

# Securing Citrus

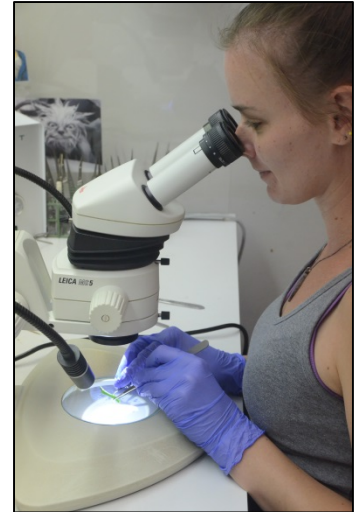
## USDA-ARS National Plant Germplasm System (NPGS)

### September 2016

**Goal: Secure the NPGS pathogen-free clonal collection of citrus using cryopreservation technologies by 2017. Develop strategies to use seeds to back-up wild species of citrus accessions valued for their allelic diversity.**

Large-scale citrus cryopreservation efforts using methods developed at the National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, Colorado are underway. This is an unprecedented collaboration between the Plant and Animal Genetic Resources Preservation (PAGRP) and the Plant Germplasm Preservation Research Unit (PGPRU) at the NLGRP and the National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) in Riverside, California.

Twelve to sixteen citrus accessions are sent weekly (Vicki Newman) from the NCGRCD to the NLGRP. The accessions are assigned inventory numbers by Renee White and then cryopreserved by technicians Remi Bonnart, Bradford Hall, and Ashley Shepherd, and Dianne Skogerboe. A subset of the processed accessions are warmed and micrografted to assess the viability.



Technician Ashley Shepherd excises buds from Citrus twigs

NCGRCD technician Brittany Moreland is cryopreserving 25 accessions in Riverside. These accessions will be sent to NLGRP for long-term storage.

When viability assessments are complete, the cryopreserved citrus will be transferred to the NLGRP storage vault and documented in GRIN-Global.

The cryopreservation portion of the project is expected to continue through February 2017. Viability assessments on the processed accessions will continue throughout 2017.

This project is supported by USDA-ARS National Program Staff, Plains Area, Pacific West Area, NCGRCD, PAGRP, PGPRU and the California Citrus Research Board.

### Cryopreservation Progress

Goal: 479 accessions

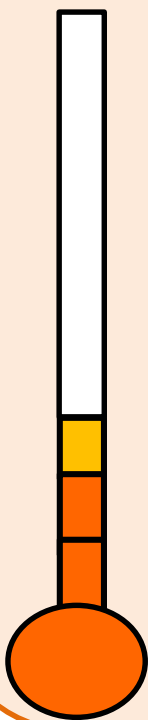
245 accn. remaining

25 accn. to be processed by NCGRCD

38 accn. processed Sept 2016

40 accn. processed July-Aug 2016

131 accn. processed 2012-2015



# Securing Citrus

## USDA-ARS National Plant Germplasm System (NPGS)

### October 2016

In October, 48 citrus accessions were cryopreserved, bringing our total number processed to 257. NCGRCD technician Brittany Moreland visited NLGRP for a 4 week leadership training. One of her training goals was to refine her cryopreservation skills to fully implement cryopreservation technologies at the Riverside location. As time permits, viability assessments (micrografting) are being performed. In addition, SOPs for citrus cryopreservation are under development.

