# ONPRC Module 4B Saturday Academy Cryopreservation & Endangered Species

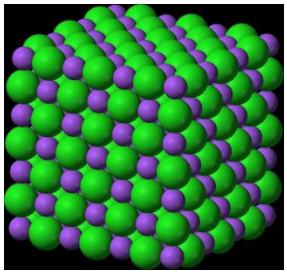
| Main Question | Laboratory Questions |
|---|---|
| What is "cryopreservation" and how can we use it to preserve fertility in endangered species? | • What are the main causes for cell or tissue damages cryopreservation?<br>• What are the 2 main techniques used in cryopreservation to minimize freezing-induced damages?<br>• What are cryopreservation agents and how do they work?<br>• How can we preserve fertility in endangered species using cryopreservation? |

## What is cryopreservation?

Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures (usually the temperature of liquid nitrogen, -196°C). At these low temperatures, any biological activity, including the biochemical reactions, that would lead to cell death is effectively stopped.

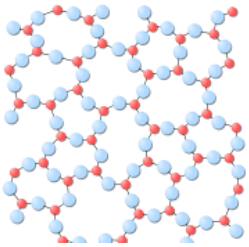
# Cryopreservation Vocabulary

**Crystalline structure:**



A solid composed of molecules or atoms arranged in a very specific and orderly way that displays perfect symmetry

**Amorphous state (glass transition):**



A solid in which there is no specific order of the atoms. Usually resulted from rapid cooling (before the molecule has enough time to organize into an orderly structure (crystal).

http://upload.wikimedia.org/wikipedia/commons/e/e9/Sodium-chloride-3D-ionic.png
http://en.wikipedia.org/wiki/Glass#mediaviewer/File:Silica.svg **Both figures - Public Domain**

**Viscous**: Thickness of a certain fluid. Water is "thin", having a lower viscosity, while honey is "thick", having a higher viscosity.

**Cryoprotective agents (CPAs):** A substance that is used to protect biological tissue from freezing damage (damage due to ice formation). CPAs are usually very thick and have high viscosity.

**Dehydration:** The removal of water from a cell, tissue, or organism.

**Liquid Nitrogen:** Nitrogen in a liquid state at a very low temperature (-196°C). It can cause rapid freezing on contact with living tissue, which may lead to frostbite (use with caution! Always use goggles and thick gloves).

**Vitrification:** The transition of a substance into a glass (amorphous state). Cells in a solution of water and CPAs can be vitrified by rapid freezing in liquid nitrogen.

**Devitrification:** The growth of ice crystals during rewarming of a vitrified solution.

**Supercooled liquid:** Liquid at normal freezing temperature without ice formation.

**Slow rate freeze:** Freezing of biological samples using programmable steps at a very slow declined rate of temperature.

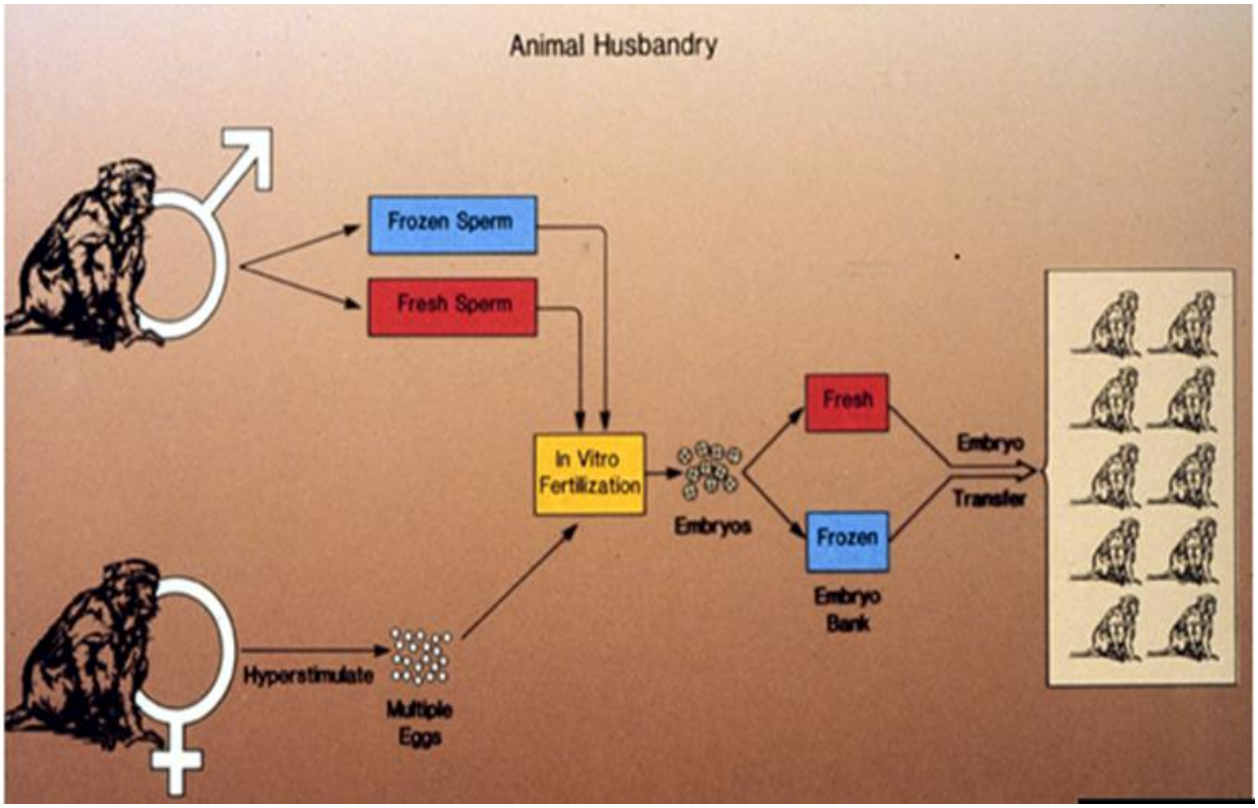
**Seeding**: Seeding is the process of inducing ice crystal formation outside the cell during slow rate freeze.

