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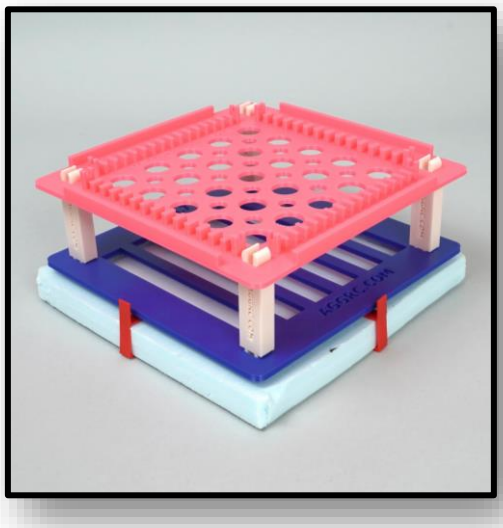
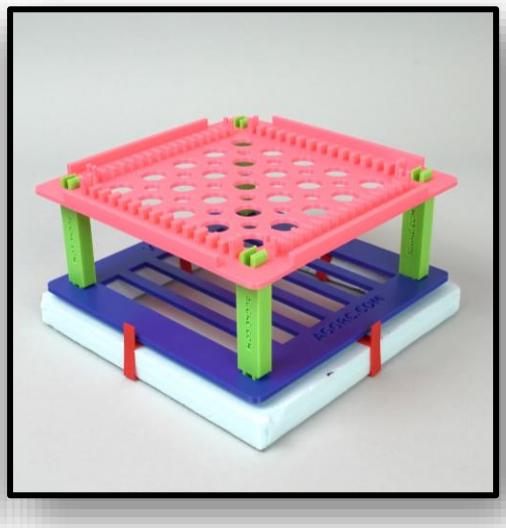
	MANUAL Open Hardware	EFFECTIVE DATE: June 2024
	SUBJECT CryoKit 2.4.7 User Manual	REVISED/REVIEW: Brittany Ratliff/Yue Liu

CryoKit V2.4.7 User Manual

AGGRC





Technical Support:

Brittany Ratliff, Fabrication and Design Lead, (bratliff@agcenter.lsu.edu)

Dr. Yue Liu, Director of Technology Development (yliu@agcenter.lsu.edu)

Please send us your comments and suggestions!

Scan to learn more about the AGGRC!

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Background

The Positional Cooling Platform Device (PCPD, known as the 'CryoKit') is a 3-D printable device that supports reproducible cryopreservation with standardizable cooling rates (5 to 35°C/min). The CryoKit accommodates French straws (0.25 and 0.5 mL), and cryopreservation vials (0.5 and 2 mL). Development of germplasm repositories to preserve genetic resources of aquatic species is impeded globally by a lack of standardized, inexpensive, reproducible, and portable cryopreservation technologies. The CryoKit can be used with a standard polystyrene container that holds liquid nitrogen for on-site cryopreservation for aquatic species and is distributed as open hardware. Creative Commons license: CC-BY-NC-SA.

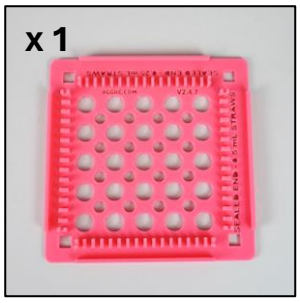
The CryoKit was developed by the Aquatic Germplasm and Genetic Resources Center (AGGRC) at the Louisiana State University Agricultural Center by E Hu, Bill Childress, Brittany Ratliff, Victoria Byrd, Yue Liu, Terrence Tiersch, and colleagues, in partnership with the Zebrafish International Resource Center (ZIRC) and USDA National Animal Germplasm Program (NAGP). This work was funded primarily by NIH Office of Research Infrastructure Program (ORIP). This device is part of a multi-year project for development of a series of open-hardware devices to support standardized and reproducible tools for research communities that use aquatic models to study human disease.

