

## Ketterson / Nolan Research Group Collection

This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

### Context Statement

This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (<https://scholarworks.iu.edu/dspace/handle/2022/7911>).

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## **Blood Samples for DNA**

### **May 13, 2006**

*Only one important change since 1998: early Spring of 2003 we collected only 25ul of blood per tube of preservative (one half of a full microhematocrit tube of blood). In 2004 returned to full tube per eppendorf (1 tube blood/0.5ml Longmire's), but blood was frequently spun first, then blown into preservative.*

For adults, take a maximum of 3 microhematocrit tubes per bird, try to get at least two; for nestlings try to get two. The first one will be the 'a' tube, the second the 'b' tube, and the third, the 'c' tube. If one tube has less blood in it than the others, make it come later in the series, i.e., either the b or the c tube (b if only 2, c if 3).

**While in the field or lab, never mix up a bird and its blood sample!** It's as simple as that; it's essential. There is no way to correct an error of this kind. To insure avoiding this kind of error, put the microhematocrit tubes in marked vials, keep notes, etc. If you find you aren't sure, please don't guess.

If the sample is for DNA only, no need to spin. Simply break off the clay tip and dispense it directly from the microhematocrit tube into an autoclaved 1.6 ml eppendorf tube containing 500 ul of Longmire's solution (using gravity or using air pressure). *(Recall that Longmire's solution has sodium azide in it as an anti-bacterial agent and it is poison. Treat it with respect by wearing gloves and washing your hands after use).*

If you plan to use the plasma for hormones, IgG etc., then cap the tubes with clay sealant and spin them down in the microhematocrit centrifuge. **Immediately after the spin, draw off the plasma and blow the packed RBCs into Longmires.** Do not delay as delay can degrade the quality of the DNA! Also be sure that the RBCs are bathed in preservative and don't remain as un-bathed worms at the bottom of the eppendorf tube).

It is **essential** that the DNA transfer be done cleanly (no human DNA from your hands, no mixing between avian blood samples), because we wish to amplify this DNA using PCR and traces of foreign DNA can become a big problem.

The eppendorf tubes should be prepared in advance and labelled by sample # and a, b, c. Throughout the summer this is a job that almost always needs doing, and life is easier when we stay ahead of this job. Also it's essential to keep the tops on the containers of longmire's and the eppendorf tubes with solution, so bacteria don't settle in the tubes and contaminate the DNA.

Please record **all** data requested on the data sheet. This seems tedious at the time, e.g., repeating the color combinations, but it helps us recover from mistakes, e.g. when the band number, nest ID and colors don't all match, we can figure out which is likely to be wrong. That means please look at the bird for its colors, don't just copy colors from another data sheet.

Add the band number to the eppendorf tube along with the sample number and date. Let sample number be clearly visible from the top as well.

Wrap tops of tubes in parafilm (also helpful if parafilm squares prepared in advance) and place the a, b, and c samples in Nunc boxes. When the boxes are full, store them in a refrigerator (not a freezer) and label the boxes with the year and the numbers of the first and last samples contained in the box.