

## Isolation of genomic DNA from feathers

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**Abstract.** The use of feathers in veterinary clinical practice simplifies the sampling of avian genomic DNA, especially when blood extraction is difficult because of the age or the size of the bird. A rapid and accurate protocol was used to isolate high-quality genomic DNA from feathers. The technique includes a lysis step of the feather quill, which differs in temperature and time of incubation depending on the feather size. Purification of genomic DNA is performed with phenol:chloroform:isoamyl alcohol extraction and ethanol precipitation. This protocol consistently provided significant amounts of high-quality genomic DNA from more than 800 birds belonging to 120 different species. Genomic DNA isolated with this method was used for Southern blotting and also in several polymerase chain reaction systems devoted to sex determination and paternity testing.

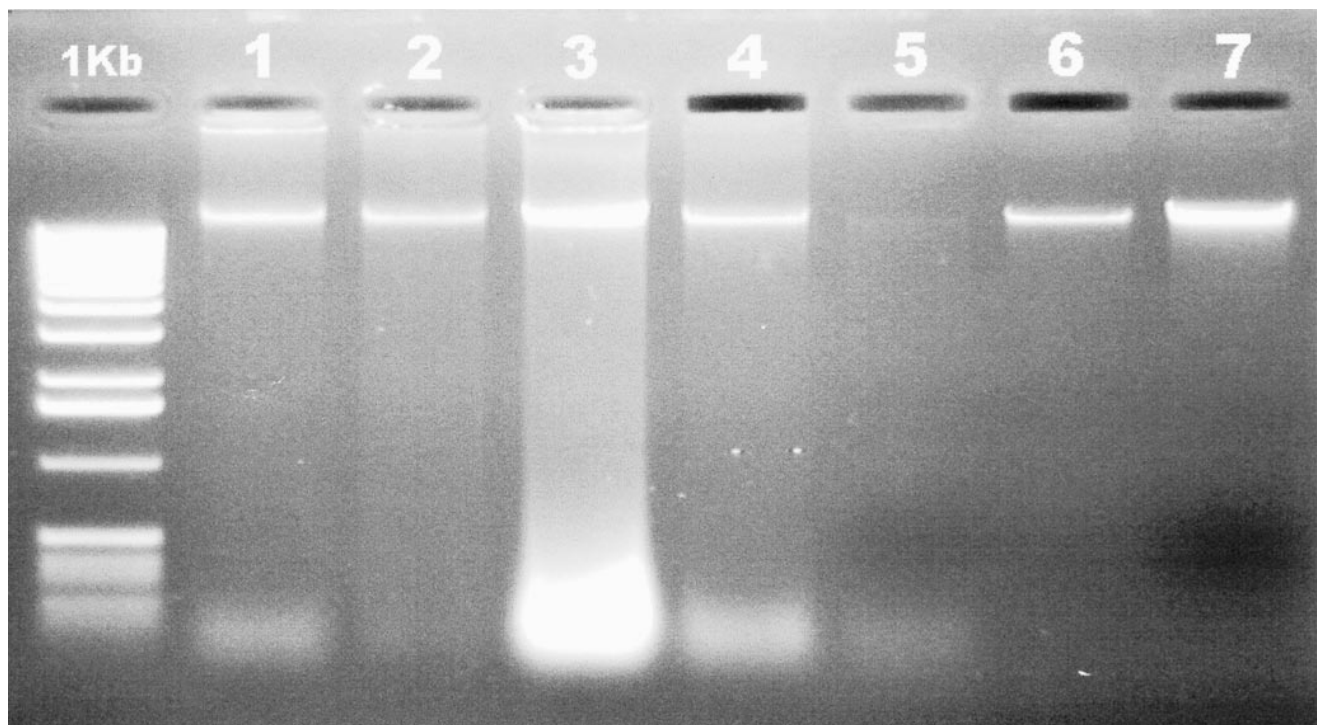
Molecular markers are widely used in veterinary clinical practice for sex identification, marker-assisted selection, parentage testing, and infectious and genetic disease diagnosis.<sup>1,4,8</sup> Using feathers instead of blood as a genomic DNA source, minimizes the stress on the bird and simplifies sampling, particularly when studying large bird species such as ostrich and emu. Moreover, in juvenile birds and small parrots, the small size of the blood vessels makes blood extraction very difficult. A rapid and accurate protocol was used

to isolate high-quality genomic DNA from fresh feathers kept for 2 weeks at room temperature or up to 1 month at 4 C.

