

# Protocolo de Preservação e Extração de DNA a partir de fezes de Bugios-ruivos (*Alouatta clamitans*)

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## Objetivos

Estabelecer um protocolo de coleta de amostras não invasivas (fezes) de bugios-ruivos (*Alouatta clamitans*) *in situ* e posterior amplificação de DNA para fins de estudo populacional.

## Métodos e procedimentos

Protocolo 1: Oklander et al. (2007), com o uso do kit de extração ReliaPrep Blood gDNA Miniprep System da PROMEGA e amplificação de DNA por PCR multiplex.

Protocolo 2: SOUZA et al. (2017), com uso do kit de extração QIAGEN Stool Mini Kit e amplificação de DNA por PCR simplex.

Protocolo 3: NAIR et al. (2003), com amostras de pesos diferentes (0,5 g e 1,2 g).

## Resultados

Protocolo 1: foram obtidas amostras de 10 indivíduos, porém, à PCR, não foram obtidas bandas satisfatórias (bem definidas).

Protocolo 2: não foram obtidos resultados satisfatórios (sem presença de bandas).

Protocolo 3: não foram obtidos resultados satisfatórios (ausência de colonócitos).

## Conclusões

Dentre as formas de armazenamento, o uso de NaCl e etanol apresentaram-se mais eficientes. Para a amplificação de DNA, o método multiplex não é recomendado. Novas tentativas devem ser feitas, a fim de

estabelecer um protocolo específico para o bugio-ruivo (*Alouatta clamitans*)

## Referências bibliográficas

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## ABSTRACT

Primates are considered as umbrella or flagship species, drawing people's attention to issues related to biodiversity conservation and, indirectly, protecting other cohabiting species. Howler monkeys (*Alouatta* spp.) are primates that resist human presence, and are often the only large mammals in certain fragments. Since they are important seed dispersers, they play an important role in the maintenance of ecosystem services and the establishment of ecological interactions in the anthropized environments they inhabit. However, *A. clamitans* recently suffered a severe population decline due to yellow fever outbreak. Genetic studies, particularly those concerning microsatellites, help to understand population structure, reproductive strategies, migration patterns and social organization, important aspects for the conservation of species. Genetic studies are frequently based on classical biological samples, such as blood and tissue, but obtaining such samples from wild animals may be unfeasible. Thus, the employment of biological surrogates such as faeces and urine have been preferred because they are easily accessible. However, faecal DNA extraction presents particularities, since it contains cell diet items, natural bacteria and secondary metabolites, requiring specific adaptations of traditional protocols. *A. clamitans* has not yet been studied at intrapopulation level and an important step is to establish a protocol for samples collection and storage, as well as to test protocols for extracting and amplifying microsatellites from non-invasive samples, which is the aim of the present project. In order to do so, we will apply protocols that are established for other species of howler monkeys, prioritizing those of non-invasive samples, such as faeces. Fresh samples from known individuals will be collected and stored in cooking salt (NaCl). A national commercial kit for DNA extraction will then be applied to extract DNA. After this step, the possibility of amplification of the microsatellites used in other species will be tested. The success of this standardized protocol is of paramount importance for the establishment of a field-friendly routine that could be applied to several studies with wild primate populations.