Freezer Bag Protocol:

This protocol is for plucking tissue and cataloging specimens that have been frozen in the -80C or -20C.

1. Set up an extraction station
   1. Bunsen burner
   2. Forceps
   3. Kim wipes
   4. Tubes (2ml or 1.5ml) and trays
   5. Permanent Marker
   6. Gloves
   7. Parafilm
2. Begin to defrost the specimen so that you can pluck tissue from the specimen (you do not want the specimen to be completely thawed because you do not want the RNA to degrade, try to extract while still a little frozen but not so that you will damage the morphology)
3. While the specimen is defrosting assign an HBG number to the specimen on a small piece of waterproof paper to include with the specimen (write using a sharpened #2 pencil) and on the extraction tube. Please use the correct RNA storage tubes (Blue cryotubes). Please check both Filemaker and the whiteboard so you know the next HBG number to assign. If you are still unsure check with Lab Manager. This is crucial; we cannot be assigning the same HBG number to 2 specimens so please be careful.
4. Take a pencil eraser sized chunk of tissue (more if a large specimen or less if small). In some cases, we will want to save 2 separate chucks of tissue in 2 separate vials if we are using it for different downstream applications (RNA extractions vs. DNA extraction) \*\*\*\*Note: Try to keep the specimen submerged or covered in RNAlater while plucking the tissue. It is important that the tissue be saturated with RNAlater at all times.
5. Preserve the tissue pluck in RNAlater ICE (blue in color) or RNAlater (clear)! (please do not preserve in ETOH, this will damage the RNA of we decide to do RNA extractions, RNAlater will allow us to extract RNA or DNA).
6. Place tissue pluck(s) directly in -80C into correct RNA storage box (blue ones)
7. Take a picture of the whole specimen with the HBG number below to document color (optional depending on application).
8. Put the specimen and waterproof paper in jar with 70% ETOH
9. Database the specimen(s) in Filemaker (you can do this after you are done plucking for the day which may be easier and more time effective)
10. Repeat for all
11. Place specimen in an area where HBG knows that they have been cataloged but need identification
12. Update the whiteboard with the date and the last HBG number assigned for the day
13. Once identified and cataloged correctly, place a NEW label (printed on Thermal Printer) in the jar and put the jar on the shelf (organized by collection locality)

Extra Notes: if there are multiple individuals of the same species from the same locality they can all be placed in a single jar UNLESS we need them separated for population genetics work. Please pluck tissue for all representatives and assign with HBG #A, #B, #C (we have tags that can be placed around the tail so that each can be identified.

If there are different species in a the same bag please separate and assign different HBGs