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).

DNA Extraction from Kits

Day 1 – Sample Set Up and Protein Digestion (1-1.5 hours)

1. ☐ Unwrap samples, record Blood # and Band # in notebook.
2. ☐ Transfer ~100 µL of each sample into clean tube labeled with assay # (1-24).
3. ☐ Turn on the H_2O bath or hot block. Add 10 µl of Pro-K to samples.
4. ☐ Incubate at 55°C in hot block or water bath overnight.

Day 2 - Step 1: Cell Lysis

1. ☐ Add **150 µl of GT Buffer** to the sample and mix by shaking vigorously.
2. ☐ Add **200 µl of GB Buffer** to the sample and shake vigorously for 5 seconds.
3. ☐ Incubate the mixture at 60°C in the heat block for 10 minutes. During incubation, invert tube every 3 minutes.
4. ☐ At this time, also place the tube containing **Elution Buffer** in the heat block and heat until Step 4.

Step 2: DNA binding

1. ☐ Add **200 µl 100% ethanol** to the sample and mix immediately by shaking vigorously.
2. ☐ Place a GD Column in a 2 ml Collection Tube**.**
3. ☐ Transfer the entire mixture to the GD Column**.** Label the lid of the column.
4. ☐ Close the cap and centrifuge at 13,000 rpm for 2 minutes.
5. ☐ Discard the collection tube (pour liquid into beaker first) and place column in new 2 ml collection tube.

Step 3: Wash

1. ☐ Add **400 µl of W1 Buffer** to the GD Column.
2. ☐ Close the cap and centrifuge at 13,000 rpm for 30 seconds.
3. ☐ Discard the flow-through (pour into beaker) and place the GD Column back into the 2 ml Collection Tube.
4. ☐ Add **600 µl of Wash Buffer** to the GD Column.
5. ☐ Close the cap and centrifuge at 13,000 rpm for 30 seconds.
6. ☐ Discard the flow-through and place **the GD Column** back into the 2 ml Collection Tube.
7. ☐ Centrifuge again for 3 minutes to dry the column matrix.

Step 4: DNA elution

1. ☐ Transfer the dried **GD Column** to a clean 1.5 ml Eppendorf tube. Label the tube first!
2. ☐ Add 50 µl of preheated Elution Buffer to the center of the column matrix. NOTE: DO NOT TOUCH FILTER WITH PIPETTE TIP!
3. ☐ Let stand for 3-5 minutes or until the Elution Buffer is absorbed by the matrix.
4. ☐ Centrifuge at 13,000 rpm for 30 seconds to elute the purified DNA.