

Amplification refractory mutation system (arms) for sex identification of Asian elephants (*elephas maximus*) using dung as non-invasive sample

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There is a demand by field biologists to establish molecular techniques to identify the sex of elephants using non-invasive samples. The present study applied a molecular method, Amplification Refractory Mutation System (ARMS), for sex identification of Asian elephants (*Elephas maximus*) using their dung samples. This technique focused on allele specific identification, targeting the zinc finger (*zf*) region in X and Y-chromosomes. Based on the unique nucleotide present in the *zfx* and *zfy* gene sequences in the intron *zf* region of Asian elephants, two sets of primers were designed manually. DNA was extracted from dung samples collected from 12 males and 16 females and PCR method was performed to amplify DNA using designed primers. To confirm the results, one step RT-PCR (Applied Biosystems) was performed using SYBR Green dye (QuantiTech-Qiagen).

The PCR method yielded 118bp and 130bp products for X and Y genes respectively. For male DNA samples both 118bp and a 130bp bands were observed while it was only 118bp band for female DNA samples. The Melt curve analysis (MCA) of one step RT-PCR method support the above results by resulting two separate peaks plotted at 74°C T_m for male samples and at 77.6°C T_m for female samples. The result concludes that the designed primers based on the Zinc finger protein encoding region of X and Y-chromosomes are highly specific for sex identification of Asian elephants using their dung as non- invasive sample.

Keywords: Asian elephant, Dung, Sex identification

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