Details about the development of the CryoKit can be found in the publication: *Hu, E., Childress, W., & Tiersch, T. R. (2017). 3-D printing provides a novel approach for standardization and reproducibility of freezing devices. Cryobiology, 76, 34–40*. The 3-D files for the CryoKit can be found on the AGGRC website and NIH 3D Print Exchange: **https://3d.nih.gov/users/aggrc.lsuac**

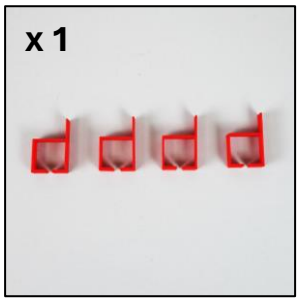
Please visit AGGRC.com to learn more about our work.

Disclaimer: Some materials (e.g., polystyrene) can heavily influence cooling rates. Other materials may be used; however, the cooling rate must be calibrated. Tracking the cooling rate every run is strongly recommended. Not all assembly configurations are described. Users are responsible for the safe handling of liquid nitrogen and samples. The cooling rate may vary slightly between runs. Tampering with thermocouples may lead to erroneous temperature data.

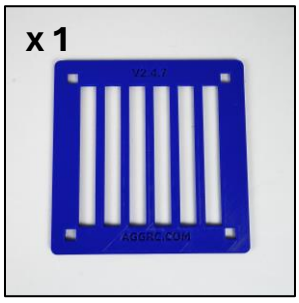
CryoKit Materials



Top Rack*
Required

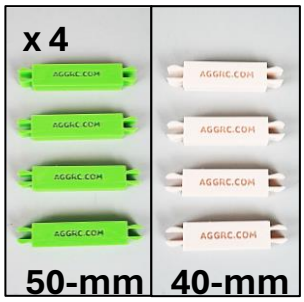


Clips*
Required

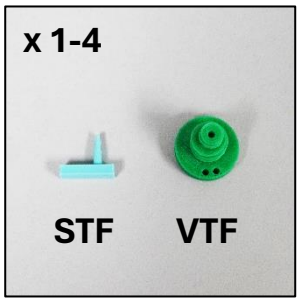


Bottom Rack*
Optional

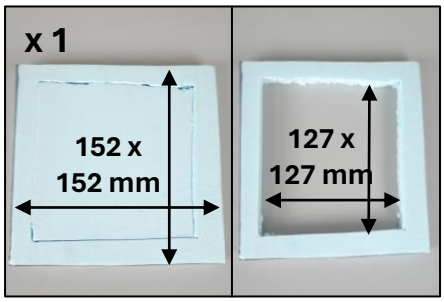
Straw Sealers*
0.25 mL or 0.5 mL
Optional



Claw Locks*
50-mm or 40-mm
Optional



Straw Thermocouple Fastener (STF)*
or
Vial Thermocouple Fastener (VTF)*
Required



Complete or Outer Polystyrene Float
Required

Lowe's (in US)
Polystyrene 0.55'' Insulation Board
(Item # 15348)
Complete Float:
152 x 152 mm
Middle Section:
127 x 127 mm

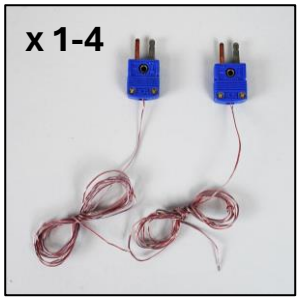
***3-D files can be found at:**
https://3d.nih.gov/users/aggrc.lsuac

AGGRC

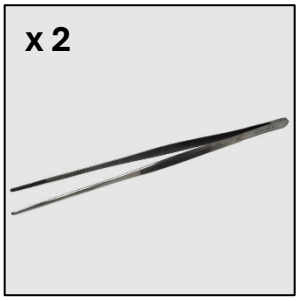
Note: Color will vary for 3-D printed parts.

