

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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HOW TO MAKE HORMONE IMPLANTS:

Ketterson Lab Protocol

Prepared by: Dustin Reichard under the tutelage of Nicki Gerlach - 01/18/12

Ketterson Lab Standard Implants

In the Ketterson lab, male implants contain 10mm (0.2 mg) of crystalline testosterone. Female implants contain 5mm (0.1 mg) of crystalline testosterone.

You Will Need:

1. Crystalline Hormone – Kept in locked drawer in back of lab
2. Silastic Tubing – Implant Supply Drawer
3. Silastic Glue (Medical Adhesive A) – Implant Supply Drawer, ***Make sure the glue has not dried out. If so, order more from Factor II, www.factor2.com (see below)
4. Forceps (Black and Blue) – Implant Supply Drawer
5. Hi-tech Packing Tool (Bent Paperclip) – Implant Supply Drawer
6. Ruler (flexible wing rulers work well)
7. Razor Blade – Implant Supply Drawer
8. Scoopula
9. Weigh Boats (x3)
10. Labcor Sterile Multi-channel Pipette Trough (to hold hormone while packing)
11. Aluminum Foil
12. 16G1½ Needles (purple) (x2)
13. 1 mL Disposable Syringes (x2)
14. Eppendorf Tubes (one for each implant)
15. Beaker (to hold Eppie tubes during autoclaving)
16. Autoclave Tape
17. Lab Tape
18. Gloves
19. Mask (optional; the hood sash does a good job of shielding your face)
20. Lab Coat – If you borrow from the lab, BE SURE TO WASH AT HOME before returning
21. Ziplock Bags (x2, for hormone contaminated waste (freezer bag) and completed implants (sandwich bag))

BEFORE YOU BEGIN:

1. You will also need to autoclave many of your materials under specific conditions on Day 1 (see step 6), so be sure to locate a suitable autoclave and schedule time in advance if necessary.

2. You will need access to a Biocontainment Hood on Day 2. In the past, we have used a hood in Malcolm Winkler's lab for hormone packing. You'll need to e-mail Malcolm (mwinkler@bio.indiana.edu) to request access to the hood or find another alternative.

DAY 1:

1. Determine how many implants you will need and plan to prepare 20-30% extra (i.e. if you need to implant 10 birds, plan to make 13 implants).
2. Using the ruler as a guide, cut your Silastic tubing with the razor into segments that are 2 mm longer than the total amount of crystalline hormone you want to fit into the tube. For example, if your implants need to contain 8 mm of hormone, cut your tubing into 10 mm segments. Try to make your cuts as straight up-and-down as possible.
 - a. For typical female implants (5mm of testosterone), cut tubing into 7mm segments, for typical male implants (10mm of testosterone), cut tubing into 12 mm segments.
3. Wrap your tubing segments in aluminum foil and tape with autoclave tape.
4. In separate aluminum foil wrap the forceps, Hi-tech Packing Tool (Paperclip), Scoopula, and 16G1½ Needles (x2) and tape with autoclave tape.
 - a. The 16G1½ needles will need to be broken off about 1 cm from the base **before autoclaving**. This has traditionally been done by inserting the needle in a drawer lock and bending it back and forth until it breaks (broken-off tip goes into sharps waste). If you choose this method be sure that there are no jagged edges and that the needle fits into the Silastic tubing.
5. Place your Eppie tubes in a beaker, cover with foil, and tape the top with autoclave tape.
6. Autoclave the items from steps 3-5. ****IMPORTANT:** Dow Corning recommends that the Silastic tubing be autoclaved at a maximum of 121°C and 15 PSI for 30 minutes. The Demas Lab has a bench-top autoclave that has been used in the past, but it may reach 123°C and 16 PSI.
 - a. NOTE: This is also an opportunity to autoclave the items you'll need to use for the implantation process including: forceps, cotton swabs, blunt probe, trochar syringe needles (1 for each subject)
7. While the autoclave is running (about 1 hr total) find the Silastic Glue, 2 Weigh Boats, 1 mL disposable syringe, gloves, and some paper towels and place them in the lab hood.
8. After collecting your materials from the autoclave, place everything in the hood and back-load the 1 mL syringe with about 0.1-0.2 mL of Silastic glue (or more if making lots of implants) and insert the plunger. The glue is too viscous to be drawn up.

