



*Methodology*

## Optimization of PCR conditions to amplify Cyt *b*, COI and 12S rRNA gene fragments of Malayan gaur (*Bos gaurus hubbacki*) mtDNA

M.K.A. Rosli<sup>1</sup>, A.S. Zamzuriada<sup>1</sup>, S.M.F. Syed-Shabthar<sup>1</sup>, M.C. Mahani<sup>1</sup>, O. Abas-Mazni<sup>2</sup> and B.M. Md-Zain<sup>1</sup>

<sup>1</sup>School of Environmental and Natural Resource Sciences,  
Faculty of Science and Technology, Universiti Kebangsaan Malaysia,  
Bangi, Selangor, Malaysia

<sup>2</sup>Agro-Biotechnology Institute, Malaysia, Serdang, Selangor, Malaysia

Corresponding author: B.M. Md-Zain

E-mail: abgadd@ukm.my / abgadd1966@yahoo.com

Genet. Mol. Res. 10 (4): 2554-2568 (2011)

Received September 9, 2010

Accepted May 9, 2011

Published October 19, 2011

DOI <http://dx.doi.org/10.4238/2011.October.19.2>

**ABSTRACT.** PCR has been extensively used for amplification of DNA sequences. We conducted a study to obtain the best amplification conditions for cytochrome *b* (Cyt *b*), cytochrome c oxidase I (COI) and 12S rRNA (12S) gene fragments of Malayan gaur mtDNA. DNA from seven Malayan gaur samples were extracted for PCR amplification. Various trials and combinations were tested to determine the best conditions of PCR mixture and profile to obtain the best PCR products for sequencing purposes. Four selected target factors for enhancing PCR, annealing temperature, concentration of primer pairs, amount of *Taq* polymerase, and PCR cycle duration, were optimized by keeping the amount of DNA template (50 ng/μL) and concentration of PCR buffer (1X), MgCl<sub>2</sub> (2.5 mM) and dNTP mixture (200 μM each) constant. All genes were successfully amplified, giving the correct fragment lengths, as assigned for both forward and reverse primers.

The optimal conditions were determined to be: 0.1  $\mu$ M primers for Cyt *b* and COI, 0.3  $\mu$ M primers for 12S, 1 U *Taq* polymerase for all genes, 30 s of both denaturation and annealing cycles for Cyt *b*, 1 min of both stages for 12S and COI and annealing temperature of 58.4°C for Cyt *b*, 56.1°C for 12S and 51.3°C for COI. PCR products obtained under these conditions produced excellent DNA sequences.

**Key words:** PCR; Malayan gaur; *Bos gaurus*; Cyt *b*; 12S rRNA; COI