

# Sex Identification of Duck Embryos by Polymerase Chain Reaction

Shiou-Chou Liu<sup>(2)</sup>, Chian-Mu Chen<sup>(3)</sup> and Yi-Hao Yu<sup>(2)</sup>

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## Abstract

A sensitive method for the sexing of duck embryos was developed using single polymerase chain reaction (PCR) amplification of the avian chromo-helicase-DNA-binding (CHD) gene on the W- and Z-chromosomes of ducks. A pair of sex-specific primers was employed for the amplification of a single fragment of 596-bp from the Z-chromosome of male ducks and two fragments of 596-bp and 477-bp from the Z- and W-chromosomes of female ducks, respectively. DNA sequencing showed about 80% homology between the 596-bp fragment amplified from CHD1Z and 477-bp from CHD1W in duck. The primers were successfully applied to duck sexing from genomic DNA isolated from day-3 duck embryos by PCR.

Key words: Duck embryo, PCR, Sex identification.

## Introduction

Identification of the duck embryo's sex prior to incubation would be very useful, from both animal welfare and practical point of view, because about 50% of the incubated eggs are unwanted gender and are discarded later. Traditional methods of sexing ducks include vent sexing, karyotype analysis and examination of gonadal morphology; they are laborious and time consuming. DNA should provide a versatile way to discriminate male and female chicks. The polymerase chain reaction has been used successfully to identify embryonic sex in chickens (Clinton, 1994; Petite and Kegelmeyer, 1995). Such diagnosis was first shown by Saitoh *et al.*(1991) with W-chromosome-specific repetitive sequence in the chicken. Meanwhile, a 2<sup>nd</sup> single copy sequence available is the chromo-helicase-DNA-binding protein gene (CHD-Z) (Griffith and Korn, 1997). Because CHD-Z occurs in both sexes, it shall always be amplified and this ensures that the PCR reaction worked. But, a restriction enzyme should be used to selectively

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( 1 ) Contribution No 1110 from Taiwan Livestock Research Institute, Council of Agriculture.

( 2 ) I-Lan Branch Institute (Duck Research Center), COA-TLRI, Wuchieh, I-Lan, Taiwan, R.O.C.

( 3 ) National Chung-Hsing University, Taichung 402, Taiwan, R.O.C.

cut a fragment from the CHD-Z version before gel electrophoresis. Females, therefore, have two bands and males only have one. Griffith *et al.* (1998) employed two PCR primers which anneal to conserved exonic regions but amplified across an intron in both CHD-W and CHD-Z. Although the result of gel electrophoresis immediately reveals one band in the male and two in the female, but higher percentage of denaturing acrylamide gel must be used for some bases different in PCR products. The objective of this study was to develop a PCR method that could be used to rapidly and easily to sex duck embryos prior to the appearance of morphological difference in the gonad.

## Materials and Methods

Fresh blood samples were collected from duck adults and from 6 to 10 embryos of Tsaiya duck, Pekin duck, Muscovy duck and Hinney duck after incubated for 3 days. Blood was collected from the extra embryonic blood vessels of embryo and from the brachial vein of adults, then put into Eppendorf tube with lysis buffer (50 mM Tris-Cl, 100 mM NaCl, 100 mM EDTA, 0.2% SDS). Crude DNA was extracted from blood samples according to a proteinase K/phenol-chloroform procedure as described previously (Chen *et al.*, 1999). A pair of primers (5' – ATTGAAATGATCCAGTGCTTG -3') and (5'-GTTACTGATTCGICTACGAGA -3') designed by Hörnfeldt *et al.* (2000) were used for PCR amplification. PCR reactions were performed in 50  $\mu$ l contains 0.25 U of *Taq* polymerase (Promega), 2.5 mM of dNTPs, 100 mM Tris-HCl, 100 mM KCl, 1.5 mM MgCl<sub>2</sub> and 10 nmole of each primer. Between 50 and 250 ng of genomic DNA extracted from adults and embryo's blood were used as template. The PCR cycling program (in PTC-100 of MJ Research Inc.) is as follows: initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C (50 sec), annealing at 55°C (40 sec), and extension at 72°C (40 sec), then a final extension at 72°C for 5 minutes. After amplification, the PCR products were separated through a 1.5% agarose gel containing ethidium bromide and visualized under UV light. These PCR fragments were cloned into PCR vector using the TOPO cloning kit (Invitrogen, CA). Sequences of these clones were determined using comparative *Taq*-cycle-sequencing procedures by the Dye Terminator technique on an automatic sequences model 373A instrument according to the manufacture's recommendations (Applied Biosystems Inc., CA). DNA alignments were performed using GCG Sequence Analysis Software (Genetic Computer Group, WI).