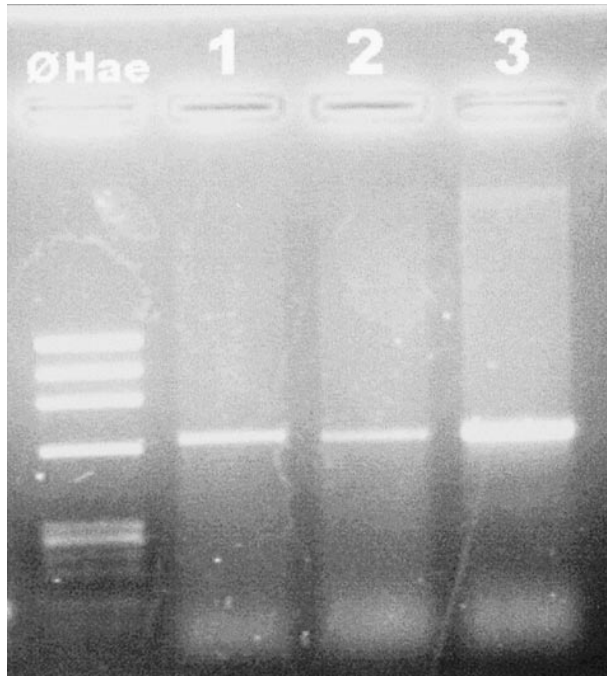
A 0.5–1-cm section was cut from the terminal portion of the feather quill and placed in a 1.5-ml Eppendorf tube containing 500  $\mu$ l of lysis buffer (50 mM Tris-HCl, pH 8, 20 mM ethylenediaminetetraacetic acid [EDTA], pH 8, 2% sodium dodecyl sulfate) and proteinase K at a final concentration of 175  $\mu$ g/ml. Lysis temperatures and incubation times were different depending on the feather size. When dealing with feathers from large birds such as ostriches or big parrots or when the feather quill contained soft tissue or blood (as in new growing feathers), the lysis was performed at 37 C overnight with gentle shaking. When using small feathers

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**Figure 1.** Electrophoresis of avian DNA extracted from feathers. Lane 1Kb: 700 ng of 1-kb ladder marker; lane 1: *Pionus menstruus* genomic DNA; lane 2: *Cacatua eleonora* genomic DNA; lanes 3–5: ostrich genomic DNA without RNase digestion (lane 3), with RNase digestion (lane 4), and at 1/10 dilution (lane 5); lanes 6, 7: 50 (lane 6) and 200 (lane 7) ng of  $\lambda$  DNA.

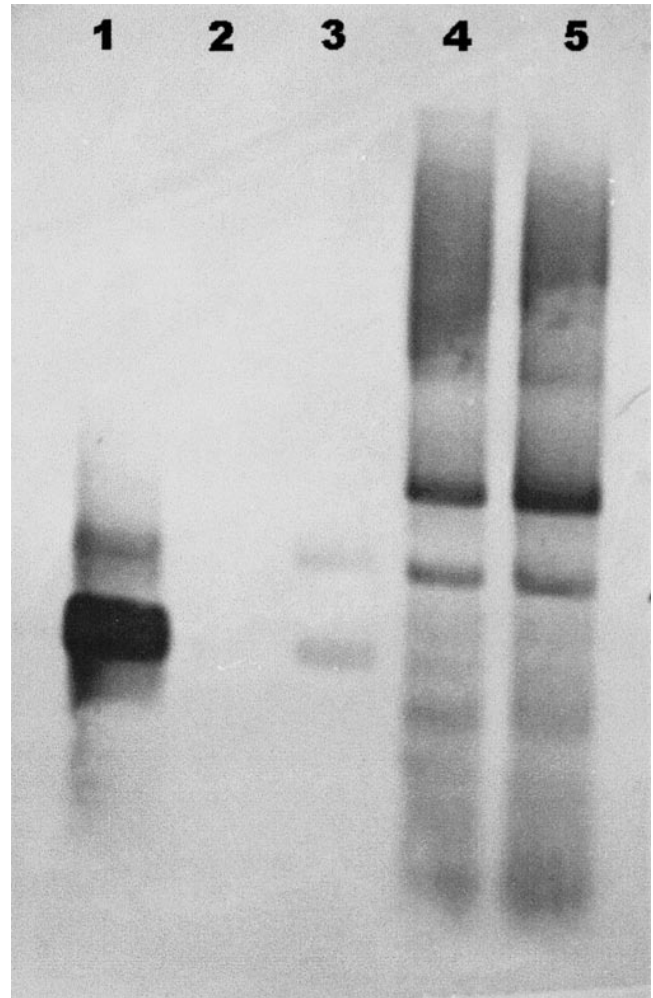


**Figure 2.** Electrophoresis of avian DNA extracted from feathers. Lane  $\phi$ Hae: 300 ng of X174 DNA  $\phi$ HaeIII marker; lanes 1–3: amplification of ostrich 648-bp sex marker in 3 females.

devoid of soft tissue and blood, samples were incubated at 56 C for 4 hours, and shaking was not necessary. After the lysis step, samples were vigorously vortexed to homogenize the lysate and then centrifuged at  $12,000 \times g$  for 10 minutes only if nondigested soft tissue was present. The supernatant was transferred to a clean 1.5-ml Eppendorf tube, and DNA was purified with phenol:chloroform:isoamyl alcohol (25:24:1) as described elsewhere.<sup>12</sup> Subsequently, 50  $\mu$ l of 2 M NaCl and 2 volumes of ethanol were used to precipitate genomic DNA. To maximize DNA recovery, this step can be performed at  $-20$  C overnight. The DNA pellet was washed in 70% ethanol and resuspended in 50–500  $\mu$ l of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) or sterile water. When the sample was a large feather or if it contained soft tissue/blood, larger volumes were needed to resuspend the DNA pellet, and usually a further RNase digestion step was required (0.1  $\mu$ g/ $\mu$ l RNase, 2–3 hours at 37 C).

