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Sex differentiation in diversified poultry species by PCR based molecular method from blood

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Accurate identification of sex is one of the key issues in poultry breeding, conservation, evolutionary studies and in improvement of *ex situ* captive breeding programs (Morinha *et al*. 2012). Sex differentiation at day-old stage is difficult and traditional methods of poultry sexing are time-consuming, expensive and in some cases, invasive and harmful. DNA based methods are more reliable and are based on either DNA hybridization or polymerase chain reaction (PCR) assay, but PCR is more accurate than hybridization (Dubiec and Zagalska –Neubaeur 2006). Females are heterogametic (ZW) and males are homogametic (ZZ) in poultry. The W-chromosome is small, filled with large repetitive arrays, mostly heterochromatic and shares many features with the mammalian Y-chromosome (Ellegren 1996). Sex can be identified by the detection of W-chromosome specific sequences. The *Xho* I and *Eco* RI family of repetitive DNA sequences on the W-chromosome are useful targets (Nakabayashi *et al.* 1998) and are clearly identifiable after a relatively small number of reaction cycles in PCR (Clinton 1994) but are reported in genus *Gallus* only (Mizuno and Macgregor 1998). A unique sequence EE0.6 (0.6 kb *Eco RI* fragment) found on the long arm of the chicken W-chromosome, is widely conserved (Ogawa *et al.* 1997); not only in non-ratite species but also in ratite species (Ogawa *et al.* 1998) and chromo-helicase-DNA (CHD) binding protein gene, also found on the chicken W-chromosome have been used for sex identification in avian species with varying degrees of success (Rahim *et al.* 2012). Purified DNA is often considered as the pre-requisite for PCR. Reports on relative efficiencies of using these DNA extraction methods in PCR are scanty. In order to reduce time, labour, cost and to avoid the use of hazardous chemicals, this investigation was carried out to develop a PCR based technique for sex differentiation in some diversified poultry species directly from blood by simple PCR and to evaluate its relative efficiency with the conventional PCR using isolated DNA as template.

Genomic DNA was isolated from 100 µl of venous blood of 3 males and 3 females from each of the 5 non-ratite species, viz. guinea fowl, duck, turkey, quail, and chicken by phenol-chloroform extraction method (Kagami *et al.* 1990). The quantity and purity of good samples were assessed and subjected to PCR analysis. Same blood samples were used to prepare blood lysate of all diversified poultry species as per Tomasulo *et al.* (2002). Lysates were used in PCR as source of template. USP1/ USP3 primer pair targeting 0.6 kb EcoR1 fragment on W-chromosome (Ogawa *et al*. 1997) was synthesized from standard manufacturer. The PCR was carried out in a reaction mixture of 25 µl containing 5 µl of 5X PCR buffer, 1.5 mM $MgCl_2$, 200 mM of dNTPs, (dATP, dGTP, dTTP and dCTP), 400 ng of each primer, 1U Taq DNA polymerase and 50 ng genomic DNA from phenol–chloroform extraction method or 5 µl diluted blood lysate. Amplification was carried out in PTC 200 DNA engine using amplification programme consisting of initial denaturation at 94°C for 4 min, followed by 35 cycles of 95°C for 80 sec, 56°C for 90 sec and 72°C for 60 sec, and final extension at 72°C for 5 min. The PCR products were analyzed along with 100bp DNA ladder as molecular size marker on 1.2% agarose gel. Molecular sizes of amplicon were estimated with the help of Inchworm software, a free internet-based software.

Simple PCR using isolated genomic DNA as template or direct blood lysate resolved only one amplicon of 370 bp in females of all the above five species and none in males. A comparative and comprehensive figure showing PCR amplification of representative samples of one male and one female from five species by both the protocols is presented in Fig.1. In all the species, the PCR resulted in similar sizes of amplicons by both the protocols i.e. using purified DNA as template or direct blood lysate as source of DNA. Observation of similar sized PCR amplified products in five non-ratite avian species with both the protocols evidenced the success of this method. Similar intensities observed in both the methods further proved

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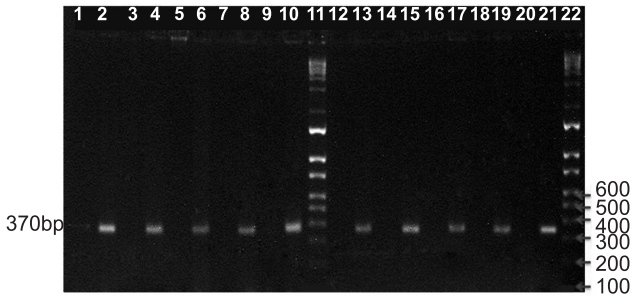

Fig.1. PCR amplification of sex-specific EE0.6 related sequence (USP1/3 primers) in diversified poultry species from direct blood lysate (lanes from 1 to 10) and genomic DNA (lanes from 12 to 21). Lanes (From left to right): 1. Guinea Fowl Male, 2. Guinea Fowl Female, 3. Duck Male, 4. Duck Female, 5. Turkey Male, 6. Turkey Female, 7. Quail Male, 8. Quail Female, 9. Chicken Male, 10. Chicken Female, 11 & 22. 100 bp DNA ladder, 12. Guinea Fowl Male, 13. Guinea Fowl Female, 14. Duck Male, 15. Duck Female, 16. Turkey Male 17. Turkey Female, 18. Quail Male, 19. Quail Female, 20. Chicken Male and 21. Chicken Female.

similar amplification efficiencies as has also been reported in chicken (Khatib and Gruenbaum 1996) and mice (Hofstetter *et al.*1997).

Present investigation evidenced that PCR-based method, directly from blood, can be successfully and accurately employed for sex differentiation in five diversified non-ratite species of poultry. It has eliminated DNA extraction step and provided a rapid and cheaper molecular sexing technique and could be vividly used in diversified poultry production systems.

SUMMARY

Sexing in diversified avian species is difficult and traditional methods have inherent limitations. PCR-based methods need isolated genomic DNA template. It was envisaged to develop PCR-based method for sex differentiation in five diversified poultry species viz., guinea fowl, duck, turkey, quail, and chicken from blood lysate. Amplification of 0.6 kb EcoR1 fragment of W-chromosome employing USP1/ USP3 primers pair in genomic DNA and blood lysate revealed only one female-specific amplicon of 370bp, with similar amplification efficiencies. Investigation provided a rapid and accurate PCR method directly from blood for sex differentiation in five non-ratite poultry species for vivid use in diversified poultry production systems.

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