

# [Genetic profile of the bvl lutra lutra population study](https://assignbuster.com/genetic-profile-of-the-bvl-lutra-lutra-population-study/)

An attempt to optimize the PCR amplification of nDNA (microsatellites) extracted from otter faeces to develop a genetic profile of the BVL Lutra lutra population

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Introduction

The determination of wild species distribution and abundance is extremely necessary for conservation biology studies since some species, such as carnivores, are elusive and secretive and mostly difficult to observe in the wild (Riddle, Pilgrim, Mills, Mckelvey, & Leonard, 2003).

Non-invasive genetic sampling is a promising technique that doesn’t interfere directly in the management of animal populations, helping researchers to study their ecology and distribution, avoiding capture complications and reducing additional costs and time of observation period (Lampa, Gruber, Henle, & Hoehn, 2008). Non-invasive samples like faeces, shed hair, urine, saliva, sloughed skin or feathers give researchers a wide variety of population genetic information once it evidences the presence of rare species, allows to estimate food habits, identify species, individuals, gender and paternity and still lead to further investigation on behavioral ecology, genetic diversity and phylogenetic relationships. Forensic applications also seem to be an important feature in this matter (L. Waits & Paetkau, 2005) shared with human forensic genetics (Jobling & Gill., 2004). The development of molecular analysis for wild species identification has been remarkably studied based on amplification and DNA extracted from the faeces (Farrell, Roman, & Sunquist, 2000; Höss, Kohn, Pääbo, Knauer, & Schröder, 1992).

To study a population’s structure and size, faeces were proven to be the most useful non-invasive samples collected when combined with microsatellite analysis. However, good results arise with high success rates of PCR amplification. Those haven’t yet been achieved for most carnivorous species, particularly in the Eurasian otter (Lampa et al., 2008), due to contamination concerns and high microsatellite genotyping errors (L. Waits & Paetkau, 2005). Genotyping success in otter faeces has reached only 20 to 40% due to low quality and quantity of DNA extracted (J. F. Dallas et al., 2003).

Microsatellites assays out of otter faeces must be optimized in all its fundamental steps, beginning in the field where the samples are collected, passing through sample storage conditions and extraction procedure, until DNA amplification by PCR (Polymerase Chain Reaction). All the previous steps are crucial for optimization procedure, so they must be correctly executed with substantial caution. Optimization success of microsatellites analysis in otter faeces may be the key to study genetic material of otter populations (Lampa et al., 2008).

The Eurasian Lutra lutra (Linnaeus, 1758) is a semi-aquatic carnivore of the family Mustelidae widespread along in Europe, existing in fresh and unpolluted water habitats as rivers, estuaries, streams, dams, lagoons and marshes usually surrounded by bankside vegetation (Foster-Turley et al., 1990; Ruiz-Olmo et al., 2008; Trindade, Farinha, Florêncio, & Sousa, 1995). This elusive and mostly nocturnal mammal feeds mainly on fish as well on aquatic insects, reptiles, amphibians, birds, small mammals, and crustaceans, whatever there is abundant prey (Gorgadze, 2013; Ruiz-Olmo et al., 2008). Frequently otters mark their territory by dropping faces, urine and anal secretions on the top of rocks and visible spots along the riverbank (Foster-Turley et al., 1990).

Although otter population is facing a regression in most Europe territory due to habitat destruction, pollution and human activity, in Portugal otters population seem to be stabilized (Trindade et al., 1995). In fact, Portugal’s classification for Lutra lutra species is data insufficient by the Red Book of Vertebrates (Cabral et al., 2005). On the contrary, IUCN Red List classification for the Eurasian otter is “ Near Threatened”(Ruiz-Olmo et al., 2008). Therefore, collecting data on its abundance and population structure is required for the development of a successful management and conservation plans (Hung, Li, & Lee, 2004). The landscape of Baixo Vouga Lagunar is an extensive area of flat landscape integrated in a vast lagoon ecosystem, Ria de Aveiro, one of the most notable Wetlands of the Portuguese coast. It is characterized by countryside territory where farming activity as rice fields and pastures are abundant, and by the transition between terrestrial and aquatic environments such as saltmarshes, reed and freshwater habitats as rivers and ditches. Such ecological environment loads an important affluence in biodiversity, gathering an extraordinary set of fauna and flora populations. As a result, the Baixo Vouga Lagunar is currently classified as a Special Protected Area of the Ria de Aveiro, under the Directive – Birds. Escaping from the populated urban territory important communities of birds and mammals have found in the BVL a safe refuge, as the otter. Eurasian otter trails and scats are frequently found in the routes along the riverside, where the samples had been collected.

