# MOLECULAR TECHNIQUE FOR GENDER IDENTIFICATION IN THE HAWAIIAN GOOSE (*BRANTA SANDVICENSIS*)

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In this project, the molecular technique of PCR amplification was used as an identification tool to determine the gender of the Hawaiian goose (*Nene*). This involves the amplification of the chromobox-helicase-DNA-binding (CHD) gene located on the sex chromosomes of most bird species. Birds have two sex chromosomes, Z and W; the females are ZW (they have one Z and one W chromosome), while the males are ZZ (possess two Z chromosomes). There are CHD genes on both chromosomes (CHD-Z and CHD-W), however they are not identical: the CHD-W gene contains an intron that is longer than that of CHD-Z. Therefore, when the genes are PCR-amplified using the same primers (P2 and P8), the females will have two products of different sizes, showing two bands on a gel, whereas the males show a single band, thus providing a method to determine the sex of the bird.

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## INTRODUCTION

Among monomorphic bird species, such as the endangered Hawaiian goose (*Branta sandvicensis*), it is difficult to differentiate between males and females, since they look physically identical. This project is designed to identify the sex of the Hawaiian goose, more commonly called the *Nene*.

Due to the decreased population of the *Nene,* it is important for every mating to succeed. Detection of sexual identity at an early age is one step that can help maintain the bird population. Using a molecular technique to determine sex of birds, we can identify whether the individual being tested is male or female, a method that is more reliable than physical observations. We believe that if we test a certain blood sample under the same conditions the results will be consistent and repeatable. This process is possible due to the CHD gene.

## METHODS

Blood samples from the *Nene* were obtained previously by the lab and stored at −20°C. Genomic DNA was extracted from the blood samples using a DNeasy Tissue Kit (Qiagen, cat# 69504). The DNA was added to a Master Mix containing the following components: PCR buffer, 1.5mM $MgCl_2$, 200μM of each dNTP, 20μM P2 primer, 20μM of P8 primer, 0.15 units of *Taq* polymerase, and 20–50ng of DNA, for a final reaction volume of 10μl. The reaction samples were then placed into the PCR thermocycler to amplify the DNA region of interest containing the CHD gene where the primers anneal. This region of the CHD gene includes a portion of non-coding region, or intron, as well as a portion of the coding region, or exon. The sequences for the primers are:

Primer P2: 5′-TCTGCATCGCTAAATCCTTT-3′
Primer P8: 5′-CTCCCAAGGATGAGRAAYTG-3′

The PCR program used was "BLAINE" and the PCR samples were evaluated using gel electrophoresis. The samples were mixed with blue loading dye (1μl of loading dye is added to 5 μl of the PCR reaction sample and then loaded onto a 3% agarose gel, which is placed in an electrophoresis apparatus with 0.5× TBE buffer). The negatively charged DNA migrates through the gel toward the positive electrode when an electric field is applied. The agarose gels were run at 40V for 5–6 hours. A 100bp ladder, which contains 12 bands of known size, was used as a molecular weight standard for the agarose gels. After electrophoresis was completed, the gel was stained in an ethidium bromide solution, in order to visualize the bands under UV light.

### Data Analysis

From the stained gel, we were then able to see single or double bands of about 370–380bp in length. Samples that had a single band were designated as male, while those that had double bands were designated as female.

## RESULTS

We tested the blood from 30 *Nene* and identified 17 males and 13 females.

The test was repeated on the same samples numerous times with consistent results. Out of 24 birds that were previously sexed using visual methods, our test confirmed the sex of 22 of them. Due to the inaccuracy of physically identifying sex in the *Nene,* the molecular technique is probably more reliable, and should be considered more accurate. We can now correct earlier errors, and re-evaluate the sex of these individuals using the PCR method. Figure 1 illustrates the high reproducibility of the PCR test, and confirmation of the visual method using the PCR test.

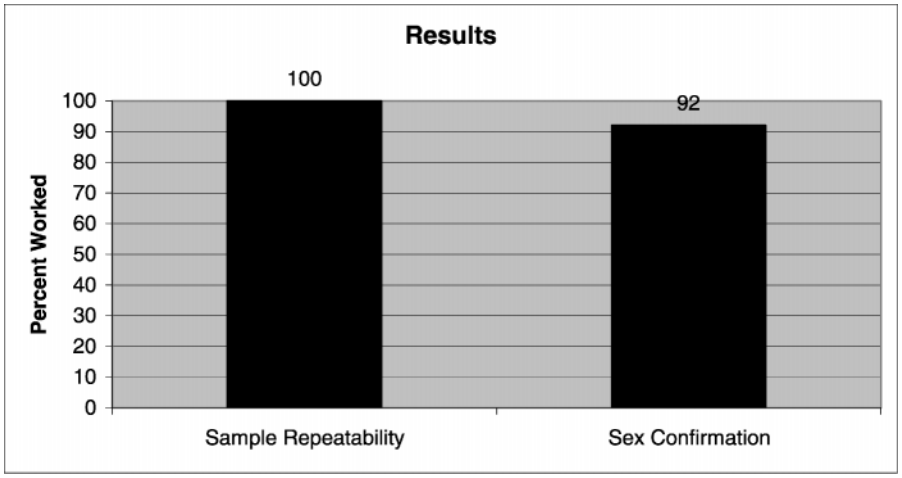


**Fig 1. High reproducibility of the results of sexing using the PCR test and confirmation of visual methods using the PCR test**

## CONCLUSION

This project involved a method of determining the sex of the nearly monomorphic bird, the *Nene,* by using PCR amplification of a region of the CHD gene. We were able to successfully identify the sex of individuals of the species using this method, which is a more reliable means as compared to visually inspecting the bird. When the genes are PCR-amplified using the same primers (P2 and P8), the females had two products of different sizes, showing two bands on a gel, whereas the males showed a single band. The data collected from this project can later be used to help conservationists develop breeding programs to help the *Nene.* This project could also be used to identify sex of all newborn *Nene* and give the conservationists more information on each *Nene*'s clutch.

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