Supporting Materials

Data Logger or Thermometer
Required



TL0021 Type-T Thermocouple
Required



12” Forceps
Required

Liquid Nitrogen
Required

Cryoprotectant Buffer Solution
Required

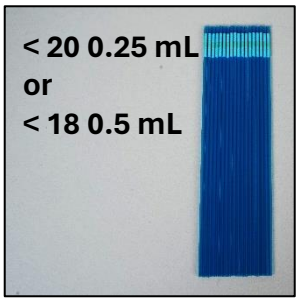
Cryogenic Gloves
Required

Safety Glasses
Required

Polystyrene Containers
Required

<u>Polar Tech Industries</u>
1) Insulated Bio Foam Container
(Item # 214 F)
2) Nestable Insulated Shipper
(Item # NS-9KD)

Supporting Materials (cont'd)

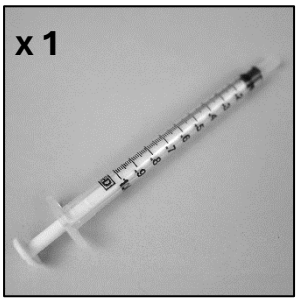


Cryopreservation French Straws

OR

Cryovials

Corning® 2 mL Cryovials
(Item # 431386)
Bank-It™ 0.5 mL Cryovials
(Item # 374075)



1 mL Syringe
Required

200 µL Pipette Tip
Optional

For Straws + Visotube

For Vials

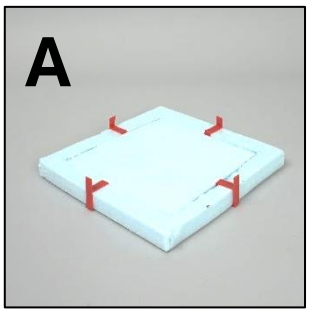
Daisy Goblet

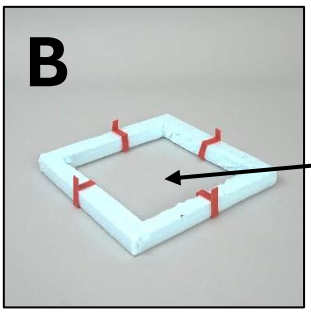
Cane

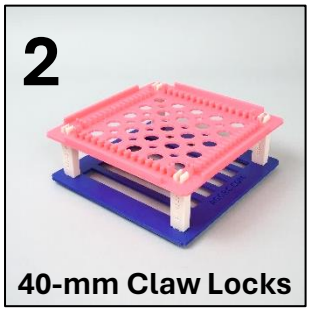
Freezer Box

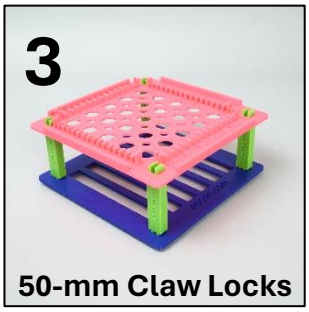
Required, depending on storage container preference

Cooling Rate Configurations









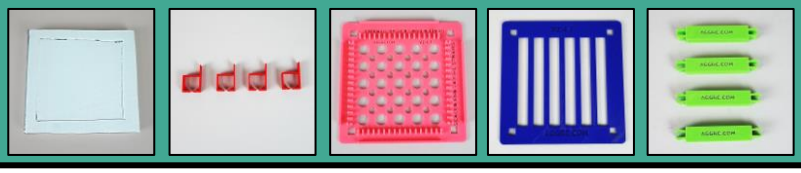
Cooling Rate (°C/min)	0.5-mL Straw Configurations	0.25-mL Straw Configurations
≈ 5	3+B	3+B
10-15	1+A	2+B
20-25	1+B	1+A

Cooling Rate (°C/min)	Bank-It™ 0.5-mL Cryovial Volume & Configurations	Corning® 2-mL Cryovial Volume & Configurations
5-7	500 µL, 2+A	500-1000 µL, 2+A
9-11	100 µL, 2+A	500 µL, 2+A
14-17	51-100 µL, 3+A	100 µL, 3+A
24-25	50 µL, 2+A	N/A

This User Manual details the assembly and cooling of configuration 1+A. However, other configurations still follow this manual.

Assembly

Materials:



1) Select Assembly Configuration

1.1) Select an assembly configuration. Refer to the chart on page 5.

Note: Not all configurations are shown. Please assess the cooling rate for your configuration and cooler arrangement.

