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Optimization of PCR conditions to amplify mitochondrial COI gene fragments of wildlife species in Kenya

Edinah Song'oro[1]*, Ann Muigai[1], Charles Kimwele[2], Erastus Gatebe[1], Mercy Mwaniki[1], Julius Kinuthia[1] and Zipporah Osiemo[1]

1 Jomo Kenyatta University of Agriculture and Technology, Faculty of Science
2 University of Nairobi, Department of Veterinary Anatomy and Physiology.

E-mail : edinahsong@gmail.com

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Abstract

PCR has been extensively used for amplification of DNA sequences. We conducted a study to obtain the best amplification conditions for cytochrome c oxidase I (COI) gene fragments of some of the targeted wildlife species in Kenya; buffalo,common zebra, grant's gazelle,warthog and common eland and domestic samples purchased from the market as 'beef', 'goat'or 'mutton'. DNA from five wildlife species and one hundred of domestic samples were extracted for PCR amplification. Various trials and combinations were tested to determine the best conditions of PCR mixtures and annealing temperatures to obtain the best PCR products for sequencing purposes. Four selected target factors for enhancing PCR, annealing temperature, concentration of primer pair, amount of *D*ream Taq™ PCR Master Mix (2x) (Fermentas) and PCR cycle duration, were optimized by keeping the amount of DNA template (2µL) and concentration of PCR buffer, MgCl2 4mM and dNTP mixture constant (Fermentas). All genes were successfully amplified, giving the correct fragment lengths of 700 base pair (bp), as assigned for both forward and reverse primer. The optimal conditions were determined to be: 0.5µl (5pmoles) for each primer, 25µl of DreamTaq ™ PCR Master Mix (2x), 30 s of both denaturation and annealing cycles and annealing temperature of 56.5°C. PCR products obtained under these conditions produced excellent bands.