Citrus cryopreservation process:



Citrus twigs are harvested in RIV and sent to NLGRP



Budwood is sectioned and sterilized



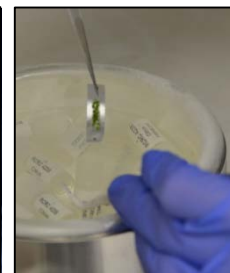
1.5 mm shoot tips (175 per accn) are excised



Shoot tips are treated with cryoprotectants



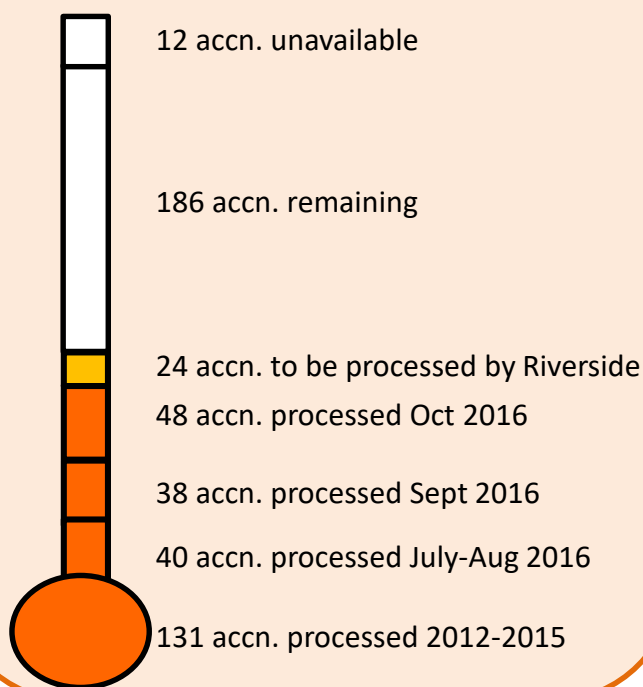
Shoot tips are placed into droplets of PVS2 on foil strips



Foil strips are plunged into LN, loaded into vials, and stored in tanks

### Cryopreservation Progress

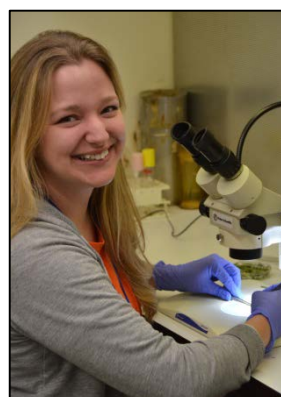
Goal: 479 accessions



The California Citrus Research Board awarded us funding for technical support for the viability assessments that will take place between March and Sept 2017.

Published:

Volk GM, Bonnart R, Shepherd A, Yin Z, Lee R, Polek ML, Krueger R. 2016. Citrus cryopreservation: viability of diverse taxa and histological observations. *Plant Cell Tiss Organ Cult*. Online first.



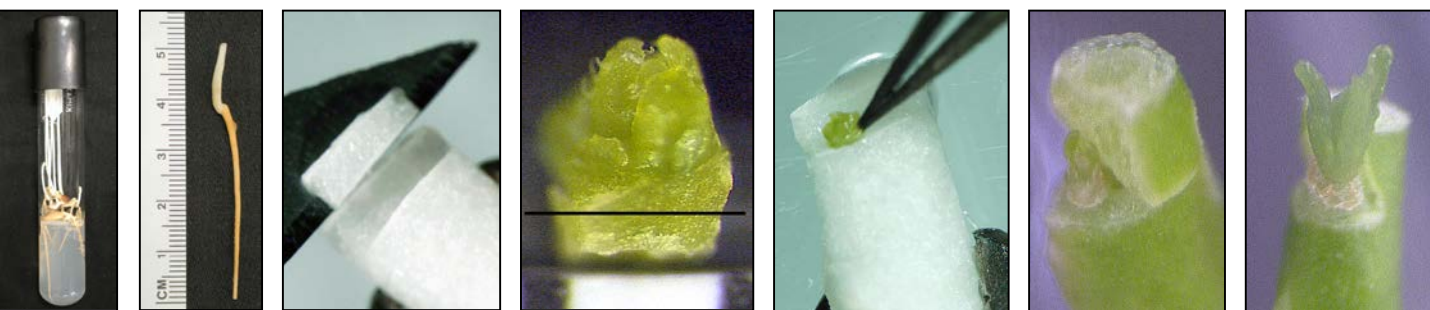
NCGRCD Technician Brittany Moreland excises shoot tips from Citrus twigs.

