bighead carp. b) lane 1 corresponds to the molecular marker consisting of a 100 bp ladder, lanes 2, 3 and 4 are amplifications of DNA extracted from heart, caudal fin and dorsal muscle of grass carp.

## CONCLUSION

Obtaining nucleic acids from different carp tissues is effective through the protocol of DNA extraction with NaCl, presenting good potential amplification by RAPD.

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