A RAPID PCR BASED METHOD USING CHELEX-100 FOR SEXING DOMESTIC CHICKEN

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Identification of the sex of chickens has important applications in transgenic technology, embryological studies as well as in agriculture industry. Though adult chickens show striking sexual dimorphism, young chicks do not show sexual dissimilarity between females and males. Sex of chicks can be identified by using simple PCR amplification of a genetic marker present on avian sex chromosomes Z and W. CHD-1 gene, which is present on both sex chromosomes provides an excellent genetic marker for sex determination as the two alleles present on Z and W chromosomes are not identical. These two alleles contain a single intronic region that has varying lengths. Thus, amplification of this intronic region specifically, would discriminate between CHD-Z and CHD-W alleles as the amplification products can be separated on the basis of their size on a gel. Female birds are heterogametic with Z and W chromosomes and male birds are homogametic with two Z chromosomes. Therefore, males can be identified by the presence of a single band while females can be identified by the presence of two bands. The amplification of the intronic region on CHD-Z and CHD-W alleles can be carried out using P2 and P8 primer pair, which has been designed to flank this intron. Chelex-100 resin, which is currently used in forensic studies to purify DNA from blood stains, hair, buccal and epithelial cells was used to develop a rapid and inexpensive method to isolate good quality genomic DNA that can be used in PCR to rapidly sex, domestic chickens. PCR conditions were optimized to facilitate amplification of target sequence from genomic DNA isolated using Chlex-100 resin. This method was compared to genomic DNA isolated with SDS/Proteinase K digestion followed by NaAc/ethanol precipitation. The objectives of this study was to establish a fast, reliable and simple method for extraction of genomic DNA from a very small quantity of blood that can be used for rapid sex determination of domestic chickens.

Blood samples were collected from four domestic chickens (*Gallus gallus domesticus*) by a puncture in the wing vein and their gender was recorded by visual inspection. All chemicals used were of molecular biological grade unless otherwise specified, and were purchased from Sigma Aldrich chemical company (St.Louis. USA). Taq polymerase, PCR buffer and dNTPs were provided from UC Biotech (Sri Lanka). Two microliters of blood was suspended in 200 µl of 6 % (w/v) Chelex-100 by briefly vortexing and then incubated at 56°C for 30 minutes and boiled for 8 minutes. Resulting suspension was centrifuged at 12,000 rpm for 5 minutes. The supernatant was directly used for PCR or stored at 20 °C until further use. The PCR primers used were P8 (5'-CTC CCA AGG ATG AGR AAY TG-3') and P2 (5'- TCT GCA TCG CTA AAT CCT TT -3')[1] PCR amplification was carried out in 25 µl reaction mixtures containing 1 x reaction buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl), 1 x dNTPs mixture (UC biotech), 0.5 µM of each primer, 2 U Taq Polymerase (UC biotech) and 5 µl of Chelex DNA extract. Three different annealing temperatures (47 °C, 48 °C and 49 °C) were used to determine the optimum annealing temperature of the P2 and P8 primers for chicken gDNA that was isolated using SDS/Proteinase K digestion method and 48 °C was found to be the optimum. Four different $MgCl_2$ concentrations were used (1.5 mM, 3 mM, 4 mM and 5 mM) to determine the optimum Mg^{2+} ion concentration necessary for PCR reactions and 3 mM of $MgCl_2$ was found to be the optimum. Thermal profile for amplification was 96 °C (5 min), 40 cycles at 96 °C (45 sec), 48 °C (45 sec) and 72 °C (45 sec) plus a final extension at 72 °C (7 min). PCR products were separated on a 3% (W/V) agarose gels stained with ethidium bromide.

