

A method using CO₂ anesthesia to collect embryos for microinjection in *Drosophila elegans*

Jonathan H. Massey¹, Jun Li^{1,2}, and Patricia J. Wittkopp^{1,3}

¹Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI

²Institute of Evolution and Ecology, School of Life Sciences, Central China Normal University, Wuhan 430079, China

³Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA

Abstract

A key step for genetic transformation of animal hosts is collecting enough embryos for microinjection. In *Drosophila melanogaster*, fertile females will often lay hundreds of embryos when placed on a grape plate with yeast. In other *Drosophila* species, however, females typically lay few if any embryos under similar conditions, hindering efforts to inject genetic reagents to create new mutants or markers for downstream genetic analyses. Here, using the species *Drosophila elegans*, we describe a method using CO₂ anesthesia to collect embryos for microinjection. This technique allowed us to collect and inject enough embryos for CRISPR/Cas9 gene editing in this non-model species, resulting in a null allele of the *ebony* gene.

Required Materials

Grape Juice Agar (e.g., Nutri-fly, Cat #: 47-102, Genesee Scientific)

Petri Dishes (Cat # 3050-00, Weber Scientific): 100 X 15 mm (83 mm Diameter)

Embryo Collection Cages - Small (Cat # 59-100, Genesee Scientific): 5.6 cm (D) x 7.6 cm (H)

Standard *Drosophila* media with added molasses (Wirtz and Semey, 1982)

At least 10 expanded vials of *Drosophila elegans* (~1000-2000 flies) on molasses media

Standard *Drosophila* CO₂ anesthesia equipment

Whatman 1004-042 Filter Circles, 42.5mm Diameter

Procedure

1. Prepare fresh grape juice agar plates following the Nutri-fly recipe from Genesee Scientific (Cat #: 47-102).
2. Expand *Drosophila elegans* population to at least ~1000-2000 flies. *D. elegans* have trouble pupating on the sides of the vials, so adding Whatman filter paper deep into the food of each vial once L3 larvae develop helps provide a greater surface area for larvae to pupate on, allowing adults to eclose (see Methods in Massey *et al.* 2020 for more details on rearing methods).
3. Age *D. elegans* males and females together in vials for ≥ 2 weeks (flipping to new food vials when L3 larvae develop) to give the adults enough time for multiple matings to occur and for embryos to develop.

4. Using CO₂ anesthesia, knock out at least 500 flies and dump them into an empty embryo collection cage (Cat # 59-100, Genesee Scientific) (Figure 1, Steps 1 and 2).
5. If the females have mated and are old enough (\geq 2 weeks), you should see several individuals begin to release an embryo from their ovipositor (Figure 1, Step 3).
6. Take a fresh grape juice agar plate at room temperature, flip it upside-down, and push the agar down hard enough onto the bottom of the embryo cage to puncture the agar and form a seal with the inside of the embryo cage (Figure 1, Step 4).
7. Flip the assembly (grape plate + embryo cage + anesthetized flies) over so that the flies come into contact with the grape agar. Next, tap the assembly down onto a hard surface repeatedly (~10 times) to force the released embryos to stick onto the agar (Figure 1, Step 5).
8. Flip the assembly back over so that the flies are again on the mesh ceiling of the embryo cage as in Figure 1, Step 4. Tap the assembly onto a hard surface to release any remaining adults stuck to the grape agar plate.
9. Place the assembly back onto the CO₂ pad to anesthetize the adults again before pulling the agar plate off of the embryo cage and dumping the adults back into their vials. We have found that adult females will continue to release embryos in this manner every 24 hours.
10. To facilitate larger (>1000) embryo collection procedures for multiple rounds of microinjection, repeat steps 2-9 in this procedure with a more expanded population of *D. elegans*. For our CRISPR/Cas9 experiment targeting the *ebony* gene (see Methods in Massey *et al.*, 2021; Figure 2 below), we expanded our *D. elegans* population to ~10,000 flies and collected ~500 embryos a day to inject ~2000 embryos.

Conclusion

Here, we present a method for collecting embryos from *D. elegans*. We also find that fertile females in other non-model species (*D. yakuba*, *D. simulans*, and *D. mauritiana*), as well as *D. melanogaster*, often release an embryo from their ovipositor when subjected to CO₂ anesthesia (Figure 3A-D). It is more difficult, however, to eject these embryos onto grape plates with the tapping method described in Step 8 above. Applying this technique to other *Drosophila* species for downstream microinjection applications, therefore, will likely require either using a paintbrush to remove individual embryos or a more forceful method to eject the embryos onto the grape plate. Finally, in our experience with *D. elegans*, we find that it is unnecessary to wash embryos in preparation of microinjection using the CO₂ anesthesia technique. Avoiding this step helped improve our embryo survival rates post injection.