9. WHILE WEARING GLOVES, pick up one end of each implant and insert the glue syringe's needle into the opposite end. Only the tip of the needle should be inside the tubing – keep in mind that our goal here is to only add 1 mm of glue to the tubing.
 - a. Slowly depress the plunger and as the glue starts to flow, rotate the implant and pull it slowly away from the syringe. Rotating the implant will ensure an even coating of glue, without holes for the hormone to leak through.
 - b. The glue should reach the end of the implant and not be bulging to the point where it will be difficult to load the implant in the trochar syringe.
 - c. Place each implant in the weigh boat to dry.
10. After gluing ONE END of every implant, cover the weigh boat containing the implants with another weigh boat and tape them together.
 - a. Label the top of the weigh boat with your initials and today's date.
 - b. Place the implants in the hood to dry overnight.
11. Throw away your gloves and paper towels.
12. Dispose of the glue syringe in the sharps container.

DAY 2:

1. KEEP IN MIND that after packing your implants you will be unable to enter the Ketterson Lab until you have showered and changed clothes. Be sure to plan accordingly as there will be NO EXCEPTIONS.
 - a. Typically you should schedule your implant packing for the afternoon and move all of the items that you'd like to take home with you (e.g., computer, jacket, etc) to the back of the lab near the back door.
2. Collect your implants, Silastic glue, forceps, Hi-tech packing tool (Paperclip), 2 new weigh boats, multi-channel pipette trough, scoopula, gloves, mask (optional), lab coat, paper towels, 2 ziplock bags, beaker of Eppie tubes, 1 mL disposable syringe, 16G1½ needle, and Hormone and proceed to the Biocontainment hood.
 - a. ****IMPORTANT** – you must keep a record of how much hormone you take from the stock. Be sure to weigh the bottle of hormone before and after packing.
3. You will need to sterilize the interior of the biocontainment hood before packing your implants. To do this:
 - a. Put on your lab coat.
 - b. Pull the hood sash the whole way down and turn on the UV light (usually a button above or to the right of the hood) for a minimum of 15 minutes. The sash will protect you from the UV light.
 - c. Turn off the UV light and raise the sash to the working height (labeled on left side of the hood). Turn on the fluorescent light and blower. Give the blower a few minutes to normalize.

- d. Spray the interior table of the hood with ethanol and wipe down with kimwipes.
 - e. The hood is now sterilized and you can put on your gloves and place your materials inside.
4. Back-load the glue syringe using the same technique as Day 1. Be sure that you save the plastic needle cover for disposal of the entire syringe.
5. Scoop or pour a small amount of hormone into one end of your pipette trough and pick up an implant with your forceps by the glued end.
 - a. Slide the implant across the bottom of the trough towards the pile of hormone. This can get messy as hormone will likely stick to the walls of the trough, the outside of the implant, and your forceps.
 - b. After some hormone has entered the implant, insert the paperclip into the implant to pack the hormone. Be sure to pack the hormone as evenly as possible in each implant.
 - c. Repeat these steps until there is about 1-2 mm left on the end of the tube.
 - d. Add glue to the end of the implant following the same procedure as Day 1 – the implant will be messy and covered in extra hormone. You can scrape the outside of the implant with your paperclip to improve visibility, but be careful not to flick hormone out of the implant.
 - e. Place the completed implant in the other weigh boat to dry while you're packing the remaining implants.
 - f. Repeat steps a-e until all implants are packed. You should be able to complete 8-10 implants per hour after you hit your stride. Plan accordingly.
6. Open the beaker of Eppie tubes and one-by-one transfer each completed implant into an Eppie tube. Place each tube + implant into a common ziplock bag for storage.
 - a. Be sure to label the outside of the ziplock bag with the hormone, your initials, and today's date.
7. Transfer any loose hormone on the weigh boat back into the hormone stock bottle.
 - a. REMEMBER to weigh the hormone stock bottle before returning it to storage. By dividing the amount of hormone used into the number of implants made, you can calculate the approximate weight of testosterone in each implant (should be 0.8-1.0 mg per 5mm implant).
8. Wipe down your tools and the exterior of the hormone bottles using the paper towels to remove excess hormone.
9. Wipe down the interior of the hood using ethanol and kimwipes. Turn off the light and blower.
10. Place all disposable materials that came into contact with hormone into the waste ziplock bag (weigh boats, paper towels, kimwipes, etc) and throw it in the **dumpster**.
11. Wash off your packing tools with copious amounts of water.
12. Return to the Ketterson Lab and knock on the back door to return the hormone and collect your belongings. Both the hormone stock bottles and the implants should be

locked in the hormone drawer. Other tools can be left in the back of the lab and put away when you return.

13. Go home, shower, and wash your clothes (don't forget your lab coat!) before returning the lab!

ORDERING SILASTIC GLUE:

Silastic glue for Implants: Silastic(R) Medical Adhesive A. Manufactured by Dow Corning, sold by the distributor Factor II:

phone: 1-800-332-8688

web: www.factor2.com

They sell 2 oz tubes for \$29.95 each

They also require an affidavit on file that you will not be using the adhesive in humans.