Benchmarks

digestion hastens the entire procedure, allows minimal hands-on work and saves resources.

REFERENCES

1. **Holmes, D.S. and M. Quigley.** 1981. A rapid boiling method for preparation of bacterial plasmids. *Anal. Biochem.* 114:193-197.
2. **Liu, Z. and N.C. Mishra.** 1995. Single-tube method for plasmid miniprep from large numbers of clones for direct screening by size or restriction digestion. *BioTechniques* 18:214-217.
3. **Majumdar, M.K. and D.A. Williams.** 1992. Minipreparations of plasmid DNA directly from cell culture by the boiling method. *BioTechniques* 13:366.
4. **Rajeevan, M.S. and C.L. Bassett.** 1994. Flexibility in the boiling method of extracting plasmid DNA directly from cell culture. *BioTechniques* 16:376-380.
5. **Xiang, C., H. Wang, P. Shiel, P. Berger and**

D.J. Guerra. 1994. A modified alkaline lysis miniprep protocol using a single microcentrifuge tube. *BioTechniques* 17:30-32.

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Isolation of Genomic DNA from Claw Clippings for Genetic Analysis in Chickens

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The chicken, *Gallus domesticus*, is one of many economically important livestock and poultry species for which there is an active, ongoing DNA marker development program to establish a high-utility genome map (2,3). Among potential practical applications of a molecular marker-based genome map is the use of markers on the Z chromosome to sex birds at a very young age. Currently, sources of DNA for marker-based genetic analysis in chickens, either by Southern blotting or the polymerase chain reaction (PCR) method, are blood and tissue. However, both of these sources have significant disadvantages because obtaining blood from young birds is stressful and often difficult, and obtaining DNA from tissues traditionally requires sacrificing the bird. Because of these disadvantages, we investigated the use of claw clippings as a source of genomic DNA for PCR amplification of Z chromosome-based microsatellites. The procedure was based on a previously reported protocol for isolation of DNA from fingernail (5).

Claw clippings were obtained from male and female birds and washed briefly in 1.0 N NaOH before snap-

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Figure 1. Polyacrylamide gel electrophoresis of products from PCR amplification using genomic DNA isolated from claw and blood of chickens. Lanes identified as TM and TF are amplified products from genomic DNA isolated from claws of male and female birds, respectively. Lanes identified as BM and BF represent amplified products from males and females, respectively, using genomic DNA isolated from blood. Amplified products from templates were obtained from blood (BM or BF) and clippings (TM or TF) of the same bird. Lanes with identical numerical subscript within each sex represent amplified products using templates from blood and claw of the same bird. A and B represent the expected alleles at the *ADL 22* locus (3). As expected, the B allele was detected only in the heterozygous males using template DNA from both blood and claw. Females, the heterogametic sex in chickens, had only the A allele in amplified patterns using template DNA from claw clippings and blood. The nonlabeled band is an artifact, probably due to the low annealing temperature used (3). Lane M is the Φ X174 *Hinf*I marker. Lane C represents no template (distilled water) control.

freezing in liquid nitrogen. Frozen claws were crushed, and 50 mg were used for DNA extraction. Lysis and extraction of DNA from the crushed claws and from 50 μ L of blood obtained from the same birds were as previously described (1). The purified DNA was dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and the concentration and relative purity were determined using a Model DU® 7400 Programmable Spectrophotometer (Beckman Instruments, Fullerton, CA, USA) based on absorbance at 260 and 280 nm, respectively.

The DNA obtained from claw clippings ranged from 0.3 to 120 μ g, with an average of 40 μ g. The yield from blood ranged from 150 to 300 μ g, with an average of 200 μ g. The DNA isolated from both claw and blood was used as a template in restriction enzyme digestion (data not presented) and in the PCR amplification of a microsatellite locus, *ADL 22* (3), on the Z chromosome (Figure 1). The PCR amplification was carried out in a final volume of 25 μ L containing 60 ng genomic DNA, 1 μ M of each primer, 200 μ M of each dNTP, 2 mM $MgCl_2$, 2.5 U of AmpliTaq® DNA Polymerase (Perkin-Elmer, Norwalk, CT, USA) and 1 $\times$ PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3). All PCR amplifications were performed in a Model PTC-100™ Thermal Cycler (MJ Research, Watertown, MA, USA) for 30 cycles (denaturation at 94°C for 45 s, annealing at 45°C for 1 min and extension at 72°C for 1 min)

after initial denaturation at 94°C for 3 min. The PCR products were analyzed on 8% nondenaturing polyacrylamide gels, followed by silver staining for detection as previously described (4).

Amplification patterns using both types of templates indicated comparable efficiency of amplification. The genotypes obtained from both claw and blood were the same for each individual. In addition, using both types of templates, individuals were accurately identified as either males or females. The results showed that claw clippings can be used as a source for DNA for restriction digestion and genetic analysis by PCR.

REFERENCES

1. Bartlett, J.R., C.P. Jones and E.J. Smith. 1996. Linkage analysis among endogenous viral element 1, blue eggshell and pea comb loci in chickens. *J. Hered.* 87:67-71.
2. Bumstead, N. and J. Palyga. 1992. A preliminary linkage map of the chicken genome. *Genomics* 13:690-697.
3. Cheng, H.H. and L.B. Crittenden. 1994. Microsatellite markers for genetic mapping in the chicken. *Poultry Sci.* 73:539-546.
4. Goldman, D. and C.R. Merrill. 1982. Silver staining of DNA in polyacrylamide gels. *Electrophoresis* 3:24-28.
5. Toshihiko, K., K. Takagi, S. Nakamura, T. Hirasawa, M. Sada and K. Uchida. 1992. Genetic analysis using fingernail DNA. *Nucleic Acids Res.* 20:5489-5490.

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