When the PCR products were separated on a 3% (W/V) Agarose gel, a single band was observed for males due to amplification of the CHD-Z allele while two bands were observed for females due to the presence of both CHD-Z and CHD-W alleles. Thus, P2 and P8 primers successfully amplified CHD-Z and CHD-W alleles producing distinctive and reproducible (tested thrice) banding pattern for males (single band) and females (two bands; the CHD-W allele longer than CHD-Z allele). Since Chelex-100 resin is a strong chelator of Mg^{2+} ions, any traces of Chelex 100 resin that remains in the supernatant may inhibit the PCR reaction. Therefore, it was necessary to determine the optimum Mg^{2+} ion concentration required for the PCR reaction when gDNA extracted using Chelex 100 resin was used in the PCR.

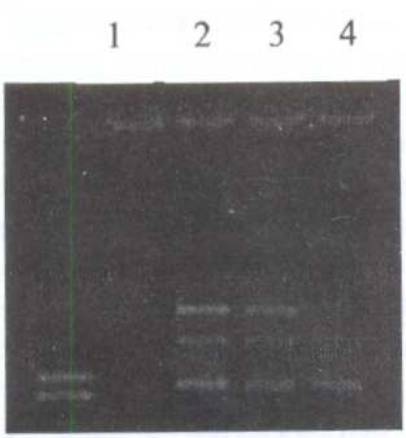

Figure 1: Optimization of the Mg^{2+} concentration used for PCR reaction when the template DNA is isolated using Chelex-100 resin. PCR products separated on a 0.8% (w/v) agaros gel. Lane 1- 300 & 400 bp DNA marker, Lane 2- 1.5 mM Mg^{2+}, Lane 3- 5 mM Mg^{2+}, Lane 4- 4 mM Mg^{2+}, Lane 5- 3 mM Mg^{2+}

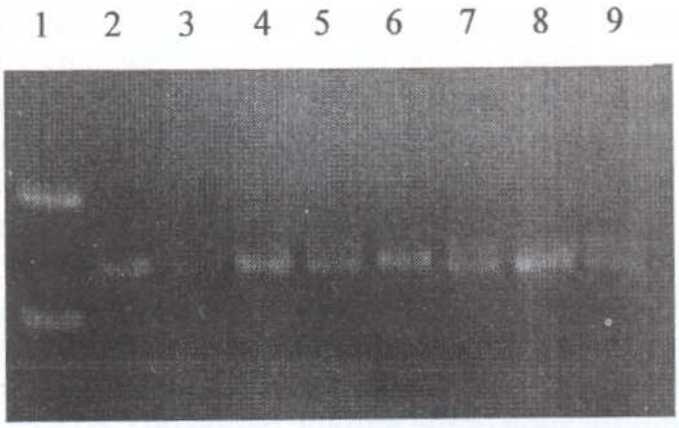

Figure 2: Gender determination of *Gallus gallus* (n= 4) by analysis of the fragment amplified with P2 and P8 primers. PCR products are separated on a 3% (w/v) agarose gel. Lane 1- 300 & 400 bp DNA marker, Lane 2-5 - Template DNA isolated using SDS-Proteinase K method, Lane 6-9 - Template DNA isolated using Chelex resin. Lane 2, 4, 6, 8- Male samples, Lane 3, 5, 7, 9- Female samples. Source for DNA for lane 2 & 6, 3 & 7, 4 & 8, 5 & 9 are same.

Compared to the gDNA isolation method using SDS/Proteinase K digestion followed by NaAc/ethanol precipitation, which requires several steps, washing and desalting procedure and multiple tube transfers, the Chelex-100 resin based DNA extraction method requires only a few simple steps that takes very little time. With Chelex-100 resin, DNA extraction could be completed in less than one hour, while it could take up to 2 days to complete the DNA extraction with SDS/Proteinase K digestion method. Furthermore, the cost of Chelex resin based DNA extraction method is about five times cheaper than the SDS/Proteinase K digestion method. Thus, using Chelex resin and P2/P8 primers, chicks can be reliably and accurately sexed at low cost, soon after their hatching within 5 hours.

REFERENCES

1. Griffiths R, Double M.C., Orr K & Dawson R.J.G. (1998). A DNA test to sex most birds. Molecular Ecology 7:10715