# Securing Citrus

## USDA-ARS National Plant Germplasm System (NPGS)

### November 2016

In November, 53 citrus accessions were cryopreserved, bringing our total number processed to 334. As time permits, cryopreserved accessions have been micrografted for viability assessments. One vial of 10 shoot tips is retrieved from the cryotank and warmed. Shoot tips are then rinsed in 1.2 M sucrose for 20 minutes to remove cryoprotectants, and then carefully trimmed and placed onto etiolated seedling rootstocks (see below). Data are collected after 8 weeks of regrowth.



Grow etiolated seedlings in vitro for 3-7 weeks, then trim before use.

Cut shelf into the top of the seedling.

Make a fresh cut on the bottom of the shoot tip.

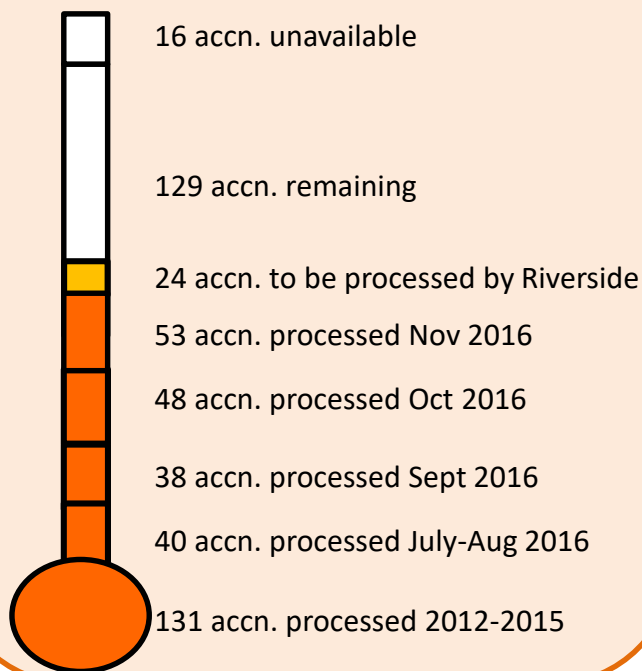
Place shoot tip on rootstock shelf and return to recovery medium.

6 days of recovery

13 days of recovery

### Cryopreservation Progress

Goal: 479 accessions



Test tubes of micrografted citrus accessions in the growth room (above).

PAGRP technician Bradford Hall cryopreserved citrus shoot tips (right).



# Securing Citrus

## USDA-ARS National Plant Germplasm System (NPGS)

### December 2016

In December, 40 citrus accessions were cryopreserved at NLGRP. The U.S. national collection of citrus genetic resources is a collaborative effort between the NCGRCD and the University of California, Riverside. The collection includes 1224 accessions of *Citrus* spp., 203 accessions represent the closely related genera *Poncirus*, *Fortunella*, and *Microcitrus*, and 71 accessions represent 26 more distantly related Aurantioideae genera. Trees are maintained as (1) duplicate pathogen-tested, protected trees maintained under screen, (2) duplicate greenhouse potted trees, and/or (3) field plantings. The pathogen-tested, protected trees are the focus of this cryopreservation project. These accessions are valued for their specific genetic combinations, and are thus cryopreserved as clones in the form of shoot tips.