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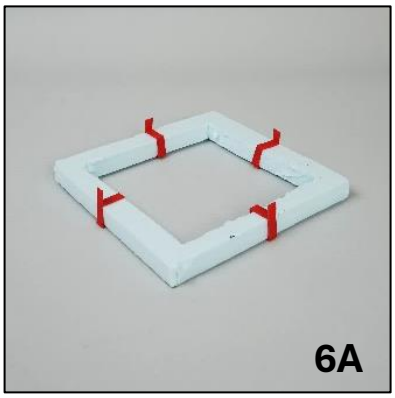
2) Assemble Polystyrene Float

2.1) Using a sharp blade or foam cutter, cut a square from the polystyrene sheet with dimensions of 152 mm^2.

2.2) Using the same piece, cut the middle section with dimensions of 127 mm^2.

2.3) Gently open each **Clip** and place them around each side of the outer **Polystyrene Float** (Figure 6A).

2.4) Place the inner **Polystyrene Float** in the center if using configuration **A.**



6A

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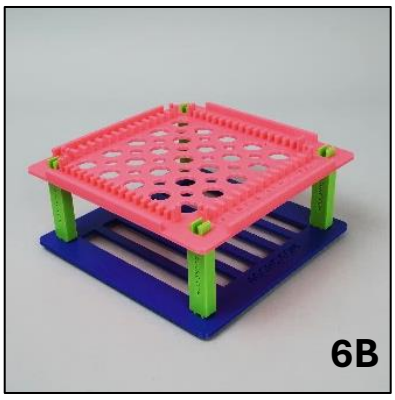
3) Assemble Cooling Platform

Note: Skip this step is using configuration **1**.

3.1) Depending on your selected configuration, insert either side of the **40-mm** or **50-mm Claw Lock** into the holes on the **Bottom Rack.**

3.2) Place the **Top Rack** on top of the **Claw Locks**. Align the holes and press each corner to snap them into place (Figure 6B).



6B

Preparation

Materials:





4) Pre-Cool

4.1) Put on **Cryogenic Gloves** and **Safety Glasses**.

4.2) With the smaller **Polystyrene Container** inside the larger, add liquid nitrogen to the smaller **Polystyrene Container** to a depth of 10-cm (9.5 cm – 10.5 cm, about 5 L). Marking the 10-cm line in the smaller container may help with filling (Figures 7A, 7B).

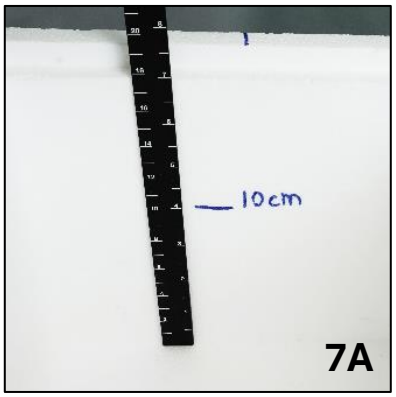
Note: The liquid nitrogen level decreases by 0.5 - 1 cm per cooling run, which does not affect performance. However, the liquid nitrogen level should be adjusted if it has dropped below 9.5-cm. Check the level after every cooling run.

4.3) Pre-cool the **Polystyrene Float**. Lower the float onto the liquid nitrogen and close both **Polystyrene Containers** (Figures 7C, 7D).

Note: If the **Polystyrene Float** is not pre-cooled, the liquid nitrogen will boil when samples are lowered which will cause variable cooling rates.

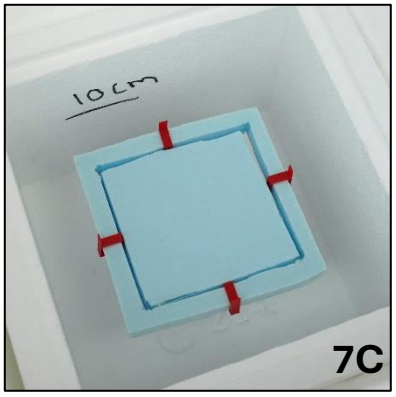
4.4) Set an equilibration timer for a minimum of 5-min after closing both **Polystyrene Containers**, ensuring a temperature gradient is established. The temperature gradient must be established before cooling samples.

Note: After equilibration, both **Polystyrene Containers** must remain closed.

