The Enhancement of the Sensitivity Of A PCR-based Avian Sex Determination Assay

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ABSTRACT

Our study attempted to decrease the number of cells required to sex externally monomorphic birds using PCR. A sequence within the chromobox-helicase-DNA-binding (*CHD*) gene, located on the sex chromosomes of all avian species, was amplified. Lymphocytes from chickens (*Gallus domesticus*) were used as the DNA source for all experiments. An initial experiment established Griffiths' assay in our lab. Results were consistent with published data. No signals were observed in assays with less than 20,000 cells. The next experiment compared the use of Griffiths' amplification buffer to a buffer we developed, Bart. Signals were produced and a working assay was established with only 10 cells needed. It was also possible to increase the number of productive replication cycles from 35 to 45 without generation of noise. In fact, use of Bart eliminated primer-generated noise, leaving only sexing bands in the gel.

INTRODUCTION

For over a decade it has been possible to externally sex monomorphic birds using PCR. A major drawback of the protocol developed by Richard Griffiths is that DNA from at least 20,000 cells is needed (Griffiths, 1996). Our study attempted to increase the sensitivity of the protocol by decreasing the number of cells required. Over fifty percent of avian species appear to be identical as well as sexually uniform in morphology (Griffiths, 1996). In avian species the female is the heterogametic sex (ZW), while the males are homogametic (ZZ), a model opposite of humans. The *CHD* (chromo-helicase-DNA-binding) genes, located on the sex chromosomes, enable molecular sex determination (Griffiths, 1996). Female birds contain both the *CHD-W* and *CHD-Z* genes which results in the amplification of both sequences during PCR. Male birds contain two copies of the *CHD-Z* gene, which in turn produces only one sequence during PCR. When the copies of the genes are run during gel electrophoresis, female samples are visually identified as having two bands, while the males have only one. An initial experiment aimed to establish Griffiths' working assay in our lab. The next experiment compared the use of Griffiths' amplification buffer to a buffer we developed, Bart.

MATERIALS AND METHODS

A sequence within the chromobox-helicase-DNA-binding (*CHD*) gene, located on the sex chromosomes of all avian species, was amplified. The sequence lengths were 362 and 354 base pairs for the *CHD-W* and *CHD-Z*, respectively. The polyacrylamide gel electrophoresis (PAGE) purified primers used were 5′-TCTGCATCGCTAAATCCTTT-3′ and 5′-CTCCCAAGGATGAGRAAYTG-3′ (2.5 µM) (IDT, Inc., San Jose, CA, USA). All assays used *Taq* DNA polymerase (2.7 U) and deoxyribonucleotides (5 µM). Lymphocytes from chickens (*Gallus domesticus*) (10 cells/2 µL) were used as the DNA source for all experiments. Assays were run with positive and negative DNA controls. The DNA was replicated in a Corbett Rapid Thermocycler in 20 µL volumes with an annealing temperature of 48°C. All of the PCR products were separated using PAGE. An 8% gel (17:1, con- to bis-acrylamide) with 10 mM TRIS (pH 8) was formed in an agarose gel chamber under Ar. The gel was placed in 10 mM TRIS (pH 8) in the electrophoresis apparatus and the PCR products were added to wells. The applied voltage was 200 and the duration was 2 h. The gel was stained for 30 min in 1.25 µM ethidium bromide in 100 mL of 10 mM TRIS (pH 8). Destaining was carried out over 45 min in 100 mL of H_2O. The gel was viewed using a transilluminator and photographed with a digital camera.

RESULTS

The experiment establishing Griffiths' assay were consistent with published data, albeit with the same troubling signal-to-noise problems. No signals were observed in assays with less than 20,000 cells. The experiment that used Bart buffer produced signals with the use of only 10 cells (Fig 1), significantly fewer cells than the 20,000 cells necessary for Griffiths' protocol. It was also possible to increase the number of productive replication cycles from 35 to 45 without generation of noise. The use of Bart also eliminated primer-generated noise, leaving only sexing bands in the gel. Interestingly, when Bart was used with 20,000 cells, no signals were observed. Assays incorporating Bart were run in triplicate and signals were consistently observed.

Figure 1: Bart buffer with 10 cells/2µL after 55 cycles.

DISCUSSION

Reduction in the number of cells required for avian sex determination provides potential applications for the sexing of embryos or sexing from a single down feather. Our assay makes sex determination prior to hormonal treatment simple. We are currently replacing the use of the *CHD* gene with a conserved *W*-specific sequence.

REFERENCES

Griffiths, R., Dan, S., and Dijkstra, C. 1996. *Sex Identification in Birds Using Two CHD Genes*. Proceedings of the Royal Society London B. 263: 1249-1254.

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