## Results and Discussion

The use of CHD primers on duck's genomic DNA produced only single CHD1Z specific fragment in male duck DNA samples and both CHD1Z and CHD1W specific fragment in female duck DNA samples regardless of which duck genomic DNA was used as template (Fig. 1). After reaction, PCR products were cloned and sequenced, the partial CHD genomic structure of duck was revealed (Fig. 2). The length of the amplified DNA for CHD1Z is 119 bases longer than CHD1W and there was about 80% homologous between amplified DNA of CHD1W and CHD1Z. Some PCR-based methods of sexing chicken or turkey embryos used only W chromosome specific primers (Saitoh *et al.*, 1989; Clinton, 1994; Petite and Kelgelmeyer, 1995) which will show some faults when no product appeared after PCR reaction. Even

together with internal control primer ( $\beta$ -actin, ATP synthase or other non sex specific gene primer), there will still have some inconvenience included copies of internal gene per genome and PCR reaction condition when combine two different pairs primers (Cassar *et al.*, 1998; Clinton *et al.*, 2001; D'Costa and Petite, 1998). The result is a laborious and tedious task when performed on large numbers of samples.

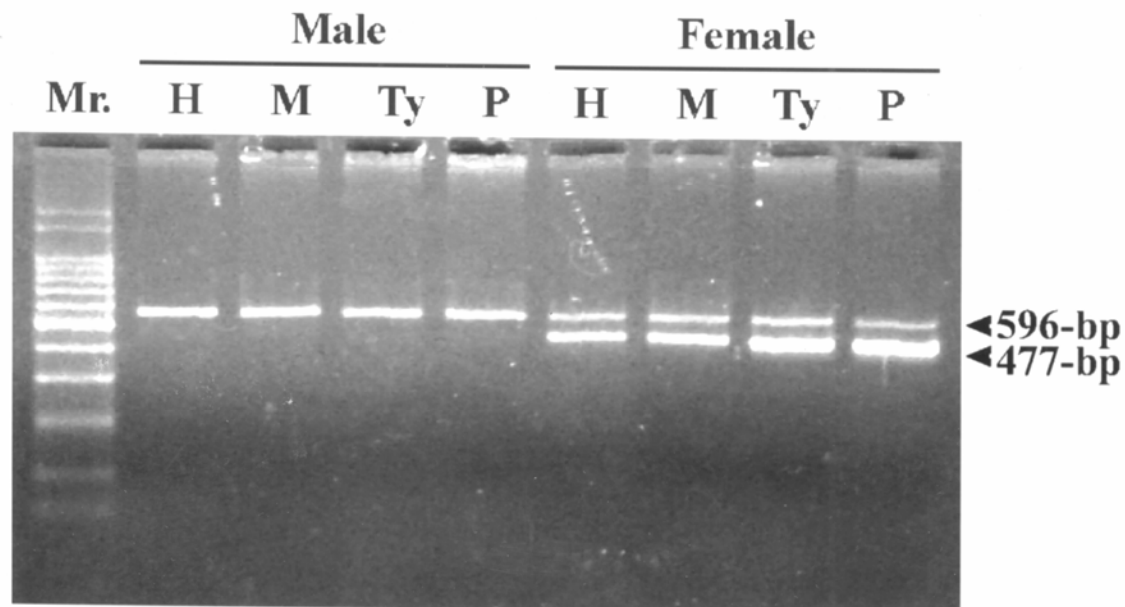


Fig. 1. Electrophoresis of PCR products from genomic DNA in different duck embryos for sexing experiment. Mr: Bio100 DNA ladder, M: Muscovy, Ty: Tsaiya, P: Pekin duck and H: Hinny duck.