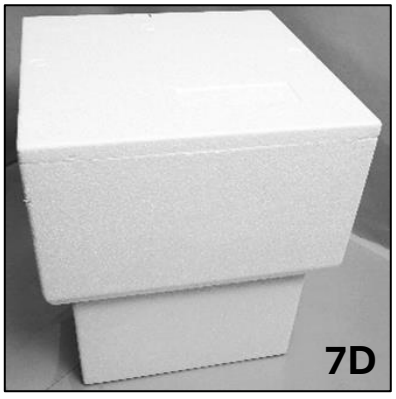


7A


7B

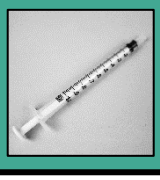


7C


7D

Preparation

Materials:




5) Prepare Samples

5.1) Set a timer and equilibrate your sample and **Cryoprotectant.**

5.2) Considering the number of **Thermocouples** planned to be used, fill up to 17 0.25-mL straws , 15 0.5-mL straws, 24 2-mL Corning® vials, or 15 0.5-mL Bank-it™ vials.

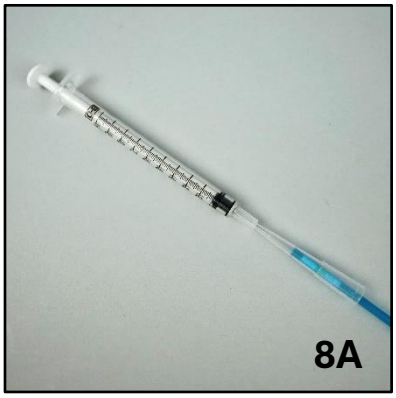
5.3A) To fill **French Straws**, place the small end of the **200-µL Pipette Tip** into the **1-mL Syringe**. Insert the cotton end of the **French Straw** into the **200-µL Pipette Tip** (Figure 8A). Insert the end of the straw into solution and pull back on the plunger to fill the straw with the appropriate volume (Figure 8B). Remove the straw from the solution and pull the syringe plunger all the way back.

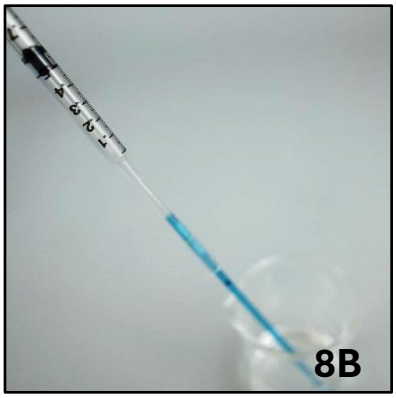
5.3B) To fill **Cryovials,** use the **Syringe** or a pipette to measure the appropriate volume of solution and fill the **Cryovial**.

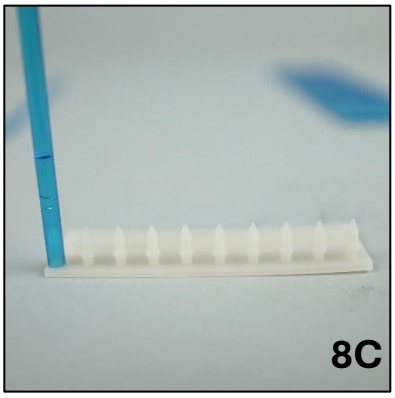
5.4A) If there is no available process in your laboratory to seal **French Straws**, insert one of the tips of the **Straw Sealers** into a **French Straw** (Figure 8C)**,** then bend the straw to break off the **Straw Sealer** and create a seal (Figure 8D).

5.4B) Close the filled **Cryovials** with their original cap**.**

5.5) Fill 1-4 **French Straws** or **Cryovials** with the approximate volume of **Cryoprotectant** solution for your **Thermocouples**. Keep these containers unsealed or uncapped and separate them from the sample-holding straws for step 6.