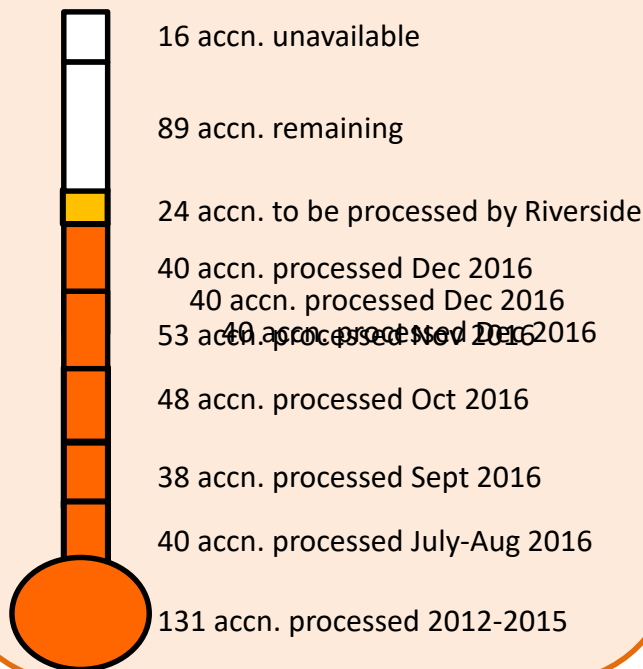
NCGRCD screenhouse *Citrus* collection.

The number of accessions of some commonly known citrus types that are being cryopreserved are listed below.

Species	Common name	Accn. (no.)
<i>C. aurantifolia</i> ,		
<i>C. latifolia</i>	lime	8
<i>C. aurantium</i>	sour orange	6
<i>C. clementina</i>	clementine	19
<i>C. limon</i>	lemon	45
<i>C. maxima</i>	pummelo	45
<i>C. medica</i>	citron	5
<i>C. paradisi</i>	grapefruit	19
<i>C. reticulata</i>	mandarin	52
<i>C. sinensis</i>	orange	125
<i>C. unshiu</i>	mandarin	43
<i>Fortunella</i> sp.	kumquat	7

### Cryopreservation Progress

Goal: 479 accessions



PAGRP technician Dianne Skogerboe prepares citrus for cryopreservation.



# Securing Citrus

## USDA-ARS National Plant Germplasm System (NPGS)

### January 2017

We only have 34 citrus accessions left to cryopreserve!

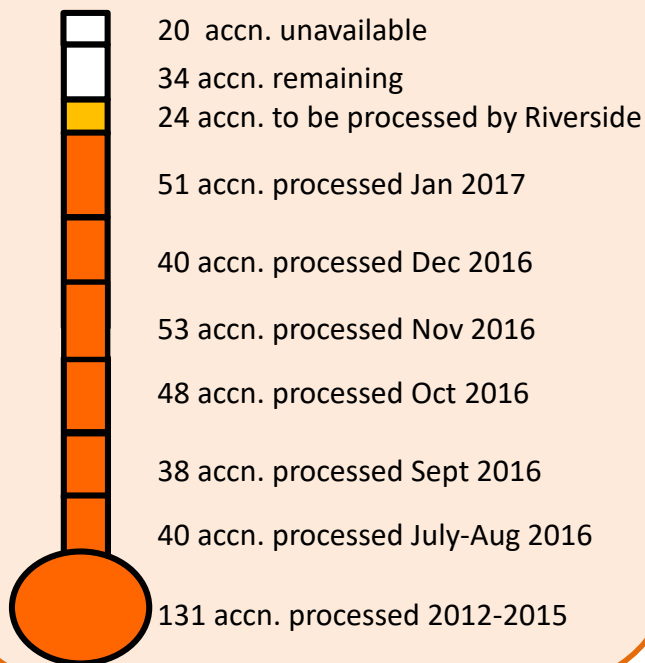
Preserved seeds and pollen provide an alternative, and likely less labor-intensive, method to secure genes of interest in citrus wild relatives. Citrus seeds are not orthodox—meaning that they can't be dried like most seeds and stored in a  $-18^{\circ}\text{C}$  ( $0^{\circ}\text{F}$ ) freezer. Our cryopreservation methods balance seed size with tolerated water content. In species including *C. medica*, *C. celebica*, *C. limon* and *C. clementina*, and possibly *C. aurantium*, *C. reticulata* and *C. sinensis*, we can successfully cryopreserve whole seeds. If seeds are too sensitive to the drying methods (e.g., *Poncirus trifoliata*, *C. reshni*, *C. sphaerocarpa*, *Aegle marmelos*, and *Severinia buxifolia*), embryonic axes can be cryopreserved after excision, desiccation, and superfast cooling at  $\sim 100^{\circ}\text{C}/\text{sec}$ . There is one caveat: the quality and age of the seeds affects how well they respond to both desiccation and rapid cooling.



