

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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EXTRACTION OF DNA FROM BLOOD:

A. Salt Extraction Method (Blood only!!) :

Set up a row of labeled 15 ml tubes

To each tube add:

1 ml **1x TNE** buffer
200 ul **1 M Tris** HCl pH 8.0
75 ul **20% SDS**

or 1 ml "**Extraction**" **buffer** (see below)

Add approx. **100-500 ul of blood sample** (i.e., the blood in lysis buffer)
(leave pipettor set at about 500-600 ul). The blood will be hard to suck up and stringy, so
**use the cap of the tube to snip off the glob of blood. Don't worry about exact amounts here
you simply want a small blob of blood/buffer.**

10 ul **Proteinase K** (20 mg/ml) **ADD LAST** and return to fridge ASAP

incubate in waterbath overnight (o/n) @ 37 Cor @ 45 C for 2 hr (o/n is better).

add 1/3 to 1/2x volume of **5M NaCl** (**750 ul in this case**)

**IMPORTANT!: THE SALT SOLUTION SHOULD NEVER BE MORE than
1/2 to 1/3 of total volume -- in other words, too much salt solution is not good;
you will end up with a lot of salt crystals at the end and no DNA.**

shake hard 15-20 seconds

spin @ 3000 for 15 mins in centrifuge (e.g., clinical desk type with 6-8 slots)
collect supernatant (try to avoid the foam at top and any crud on the bottom)

Add at least 2X volume of 'frozen' absolute **Ethanol (EtOH)** - **you can just pour ETOH in,
You do not need to measure it**

Mix by inverting => DNA appears fluffy white

After DNA precipitates:

spool precipitated DNA on glass rod--dry 3-5 min. until somewhat "clear" (translucent)
(use pasteur pipettes with tip flame sealed to make glass rods)

resuspend DNA in 100 - 300 ul **doubly-distilled H2O (ddH2O)**
(depending on amount of DNA) for at least 30 min. @ 37C.

Freeze for long term storage; you may keep at 4C if used within a few days

Original reference for salt extraction:

Miller, S., D. Dykes, et al. (1988). "A simple salting out procedure for extracting DNA from human nucleated cells." Nucleic Acids Research **16**: 1215.

Extraction Buffer for about 100 samples add:

100 ml of 1xTNE₂ solution
20 ml of 1M Tris solution

7.5 ml 20% SDS solution