http://www.biopoliticaltimes.org/img/original/egg%20freezing.jpg

# Cryopreserved Endangered Species

Cheetah
http://nationalzoo.si.edu/SCBI/EndangeredSpecies/Cheetah/

Cryobiology helped Zoo scientists breed black-footed ferrets, once thought to be extinct. (*Mehgan Murphy/NZP*)
http://nationalzoo.si.edu/publications/zoogoer/2010/1/FrozenAssets.cfm




**Cryopreservation of sperm and embryos is being used to preserve endangered species. They are stored in liquid nitrogen – "a zoo in a freezer". Sperm can be thawed and used for mating with a female. Embryos can be thawed and transferred to a female for live birth.**

Drawing: Joel Ito, Medical Illustrator ONPRC



**Timu -** baby lowland gorilla, Photo Courtesy of Cincinnati Zoo

Baby Panda Bao Bao Photo: Abby Wood, Smithsonian's National Zoo
http://nationalzoo.si.edu/Animals/GiantPandas/PandaUpdates/

# Cryopreservation Laboratory Experiments

## Vitrification Experiment

- Label 1 round bottom plastic tube with "0%."
- Label 4 round bottom glass tubes with "20%, 40%, 60%, 80%." Place all labeled tubes in a rack.
- Add 3ml water to a round bottom plastic tubeabeled with "0%" glycerol.
- Make up 20, 40, 60, and 80% glycerol in water for a total volume of 3ml (First do the calculation (table below) and try to figure out how much glycerol and water you will need to make up each solution.

| | 0% | 20% | 40% | 60% | 80% |
|---|---|---|---|---|---|
| Glycerol (Gly) (ml) | 0.0 | | | | |
| Water (ml) | 3.0 | | | | |
| Total Volume (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |

- Mix each solution thoroughly with a transfer pipette (pipette the solution up and down 20 times).
- Set the timer for 30 seconds. Wear safety goggles and thick gloves and hold one tube (starting with the lowest %) with forceps. Submerge the tube into liquid nitrogen for 30 seconds. Make sure that all the solution is submerged below the surface of liquid nitrogen.
- At 30 seconds, carefully take the tube out of liquid nitrogen and observe the solution. Ask yourself, "is the solution vitrified?" How are you tell? Record your results in the table below.
- Repeat the last 2 steps for all your tubes.

## Results

| Glycerol | 0% | 20% | 40% | 60% | 80% |
|---|---|---|---|---|---|
| Evidence of Ice? Yes or No | | | | | |
| Vitrified? Yes or No | | | | | |

**Students Notes or Questions:**

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# Cryopreservation Laboratory Experiments

## Exercise

**Question 1**
**How can you tell that a solution is vitrified successfully?**

**Question 2**
**What is the percentage of glycerol (CPA) needed for successful vitrification?**

**Question 3**
**Give an example of cryopreservation in nature.**

**Question 4**
**How can cryopreservation be used to help endangered species?**

**Question 5**
**Why is cryopreservation important for preservation of endangered species?**