8A


8B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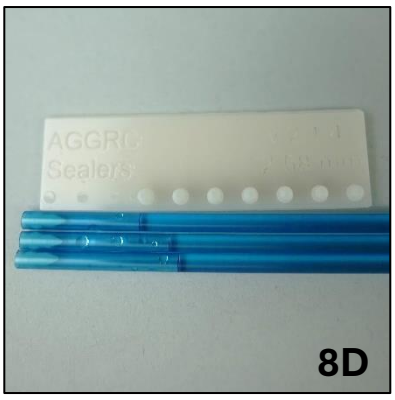
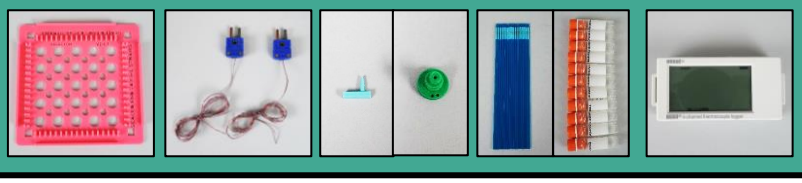

8D

Preparation

Materials:



6) Fasten Thermocouples

6.1A) Identify which side of the **Top Rack** holds 0.25 or 0.5-mL straws. Insert the end of the **Thermocouple** through any slot on the correct side of the **Top Rack**. Insert the end of the **Thermocouple** through the hole on the **Straw Thermocouple Fastener (STF)** (Figure 9A).

6.1B) Insert the end of the **Thermocouple** through any slot on any side of the **Top Rack**. Insert the end of the **Thermocouple** through the top and into a hole on the edge of the **Vial Thermocouple Fastener (VTF)**, then insert the end back through the other hole on the edge of the **VTF**. Then insert the **Thermocouple** through the hole in the center of the **VTF** (Figure 9B).

6.3) Insert the **Thermocouple** into the **French Straw** or **Cryovial** filled with **Cryoprotectant** solution that was set aside in the previous section. Position the **Thermocouple** so the tip is centered in the straw or vial.

6.4A) Align the tip of the **STF** with the **French Straw** and gently press the **STF** into the straw to fasten the **Thermocouple** (Figure 9C).

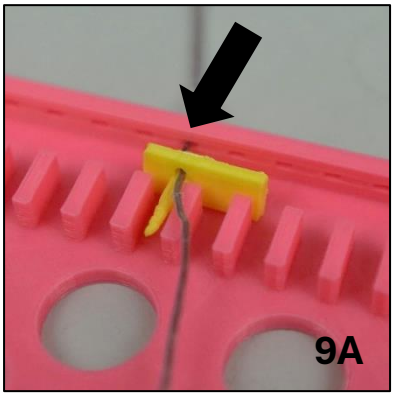
6.4B) Screw the **VTF** onto the **Cryovial**. Gently pull the **Thermocouple** to fasten it to the **VTF**.

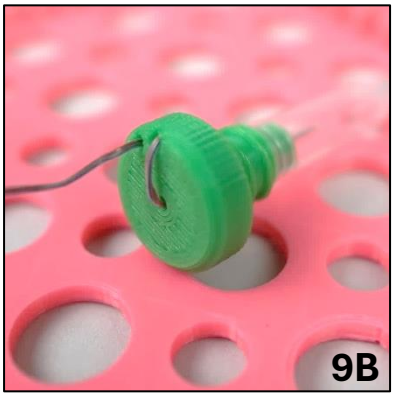
6.5) Repeat the above steps for any additional **Thermocouples,** then connect the **Thermocouples** to the **Data Logger.**

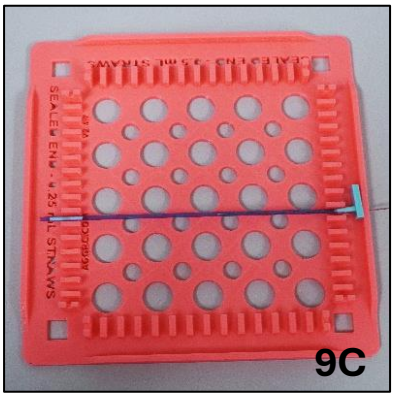
6.6A) Place all **French Straws** on the **Top Rack** and secure them by placing two additional unfilled **French Straws** into the straw locks (Figure 9D).

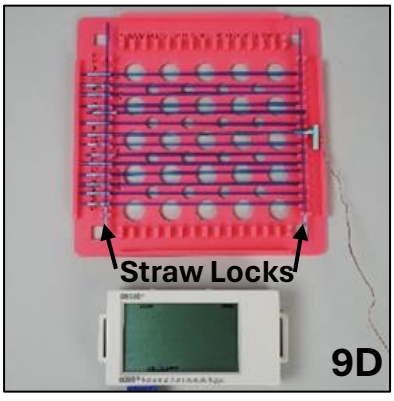
Note: It's recommended to use different colored straws for the locks so it's easier to identify them after cooling.

