	<b>Cryogenic Seed Storage</b>		
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	SOP Owner: Greg Holman	SOP Approver: Dr. Stephanie Greene	

## **1. Introduction & Purpose**

NLGRP receives seed from the National Plant Germplasm System (NPGS) which consists of 25 gene banks throughout the United States. We also receive seed from public institutions, non-governmental organizations, private companies and other agencies from all over the United States and around the world.

The packaging group is responsible for handling, packaging and storage of received seed.

## **2. Scope**

The following instructions describe packaging and storage procedures for seed shipments to be stored in liquid nitrogen.


## **3. Definitions & Abbreviations**

The following definitions and key terms are pertinent to this SOP:

Cryogenic storage	Storage in or over liquid nitrogen
GRIN-Global	Germplasm Resources Information Network
Tare, On Tare	To "tare" a scale means to reset the scale to zero after an empty container is placed on the weighing program, in order to subsequently display on the weight of the contents of the container.
On Tare	Tare weight is accounted for in analytical (scientific) scales which include a button that resets the zero of the scale display when an empty container is placed on the weighing platform, in order to subsequently display only the weight of the contents of the container.
Liquid Nitrogen, LN <sub>2</sub>	Liquid nitrogen is nitrogen in a liquid state at an extremely low temperature (-196°C, -320°F). It is a colorless clear liquid that can cause rapid freezing on contact with living tissue.

## **4. Occupational Health & Safety**

**Liquid nitrogen** is a very cold, colorless, clear liquid with a boiling point of -196° Centigrade (-320° Fahrenheit). It can cause rapid freezing on contact with living tissue, which can result in cold burns or blindness. Liquid nitrogen as a gas can result in extremely low oxygen levels which can cause unconsciousness or death. Adherence to all safety regulations is required. Gloves and face shields need to be used when working around Liquid Nitrogen due to extreme cold temperatures which can burn exposed skin.

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## **5. *Materials and Equipment***

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### **Materials:**

- Lab tape of various colors
- Heat shrinkable flexible polyolefin tubing of various sizes (1/8, 1/4, 1/2, 3/4, 1 inch)
- Permanent Marker
- Cryo canes
- Sleeves for Cryo canes
- Round bottom, polypropylene biofreeze vials, 5 ml.


### **Equipment:**

- Liquid Nitrogen Tanks
- Aluminum Cryogenic Boxes and Lids (11 in x 2.5 in x 2.5 in)
- Heat Sealing Machine
- Honeywell Bar Code Scanner, Model 1400G
- Ohaus Analytical Plus Scale, Model AP250D
- Computer with access to Grin-Global and Grin Local

## **6. *Procedure***


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- 6.1** Check Room **223** “Ready to Package” cart for a tray of samples (order). Each tray has one or more tape labels with seed shipment information that will remain on the tray until the order is completely packaged. Seed Samples for cryopreservation are labeled with a NSSL number which has a suffix of .51.
- 6.2** Each Seed Sample identified as going into Liquid Nitrogen (or Cryo) is given a Liquid Nitrogen Tank location, weighed, and the seed number is calculated.
- 6.3** Liquid Nitrogen tanks contain aluminum Cryo-Boxes that are arranged in sections. Each section within the tank has a different colored lid:
- Section 1 – Red
  - Section 2 – Blue
  - Section 3 – Gold
  - Section 4 – Green
  - Section 5 – Silver
  - Section 6 – Black
- 6.4** Each tank is also assigned a different lab-tape color for writing locations on the lids of the boxes. Ex: Tank 6 has yellow lab tape; Tank 15 has pink lab tape. PVPO will always be in Section 6 of the designated tank. If the section is marked for PVPO, lids will be purple. PVPO locations will always have yellow lab tape with purple lids.

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Noxious weeds will always have silver lids with red tape and will be placed in a designated marked tank.

