

systemic infection in *Drosophila*

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Abstract

This protocol describes how to systemically infect fruit flies with either a bacterial or viral infection through pricking the mesopleuron of the thorax

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Protocol

Bending pins

Step 1.

Using tweezers, carefully pick up a 0.14mm pin (Watkins & Doncaster; E6873) and check tip is not broken under microscope.

Step 2.

Hold pin in tweezers under the microscope at the point you want to bend, the bent tip should be equal in length to half that of the fly thorax (0.5mm). Carefully bend pin until a 90 degree angle is achieved.

Step 3.

Prepare approx. 5-10 pins as above to allow spares in case of tip snapping during systemic pricking.

Step 4.

To safely store pins until required, place in blue-tac in enclosed tube, i.e. falcon tube.

Systemically pricking flies

Step 5.

Blue-tac an eppendorf lid to the base of the microscope and into it pipette 50µl of infectious suspension/control solution using a filtered tip/tip respectively.

Step 6.

Loosen end of syringe rod, using tweezers place a bent pin in the end of rod and tighten the syringe. Straighten the pin and ensure it is secure.

Step 7.

Knock-out selected flies and place on CO₂ pad. Cover the CO₂ pad with a Kim wipe to avoid contamination. Place pad under microscope and with tweezers (or paintbrush if preferred) line up 10 flies in a row.

Step 8.

Before stabbing, locate the mesopleuron on flies – area under wing to the left of the pleural suture (<http://www.ento.csiro.au/biology/drosophila/melanogaster.html#>) -will be soft to pierce with pin with little resistance compared to surrounding area.

Step 9.

Dip the tip of pin into the infectious suspension/control solution, making sure the tip submerges fully but does not snap on bottom of container.

Step 10.

Gently stab fly in the mesopleuron, on the left side of the pleural suture. Use your other hand to hold the fly in place with the tweezers/paintbrush. To remove pin, gently slide out while continuing to hold fly in place

Step 11.

Move along row of flies, re-dipping the pin tip in infectious suspension/control solution between each fly. When all flies in row stabbed, move these individuals to one side and make new row and continue until all selected flies are stabbed. Do not leave flies on the CO₂ pad for more than 15mins.

Warnings

1. Always keep pins in a safe, enclosed place when not in use.
2. Always point pin end of syringe rod away from person when placed on bench.
3. All pins should be disposed of in a yellow sharps bins.
4. Appropriate Health & Safety guidelines for the virus/bacteria speices being used should be followed.