6.6B) Place **Cryovials** on the **Top Rack.**



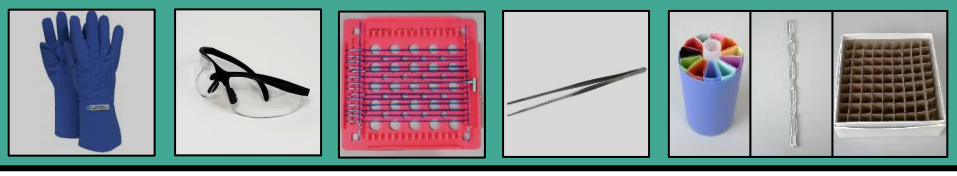




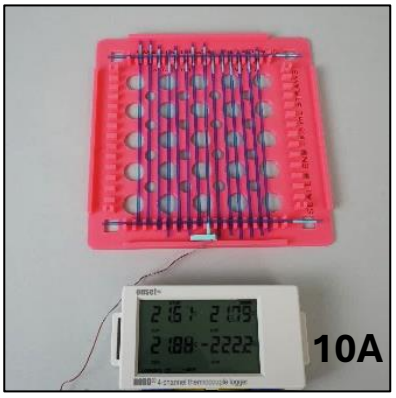


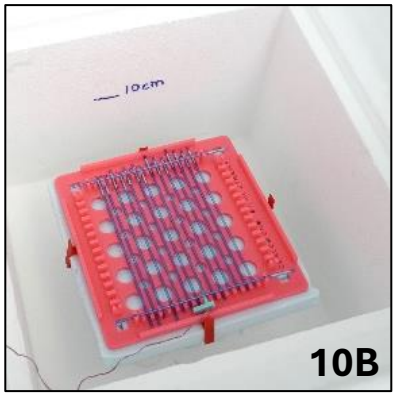
Cooling

Materials:



7) Cool Samples





7.1) Turn on the **Data Logger/Thermometer** (Figure 10A).

7.2) Open the **Polystyrene Containers** and gently place the CryoKit on the **Polystyrene Float** without leaving the containers open for longer than 10 seconds (Figure 10B).

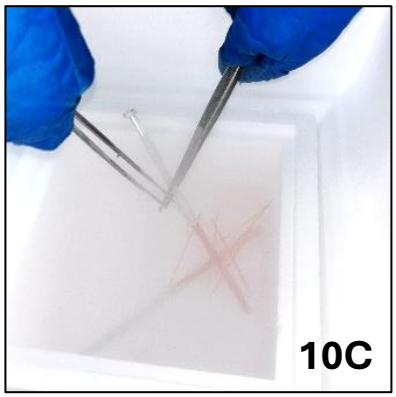
> **Note:** Do not remove the **Polystyrene Float** from the containers. Ensure the CryoKit is centered and placed flat on the **Polystyrene Float**.

7.3) Close the **Polystyrene Containers**.

> **Note:** Ensure both **Polystyrene Containers** are completely closed, and that the temperature gradient is not disturbed.

7.4) Continue to step 8 once the temperature has reached -80°C.

8) Sort Samples



8.1) Wearing **Cryogenic Gloves** and **Safety Glasses**, open the containers and use two **12" Tweezers** to plunge the CryoKit into the **Liquid Nitrogen**.

8.2) Remove the straw locks (if applicable) and flip the **Top Rack** to dump the **French Straws** or **Cryovials** into the **Liquid Nitrogen**. Remove the **Polystyrene Float** and CryoKit and let the **French Straws** or **Cryovials** submerge into the **Liquid Nitrogen**.

8.3) Place the **Daisy Goblet**, **Cane** with or without the **Visotube**, or **Freezer Box** in the liquid nitrogen.

8.4A) Sort the **French Straws** into a **Daisy Goblet** or **Canes** with a **Visotube** for storage (Figure 10C).

8.4B) Sort the **Cryovials** into **Canes** or a **Freezer Box**.