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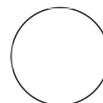
Protocol status: Working
We use this protocol and it's working

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Squishing insects for preservation of HMW DNA in the field

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ABSTRACT

Ideal preservation for maximum recovery of high molecular weight (HMW) DNA from insects is to snap freeze living insects on dry ice and store at -70C. However, dry ice or liquid nitrogen for snap freezing is not always accessible in the field. When snap freezing and ultra cold storage is not an option, gently compromising the cuticle by squishing the insect can improve the penetration of any preservation solution and thereby increase both the quantity and the quality of recovered DNA. Here we present a step by step protocol for how to carry out the squish procedure for mosquitoes. While we use mosquitoes as an example, this protocol should improve HMW DNA recovery for most adult arthropods when used in conjunction with an appropriate preservative such as 100% ethanol in excess, EDTA, or DMSO salt solution (DESS). It is advised to move room temperature squished insects in excess preservative to ultra cold storage as soon as possible. If the excess preservative will freeze solid (like DESS), and there is no further risk to cold chain before processing for HMW DNA extraction, we advise to remove the preservative solution before freezing. In our experience, we are able to retrieve good quality HMW DNA from single mosquitoes held in excess 100% ethanol or DESS at room temperature for 1 week and sequence this DNA using PacBio HiFi. Mosquitoes stored this way also resulted in good quality Hi-C data.

IMAGE ATTRIBUTION

Petra Korlević drew the mosquito in the bathtub

PROTOCOL MATERIALS

	Ethanol 100% Contributed by users	Step 1
	100% Ethanol Contributed by users	Step 2

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BEFORE START INSTRUCTIONS

Ensure all surfaces have been cleaned with 70-80% Ethanol (and ideally bleach before that). Have cleaning wipes for forceps.

1

Transfer your live caught mosquito into  Ethanol 100% Contributed by users to kill it.

2

With clean forceps remove the mosquito from  100% Ethanol Contributed by users and

gently dab it on a clean wipe (e.g. Kimtech wipe) to dry off any remaining ethanol. Transfer it into a 0.5 mL Eppendorf tube (or similar) filled with  400 μ L of preservation liquid of your choice.

[If you are using ethanol for preservation, you can skip this step and preserve directly in the ethanol that you killed the specimen in. Ensure there is sufficient ethanol in the tube to completely submerge the mosquito and not so much that you spill ethanol when you insert a pestle.]

Equipment

Safe-Lock Tube	NAME
microcentrifuge tube	TYPE
Eppendorf	BRAND
0030121023	SKU
https://www.eppendorf.com/gb-en/eShop-Products/Laboratory-Consumables/Tubes/Eppendorf-Safe-Lock-Tubes-p-0030121023	LINK

Note

Preservation liquids are there to ensure that DNA is ideally preserved until it can be extracted. 100% EtOH, EDTA, DESS, or commercial solutions are all options.

Note

Other sizes and brands of tubes can be used, but the liquid volume to insect volume ratio should be high (excess preservation liquid) and there should be enough liquid to keep the insect fully submerged during transport when tubes may end up upside down.

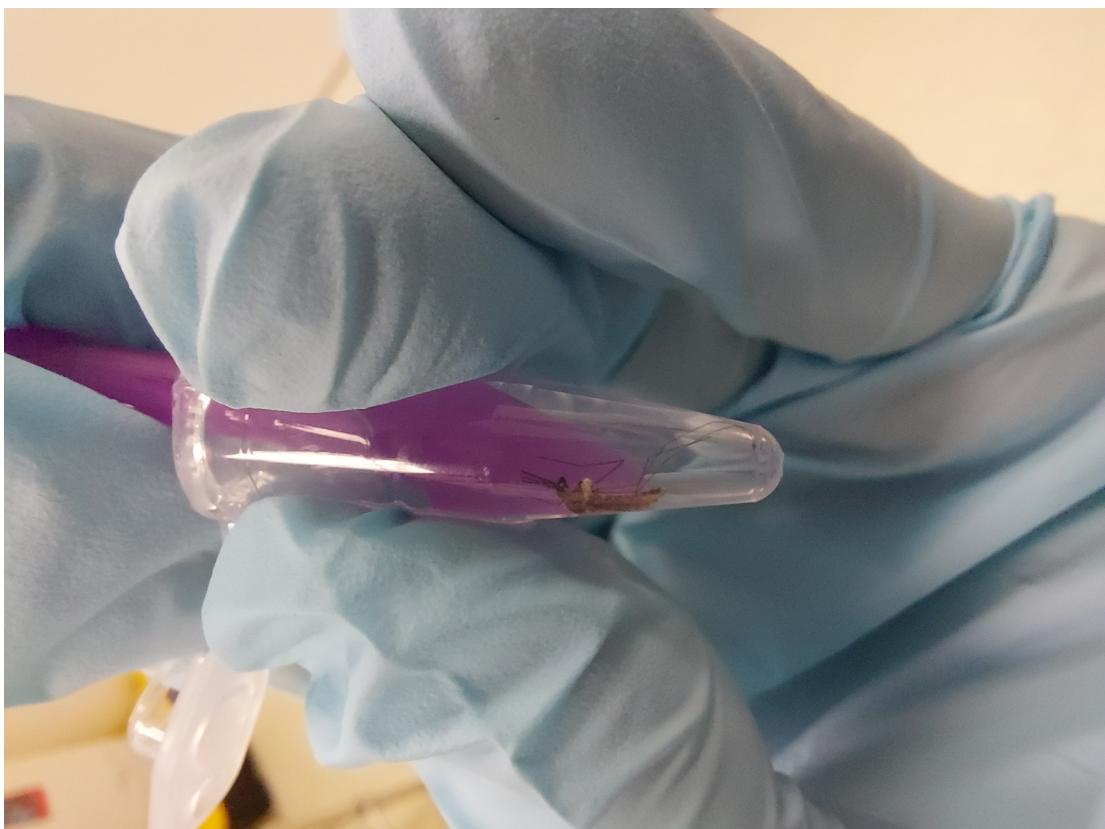
- 3 With a clean pestle appropriate for the size of tubes that you are using and insects you are preserving, gently press the insect into the liquid and against the side of the tube until you feel the cuticle break. This ensures that the preservation solution can penetrate the tissue and increase the quality and quantity of DNA later extracted.



Mosquito before it has been "squished"



Insert the pestle into the tube and position the tip of it next to the mosquito



With the tip of the pestle press the mosquito against the wall of the tube until you can feel the cuticle crack under pressure



The mosquito appears flattened but is still in one piece and can be easily removed from the liquid preservative. Try to minimize the tissue leaking into the fluid as these cells will not be easily recovered.

Note

There are different sizes of pestle available - choose the appropriate size or smaller based on the tubes you are using.

Equipment

Microtube Pestles for 0.5 mL tubes

micropesle

NAME

Starlab

TYPE

I1405-4390

BRAND

SKU

<https://www.starlabgroup.com/GB-en/product/microtube-pestles-for-0-5ml-tubes-i1405-4390.html> LINK

- 4 The mosquito is now ready to be shipped at room temperature or stored. In our experience, it is best to keep specimens at the coldest available temperature at all times so once you have access to a -70C freezer, remove the liquid (unless it is ethanol, which won't freeze) and snap freeze the specimen. This will make it easier to prepare the specimen for extraction as you won't have to wait for the preservative to thaw, potentially risking the quality of the DNA.