Through the use of controlled pollinations (to avoid hybridization), citrus germplasm valued for its genetic diversity can be cost-effectively conserved as seeds. This strategy could be implemented for many of the remaining 1000 accessions in the Riverside citrus collection.

### Cryopreservation Progress

Goal: 479 accessions



Seeds of A) *P. trifoliata* B) x *Citroncirus* sp. and C) *C. moi* prior to embryonic excision (above). Plants recovered after x *Citroncirus* embryos were excised and desiccated (above right).

Technician Jeff Zdunek (CSU) excises embryos from citrus seeds (right).



# Securing Citrus

## USDA-ARS National Plant Germplasm System (NPGS)

### February 2017

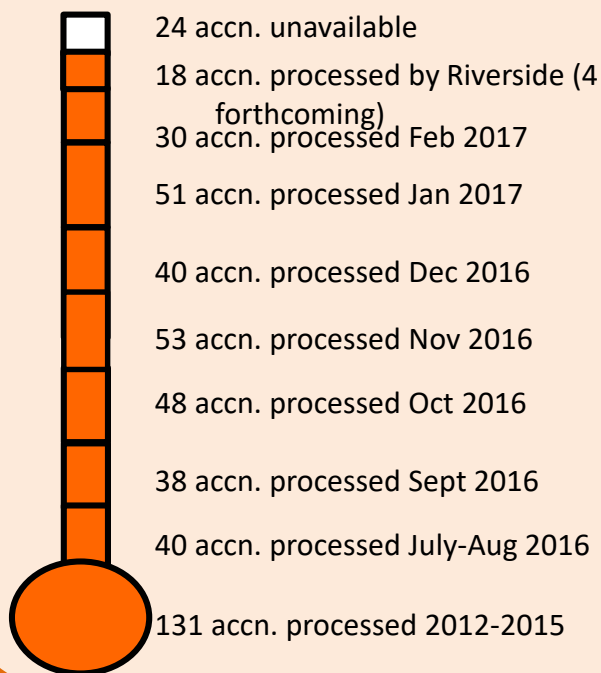
We did it! This has truly been a collaborative effort. The Fort Collins team finished cryopreserving their remaining citrus accessions on Feb. 23. Our Riverside contingent will finish processing her last 4 accessions by March 10. This completes the 2016-2017 cryoprocessing season of the NPGS pathogen-tested, clean, clonally propagated citrus accessions! We have already micrografted 60 accessions (average viability of 60%). Two PGPRU technicians will continue to work full-time on the project to micrograft the remaining accessions over the next few months. We expect to have our results for the first replicate of the viability assessments by summer 2017. "Unavailable" accessions will be processed Fall 2017.

#### 2016-2017 Securing Citrus Project Summary

- 322 Citrus accessions cryoprocessed
- 53,229 1 mm shoot tips manually excised
- 1661 hours spent excising 1 mm shoot tips
- 644 hours spent cryopreserving shoot tips
- 60 accessions already micrografted for viability assessments
- 3220 total shoot tips to be micrografted to assess viability (rep. 1)

### Cryopreservation Progress

#### Goal: 479 accessions



A. Citrus cryopreservation team: Dianne Skogerboe, Ashley Shepherd, Brittany Moreland, Remi Bonnart, and Bradford Hall.

B. Many members of the NLGRP citrus "Securing Citrus" project effort.

C. Riverside collection maintenance and cryoprocessing team.

D. Remi Bonnart continues to micrograft citrus.