This protocol has been used to isolate genomic DNA from more than 800 birds belonging to 120 different species, including parrots, raptors, and ratites. The technique consistently provided significant amounts (1–100  $\mu$ g) of nondegraded genomic DNA (Fig. 1). These DNA preparations can be used as template in several polymerase chain reaction (PCR) systems.<sup>11</sup> A 110-bp fragment from the chromodomain-helicase-DNA-binding protein (CHD) gene, which is a sex marker in nonratite birds,<sup>6,7</sup> and a 648-bp PCR product from ostrich feathers for sex identification<sup>2</sup> (Fig. 2) are routinely amplified. Moreover, DNA isolated from feathers was successfully used to perform Southern blotting and to amplify microsatellite markers for paternity testing.

Although there have been published protocols dealing with the isolation of viral DNA from feather follicle



**Figure 3.** Southern blotting on ostrich genomic DNA (female and male). Lanes 1, 3: with a sex-specific probe; lanes 4, 5: amplified DNA. Lane 2 is empty to avoid transfer of the colored signal between the female (lane 1) and the male (lane 3).

tracks,<sup>3,10</sup> only one other author has described extracting genomic DNA from feathers.<sup>5</sup> In that study, a chelex-based technique that allows denatured genomic DNA to be obtained in minute amounts was used with ancient samples. The protocol described here has the advantage that the quantity and quality of the DNA preparation can be easily visualized by agarose gel electrophoresis and ethidium bromide staining because the DNA yield is of sufficient quantity and the DNA remains double stranded. This feature allows checking of the amount and integrity of the isolated DNA, 2 parameters that are essential for the successful optimization of PCR protocols. Degradation of genomic DNA does not necessarily inhibit PCR amplification, particularly when small regions (100–200 bp) are amplified, but usually leads to nonspecific amplifications.<sup>9</sup> Moreover, the integrity of genomic DNA is crucial when targeting large DNA fragments (e.g. several kilobars) or when more demanding DNA analysis techniques are performed (e.g., Southern blot). Two different lysis procedures were used depending on the feather size because large feathers contain much more tissue than

small ones, thus requiring a longer digestion time. The presence of contaminating RNA in the DNA samples had an inhibitory effect on PCR amplification. This problem was particularly insidious when using large feathers as a source of DNA, but the addition of an RNase digestion step eliminated this problem. In summary, the nucleic acid extraction protocol described here is a useful tool for extracting genomic DNA from feathers for the purpose of sex determination and other genomic investigations.

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- b. Sigma, St. Louis, MO.

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## Comparison of two enzyme-linked immunosorbent assays for serologic diagnosis of paratuberculosis (Johne's disease) in cattle using different subspecies strains of *Mycobacterium avium*

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**Abstract.** Serologic diagnosis of bovine paratuberculosis (Johne's disease) with currently available tests may give false-positive results due to cross-reactions with avian and bovine tuberculosis viruses and other infectious agents. Indirect enzyme-linked immunosorbent assays (ELISA) for detection of antibodies against paratuberculosis based on antigens from *Mycobacterium avium* subsp. *avium* (A-ELISA) and *M. avium* subsp. *paratuberculosis* (P-ELISA) were compared. Despite an expected higher specificity for *M. a. paratuberculosis* in the P-ELISA, the 2 antigens were equally suitable for demonstration of antibody to *M. a. paratuberculosis* in cattle. Receiver operating characteristic (ROC) curves was used to demonstrate the possible antigenic relationship. The area under the curve (AUC) was calculated for each of the 2 ROC curves. The AUC for the P-ELISA ROC curve was 0.9197, and the AUC for the A-ELISA ROC curve was 0.9149, demonstrating a negligible difference in efficiency of the 2 tests ( $\alpha = 0.182$ ).

Paratuberculosis (Johne's disease) generally appears as a chronic enteritis in cattle and other ruminants.<sup>3</sup> The etiologic agent of paratuberculosis is *Mycobacterium avium* subsp.

*paratuberculosis*, previously named *M. paratuberculosis*. Two other *M. avium* subspecies have been described: *M. a. avium* and *M. a. silvaticum*.<sup>18</sup> For differentiation of *M. a. paratuberculosis* from the 2 other subspecies, detection of the specific IS900 insertion sequence by molecular techniques currently is the only option.<sup>8</sup> The other 2 *M. avium* subspecies also appear to have 1 or 2 unique sequences (IS901 and IS902) although these do not always seem to be present.<sup>12,14</sup> A general prerequisite for a sensitive and specific serologic test is a specific test antigen. *Mycobacterium a. paratuberculosis* has a wide range of antigenic determinants,

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