CHC protocol

Couples are sexed under CO_2 anaesthesia, and put individually in labelled eppendorfs. They are left in the -20ºC freezer for 15-60 min to allow for easy manipulation of the flies in the following steps.

For the remaining protocol, work in a fume hood.

Each fly is inserted with forceps in 500 µl heptane/C26 (made already and stored at -20ºC) in a glass tube.

The glass tube is 12mm x 32mm clear screw cap vial with polypropylene open hole caps and with white Teflon liners (Alltech).

The time the fly spends in glass tube needs to be precise. It can be 10-20 min, we do 10 min.

The flies are removed with forceps from the glass tubes (10 at a time to make sure timing is accurate). The forceps are swirled in the ethanol eppendorf the fly is trasfered into. 700 µl 100% ethanol were used for each fly, and they were kept at -20ºC in the end of the day in a bag labelled with the population and extraction date (e.g. 'Oulanka F1 05/08/2008').

The action order is start timer, put flies in heptane glass tubes, label eppendorf vial tops & heptane glass tubes, fill eppendorfs with ethanol, dilute next round of working heptane C26 (below).

All glass vials are left in the fume hood for the heptane to evaporate for 1-2 days. Once evaporated the lids are put on the glass vials, and they are kept at -20ºC.

For postage the vials can be sent at room temperature.

Reagents

Both kept at -20ºC.

Heptane/C26 standard

This is concentrated. At room temperature C26 dissolves.

It is 1mg/ml C26 dissolved in heptane.

Heptane/C26 working

The working concentration is 5 µg/ml. If 10 flies are extracted per round, add 25 µl Heptane/C26 standard into 4975 µl heptane.