

Comparison of DNA Isolation and Dominant and Co-dominant Molecular Markers to Reveal the Genetic Sex of *Gallus domesticus* (Domestic Chickens)

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The whole blood of chickens contains nucleated erythrocytes and thrombocytes which yield high amount of DNA thus cause many troubles during DNA extractions. Optimization of DNA extraction from avian blood is important to yield high quality DNA and is vital for the success of all downstream applications. Determination of genetic sex of chickens is an important aspect in the avian research as well as in layer industry. Among the various methods of sex determination, Polymerase Chain Reaction (PCR) based methods are considered as most accurate and inexpensive. PCR methods have been developed based on the amplification of sex chromosome linked dominant and co-dominant loci in the chicken genome to distinguish between sexes. Success of this PCR based genetic sexing depends on the optimization of the PCR conditions and the reliability and reproducibility of molecular markers. Therefore this study was aimed at investigating the optimum conditions of DNA isolation from chicken blood and to compare the reproducibility of one co-dominant and two dominant sex markers to be validated as a tool for sex determination in avian research. Six different extraction procedures including manual and solution based commercial purification kit were evaluated. Efficacy of procedures was assessed with different combinations of initial blood, lysis buffer, and protein denaturant in related to the DNA yield and purity. Three primer sets namely CHD1, HUR 0423 and HUR 0424 were evaluated for the genetic sexing of chicken by polymerase chain reaction. The study results showed that an initial volume of 10 µL blood yields a significantly high DNA with high purity. Dominant marker HUR0424 showed to be a reliable marker system for the genetic sexing of domestic chickens over co-dominant markers.

Keywords: Dominant markers, Co-dominant markers, *Gallus domesticus*, Genetic sexing, Protocol optimization

Acknowledgements: Financial assistance by the Institute for Research and Development, Colombo, Sri Lanka.