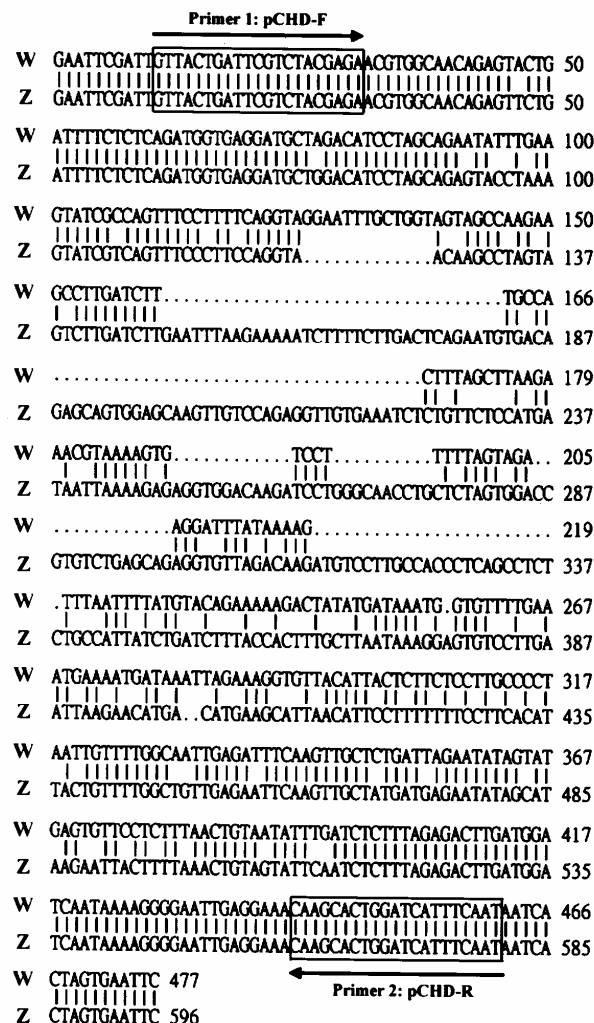


Fig. 2. Alignment of partial sequences of the CHD-Z and CHD-W in ducks.

Vent sexing is based on the anatomical difference in the cloaca of newly hatched male and female ducklings, but this method cannot be used to sex embryos. Although a relatively simple tool for sexing birds, karyotyping still need more time to culture cells and to identify. Sexing duck embryos by gonadal morphology is not applicable to all stages of development. This PCR technique is advantageous over separate uniplex PCR conserving reagents, templates and reducing sample preparation time. The additional advantage of this method is that entire procedure from blood collection to analysis of PCR products can be completed in the same day, thereby permitting the sexing of many embryos in a short time. We also apply this method to sex ducks of different species (Fig. 1), these primers worked equally well with DNAs from Tsaiya duck, Pekin duck, Muscovy duck and Hinney duck ( hybrid duck of Tsaiya and Muscovy ), and the length of PCR product from these ducks were the same. Because the CHD gene is highly conserved, therefore this system may be useful in various popular ducks.

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# 利用聚合 連鎖反應判定鴨胚之性別<sup>(1)</sup>

劉秀洲<sup>(2)</sup> 陳全木<sup>(3)</sup> 胡怡浩<sup>(2)</sup>

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## 摘 要

以鴨性染色體上 CHD 基因為模板，與合成之特定引子進行單一之聚合酶連鎖反應，進而判定鴨胚性別的方法被建立。這對具性別專一性之引子能自雄鴨的 Z 染色體擴增出 596 鹼基對之片段：而於雌鴨則會同時擴增出 596 及 477 鹼基對(源自 W 染色體)的二條片段。將 PCR 產物經選殖後進行序列分析，顯示 596 與 477 鹼基對約具有 80% 鹼基相似性。此法對於番鴨、菜鴨、北京鴨及駝鵝鴨皆能成功鑑別出性別。

關鍵詞：鴨胚、PCR、性別鑑定。

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(2) 行政院農業委員會畜產試驗所宜蘭分所。

(3) 國立中興大學。