



Dec 16, 2025

SOP – Snap-freezing fly heads

DOI

<https://dx.doi.org/10.17504/protocols.io.ewov1kjqkgr2/v1>

Alfonso Martín-Peña¹

¹College of Medicine | University of Florida



Janna Jernigan Posey

Indiana University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.ewov1kjqkgr2/v1>

Protocol Citation: Alfonso Martín-Peña 2025. SOP – Snap-freezing fly heads. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.ewov1kjqkgr2/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: December 16, 2025



Last Modified: December 16, 2025

Protocol Integer ID: 235169

Keywords: ASAPCRN, freezing fly heads snap freezing, heads with liquid nitrogen, liquid nitrogen, sop, head

Funders Acknowledgements:

Michael J. Fox Foundation for Parkinson's Research (MJFF) and the Aligning Science Across Parkinson's (ASAP)

Grant ID: ASAP-020527

Abstract

Snap Freezing fly heads with liquid nitrogen.

Guidelines

9) Using a microscope and forceps, collect fly heads into the Biomasher tube. Make sure they are below the top line before closing the tube to avoid mashing the heads.

TIP: Sometimes, especially upon warming up, heads stick to the forceps. The easiest way to get heads into the tube in that case is to "wipe" the heads off the rim of the tube. After you have the desired amount of heads on the rims, "poke" the heads into the tube.

10) Clean the glass petri dish with ethanol.

Materials

- Eppendorf tubes (1.5 or 2mL)
- Biomasher II Tube/Pest, Sterile – KIMBLE (Cat #9749625002)
- Ice bucket
- Glass vial
- Small funnel
- Liquid nitrogen
- Vortex
- Bigger plastic petri dish
- Smaller glass petri dish
- Forceps
- Microscope
- Marker

Procedure

- 1 Label each Eppendorf tube and each Biomasher tube.
- 2 Pour ice into a plastic petri dish, and rest the glass petri dish on the ice, rim up. *This will ensure that all the fly heads stay on a glass surface during step 8.*
- 3 Put a glass vial with a small funnel in ice. Make sure it's very cold before the next step.
- 4 Tap down the vial with flies so that all the flies are at the bottom of the vial. Open the vial and invert it onto the funnel+glass vial. Tap the whole thing down to ensure all the flies are transferred from their original vial into a cold glass vial. Flies should quickly fall asleep.
- 5 Funnel the anaesthetized flies into their respective Eppendorf tubes.
- 6 Freeze the Eppendorf into liquid nitrogen. TIP: Swivel and shake the Eppendorf to ensure even freezing. Keep the tube in liquid nitrogen until it stops bubbling to ensure that the flies are fully frozen.
- 7 Switch the vortex into the ON mode (not the TOUCH mode) at its highest speed. Vortex the frozen Eppendorf. This will separate the fly heads from their bodies. TIP: Aim to get rough, abrupt, and violent contact between the Eppendorf and the vortex and minimize the delay between having the tube in the liquid nitrogen and on the vortex to achieve effective decapitation.
- 8 Dump the contents of the Eppendorf onto the iced glass petri dish. Check the Eppendorf under the microscope to ensure that all the heads are out of the tube. TIP: The colder the tube, the easier it will empty out, so do not vortex for too long.
- 9 Using a microscope and forceps, collect fly heads into the Biomasher tube. Make sure they are below the top line before closing the tube to avoid mashing the heads. TIP: Sometimes, especially upon warming up, heads stick to the forceps. The easiest way to get heads into the tube in that case is to "wipe" the heads off the rim of the tube. After you have the desired amount of heads on the rims, "poke" the heads into the tube.
- 10 Clean the glass petri dish with ethanol.