- 6.5** Open the GRIN-Global curator tool using ARS email address as user name and current password.
- 6.6** The data base will complete an update. A green status bar at the bottom of the screen will show the progress of the update.
- 6.7** Click the Check-in Wizard to begin the weighing process.
- 6.8** Click the Check-In button a drop down menu will appear with an option for *Weight*.
- 6.9** Click the *Weight* button to check in regular seed, JPR, PVPO, or GMO.
- 6.10** There should be barcode labels in the tray with corresponding Germination Cards and samples. Use the sticker for the sample about to be checked in.
- 6.11** Scan the barcode label for that sample. GRIN-Global will take a couple of seconds to load the barcode information.
- 6.12** The seed sample is now ready to check into GRIN-Global. Enter weight information.
- 6.13** Pour the entire sample onto the Mettler scale.
- 6.14** Put 10 or 20 seeds (depending on size) onto the Ohaus scale. Smaller seeds use 20 seeds (Ex. Tobacco) or 10 seeds for larger seeds. (Ex. Peanuts) Enter the sample size into GRIN-Global.
- 6.15** Wait for the "S" (stable) to appear on the Ohaus scale with the weight measurement.
- 6.16** Press the print button on the Ohaus scale to send/fill the 10 or 20 seed weight into *Known Weight* field in the program.
- 6.17** Empty seeds from the Ohaus scale back into the Mettler scale.
- 6.18** Press the print symbol on the Mettler scale to send/fill the *Total Sample Weight*, *Total Seeds*, and *Hundred Seed Weight* fields on the program. The GRIN-Global database calculates these weights based on the seed amount and weight entered during the first weight measurement.
- 6.19** Every tank section has approximately 55 boxes. The technician will always be working in the next available box. Enter the tank and section of the location where the Cryo-Box samples will be going. The GRIN-Global database will keep that location in the selected field until a new location is entered. When there is a PVPO sample to check in, go downstairs to Room **148** and pull the last partial Cryo-Box of PVPO from the Liquid Nitrogen Tank. Once the PVPO is checked in, those samples will be added to the partial Cryo-Box and returned to the appropriate Liquid Nitrogen Tank.
- 6.20** Press the *Save/Print* button on the program.


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- 6.21 A pop up will ask for a Serial Number. Scan the Serial Number label to print and save to GRIN-Global.
- 6.22 The seed sample is sealed in a cryo tube. Pour seeds from Mettler scale into the Cryo-Tube using funnels on a lab stand. Put the serial number label in the tube with the seed. Very small seed goes into mini-tubes (or cryo-vials) and then onto Cryo-Canes which are covered by a sleeve to protect them from being misplaced.
- 6.23 The *Location Cryo Label* and *Germination Card Label* will print from the Zebra printer.
- 6.24 A location Cryo-Label with tank location and serial number label (the NSSL number for that shipment) is applied around each Cryo-Tube.
- 6.25 Place the *Germination Card Label* on the right side of the *Germination Card* under the *STAT CMT* field of the card.
- 6.26 Click the *Next Weight Field* to go on to the next sample. Put Cryo-Tube in the aluminum Cryo-Box.
- 6.27 When saving for the day, click *Done*. Look at the data to confirm its accuracy. A prompt will appear to *Save*. Click *Yes* to save the data to GRIN-Global.
- 6.28 A grey screen will come up on the monitor showing the new samples scanned. (This may take a while to load if many samples were checked in).
- 6.29 Once the screen has popped up with new samples, click the *SAVE* button.
- 6.30 A prompt will appear indicating the *SAVE* was successful.
- 6.31 Close the program to complete.
- 6.32 When orders are fully checked in and no more samples need to be checked in for that order place labeling tape with information from front of the tray on **Greg's** desk to be entered into a database (or spreadsheet) as packaged and placed in cold storage. The Check Database is located at T\GRIN\Check.
- 6.33 For location accuracy, a final check is done on the samples and the cards. Then the samples are put into the designated Liquid Nitrogen Tank location.
- 6.34 At the end of the day, place tray in Room **223** on a 3 tiered cart to store until the end of the week.
- 6.35 At end of the week put the Cryo-Boxes in Room **148** in specified location designated on aluminum Cryo-Box lids.

## **7. *Related Documents, flowcharts and Links***

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The following **flowchart** ([provide links](#)) are pertinent to this SOP:

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## ***8. Staff Training and Competency***

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Greg Holman, Biological Sciences Technician.

Training is required to work safely around LN2 tanks and in LN2 vaults.

## ***9. Infrastructure & Work Environment (I&WE)***

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Access is restricted in Vaults. Vault Access (Link to spreadsheet showing who has vault access)

## ***10. Proactive Management***

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## ***11. References***

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## ***12. Revision History***

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<b>Effective Date of the SOP</b>	<b>Version #</b>	<b>Description</b>	<b>Reviewed By</b>

### **Citation**

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