# Comparison of Cryoprotectants in the Cryopreservation of Drosophila Salivary Glands

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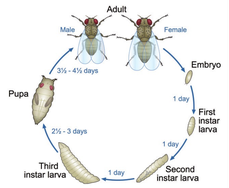
## 1. INTRODUCTION

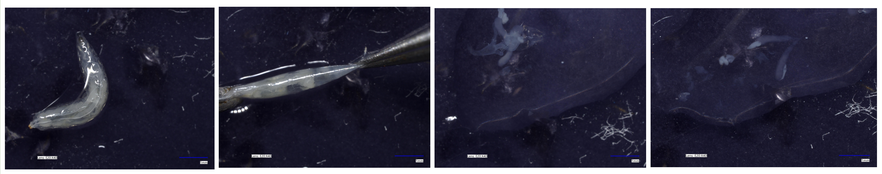
- The common fruit fly, or *Drosophila melanogaster* has been used as a model organism for over a century in the field of biology.
- Cryopreservation is a process where biological material is preserved at very low temperatures using liquid nitrogen for an extended period.
- Used to keep a genetic line, or for techniques like artificial insemination.




- Cryoprotectants are a substance used to protect tissue from freezing damage.
- The salivary glands are a good starting point when furthering understanding of the process of cryopreservation. They are fast and easy to dissect, and have large, visible nuclei.
- Proline is a nonpermeating organic amino acid that naturally aids in freeze tolerance in various insects.
- Trehalose is a nonpermeating disaccharide cryoprotectant that aids in freeze tolerance
- The **objective** is to determine whether feeding Proline or Trehalose is a more effective cryoprotectant in *Drosophila melanogaster.*

## 2. METHODS

1. Prepare Bloomington diet (cornmeal)
   - Control
   - Proline (100)
   - Trehalose (100)
3. Place diet and flies in vials with 15 morsels of dried yeast for adults.
5. Dissect salivary glands from 3rd instar larvae in saline.

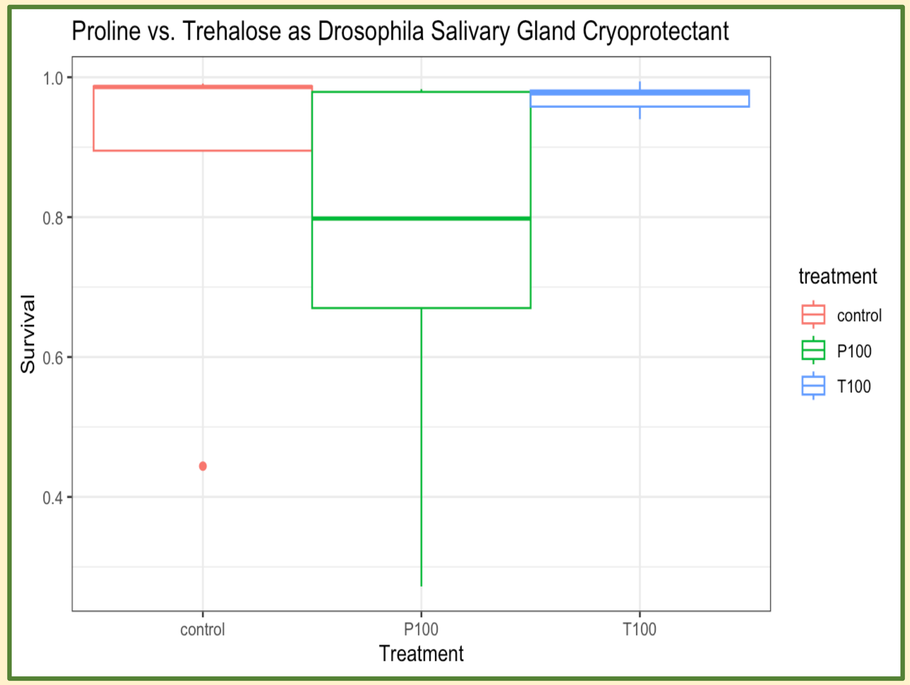


6. Transfer salivary glands to 10% DMSO in Snyder's medium, a permeating cryoprotectant.
7. Transfer on a copper loop to the Cryobath machine for slow freezing protocol at 0.5°C per minute down to -40°C.
8. Remove and store in liquid nitrogen for storage until ready to thaw.
9. Thaw by quickly placing the loop in 3 drops of saline in a cavity slide, the salivary glands should fall off into the saline.
10. Add 1 microliter of Sybr green, then 1 microliter Propidium Iodide, and cover slip.
12. Take images on the green and red settings for later analysis.
13. Count the green (live) cells and red (dead) cells.

## 3. RESULTS

| Treatment | Survival |
|---|---|
| Control | 0.86 |
| Proline (100) | 0.74 |
| Trehalose (100) | 0.97 |

There was no significant difference in the proportion of live cells between the treatments ($F_{1, 12}$=1.39, p=0.286)


## 4. CONCLUSIONS

Although there is no significant difference, trehalose had the highest survival out of the three treatments. These results are very interesting, because neither of the cryoprotectants significantly increased the survival of the cells. We would have expected the survival to be much higher when exposed to such low temperatures. More research will be needed to determine why this could be.

## 5. FUTURE DIRECTIONS

Complete 3 more trials of 5 samples for this concentration treatment, then run the experiment again with a higher and a lower concentration for each cryoprotectant. This could give us more answers as to why there was no significant difference between treatments at this concentration.
Following the testing on salivary glands, we will want to move forward with more complex whole organs in insects. The results of this experiment will be factored into the steps of future experiments.

## 6. ACKNOWLEDGEMENTS

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## 7. REFERENCES

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