References

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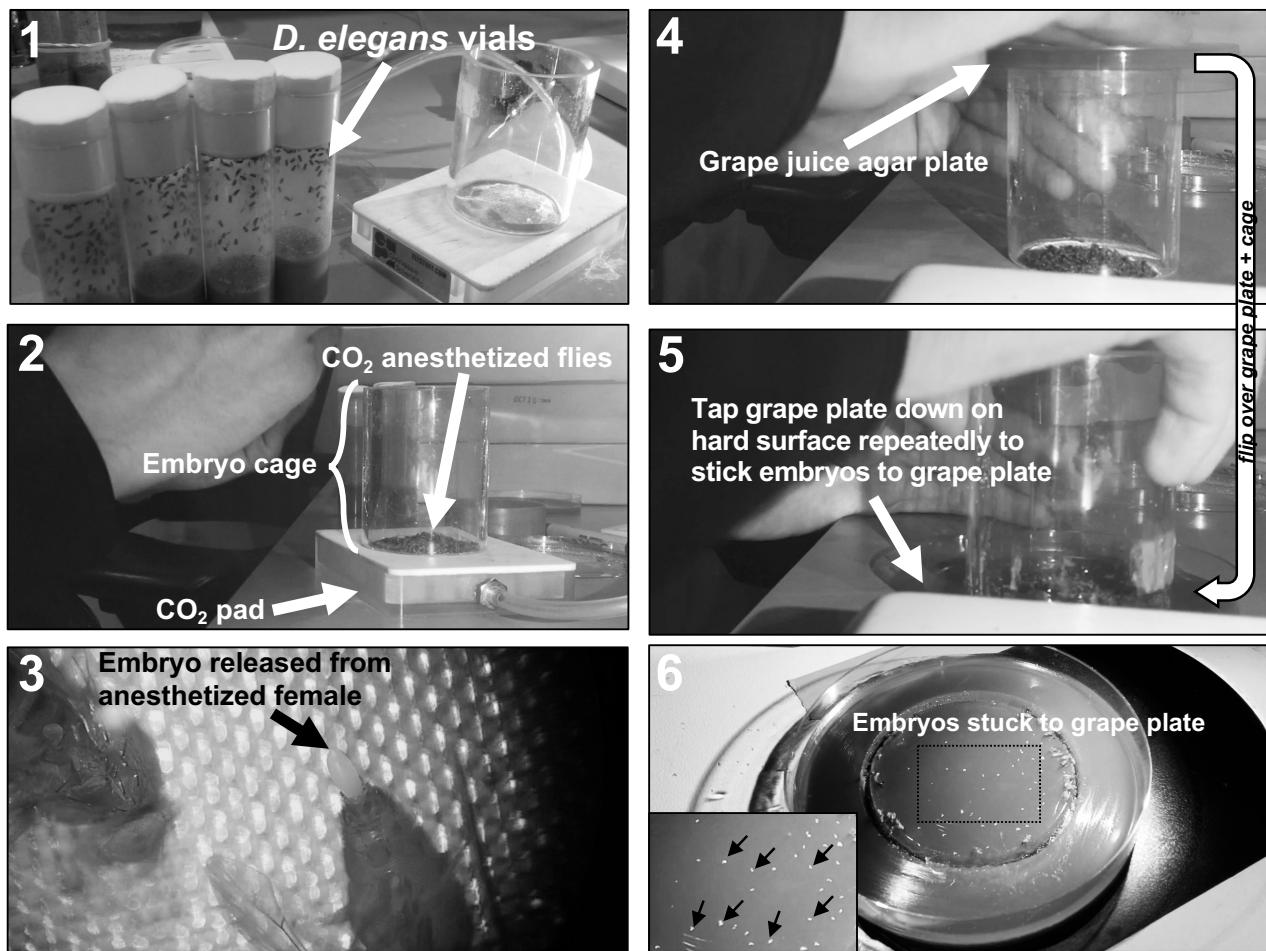


Figure 1. Steps for using CO₂ anesthesia to collect *Drosophila* embryos for microinjection

D. elegans *D. elegans ebony null*



Figure 2. *Drosophila elegans* (Hong Kong strain) wild-type (left) and *ebony* knockout (right)

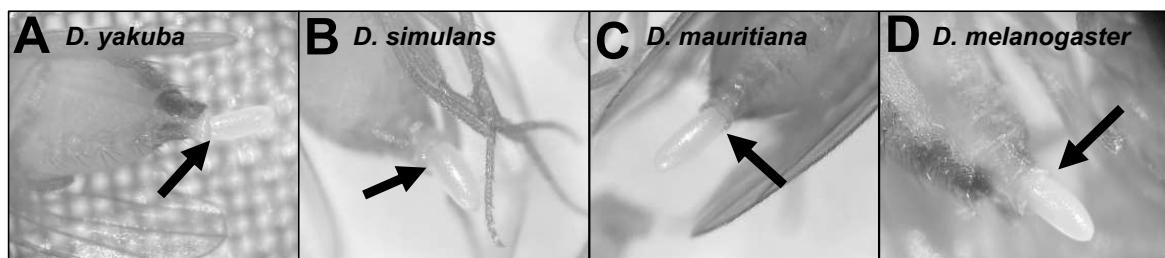


Figure 3. Embryo release from *Drosophila* species after CO₂ anesthesia