# Polymerase Chain Reaction (PCR) Optimization of Sex Chromosome Linked CHD Gene for Genetic Sexing of Domestic Chickens (*Gallus domesticus*)

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Chromodomain Helicase DNA (CHD) binding protein loci is one of the sex chromosome linked gene found in most avian species including domestic chickens. CHD genes are preserved within avian Z and W sex chromosomes. The size of the gene is slightly different in Z and W chromosomes due to the variance in the intron regions, thus, making female (ZW) to have two different sized CHD genes and male (ZZ) to have only one sized CHD gene in their genome. Primers were designed to co-amplify these regions therefore give two bands are given for female and one band is given for male in gel electrophoresis enabling them to be used as gender identification molecular markers. Although different primer sets have been designed and published in previous studies, most of them have failed to present the optimum conditions for the precise amplification of CHD loci. Therefore this investigation was aimed to optimize the amplification of the genes CHD1Z and CHD1W. Genomic DNA extraction from whole blood of mature male and female animals was performed by a commercial DNA purification kit. The primer set used for the study was selected from previous studies on domestic chickens. PCR conditions; concentration of genomic DNA, annealing temperature, annealing time, extension time, and PCR cycle number were optimized. Gel electrophoresis conditions; concentration of the gel, concentration of buffer, running time, and electrophoretic voltage were optimized. Staining procedure was optimized by using different staining techniques such as pre staining, in-gel staining, and post staining to obtain a clear resolution in the electro-gram. A DNA concentration of 10ng/ul in the final reaction volume of 20ul, and PCR conditions of an initial denaturation of 94°C for 3 min, a denaturation of 94°C for 20 sec, an annealing of 55°C for 30 sec, extension of 72°C for 1 min, cycle number of 40, and a final extension of 72°C for 7 min gave the best amplification for the primer set. Modifying the standard protocol for annealing temperatures, annealing time and extension time increase the success of co-amplification. A loading volume of 20ul DNA at a concentration of 10ng/ul, 1.5% agarose, 1X Tris base, boric acid and EDTA (TBE) buffer, 60 Voltage for 2.5 hours of running for gel electrophoresis, and post staining of the gel with 1:10000 mixture of Diamond dye and 1X TBE buffer for 30 min were observed to be the best for co-amplification of selected CHD1Z and CHD1W primer set on domestic chicken.

**Keywords**: CHD loci, *Gallus domesticus*, Genetic sexing, PCR optimization