The faecal samples used in this study were all fresh and collected in November when the weather was cooler and dry, providing ideal weather conditions for sample collection once DNA degrades over time and higher temperatures (Piggott, 2004). Otters anal jelly overcome faecal samples in DNA concentration and quality, concentrating less bacteria, enzymes, nucleases and PCR inhibitors (Hajkova et al., 2006; Lampa et al., 2008). Merely adult individuals produce the anal scent glands secretion making it quite rare to find and less interesting to estimate the population size, once not all individuals secrete anal jelly. Although these secretions reveal great genetic data on otters there isn’t much information about the chemical properties and function of these scent marks (Hajkova et al., 2006; Kean, Müller, & Chadwick, 2011).

Genetic analysis on this matter have been improving since the past few decades developing new forensic strategies like genetic markers to study world populations, including humans. Microsatellites, SNPs, mtDNA and other techniques were designed following the advances in scientific and analytical methods, user-friendly software and laboratory equipment automation (DeYoung & Honeycutt, 2005; L. P. Waits & Paetkau, 2005). Scientists recognize the feasibility of Mitochondrial DNA (mtDNA) in molecular methodology for species identification once species have distinctive mtDNA with higher success rates. Also mtDNA genetic samples have higher number of copies than nDNA and are relatively easy to extract from non-invasive tissues and faeces. For species identification mtDNA analysis is fairly recommended while microsatellites from nDNA are most required for individual identification, sex determination, parentage and other genetic studies on rare and elusive species (Allendorf & Luikart, 2007; L. Waits & Paetkau, 2005). Eurasian otters are practically monomorphic because the hypervariable control region of mtDNA has shown very low levels of sequence polymorphisms and therefore population genetic structure analysis represents no viability in this species. On the other hand, microsatellites are reasonably polymorphic in most populations of Eurasian otters (J. F. Dallas et al., 1999; J. Dallas & Piertney, 1998).

Microsatellite loci are short tandem repeats of DNA sequences that arise throughout the genome of most eukaryotes. Microsatellites can be classified as mono-, di-, tri- and tetra-nucleotide but penta- and hexa-necleotides are still considered. Longer repeats form minisatellites and satellite DNA. Microsatellite polymorphism result from variation in the length of the repeated sequences mainly caused by addition or deletion of the repeated sequences and analyzed via electrophoresis of PCR-amplified fragments(DeYoung & Honeycutt, 2005; Ellegren, 2004). Furthermore, microsatellites evaluate individuals heterozygosity, the presence of different allelesat one or more loci on homologous chromosomes, and also detect the presence of multiple alleles. As a result, microsatellites become the more effective markers to study intraspecific variation and polymorphisms in wild population’s genetic structure and relatedness as well as population size (Chambers & MacAvoy, 2000; Goldstein et al., 1999).

To optimize otter DNA amplification success from faecal samples it’s necessary to test different reagents concentrations and PCR parameters in order to demystify the best combination for Lutra lutra specie, benefit from laboratory costs reduced and increase genetic analysis efficiency for microsatellites studies. The PCR or polymerase chain reaction is a process based on three major steps: the denaturation of the double-stranded DNA molecule, the attachment of primers at a particular locus at the genome, and the extension of the primers to produce a copy of the original locus. PCR amplification success also depends on the concentration of reagents and isolated DNA quality of samples. The quality of the samples varies between same species depending on the study area, diet and time of the year as well as the microsatellite loci and amplification protocol used (Hajkova et al., 2006; Lampa et al., 2008). When optimizing PCR those steps must be considered altogether so one modification may affect the whole process.

High concentrations of DNA reduces the specificity of the amplicon, especially when the thermocycler is programmed for a large number of cycles. Primer concentration is crucial for PCR optimization so higher concentrations may lead to secondary priming and create spurious amplification results and small concentrations may not amplify at all. Magnesium concentration must also be carefully measured since higher concentrations create unwanted products and lower concentrations will not amplify. As for deoxynucleotides the higher the concentration the better de yield of amplification but smaller quantities decrease fidelity. Taq DNA Polymerase is the most important figure in PCR amplification being the responsible for the process to work.

The aim of this paper is to make an approach to optimization of extraction and PCR amplification of nuclear DNA (microsatellites) by using collected samples of Eurasian otter, Lutra lutra , faeces in order to increase the efficiency and the reliability of the considered results. Then, we’ll apply molecular analysis procedures to study the genetic diversity of a population of Eurasian otters in the region of Baixo Vouga Lagunar, Aveiro, Portugal, as an application of the methods mentioned above.

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