Generating and Rearing Axenic Drosophila

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Abstract

This is a protocol to generate axenic fly cultures by sterilizing embryos. It is part of the manuscript: Gonçalves et al. Commensal bacteria and essential amino acids control food choice behavior and reproduction. Plos Biology. 2017 Apr 18.

The protocol was adapted from:

Wayland MT, Defaye A, Rocha J, Jayaram SA, Royet J, Miguel-Aliaga I, et al. Spotting the differences: Probing host/microbiota interactions with a dedicated software tool for the analysis of faecal outputs in Drosophila. J Insect Physiol. 2014;69: 126–135. doi:10.1016/j.jinsphys.2014.05.023

with the help of the Leulier laboratory.

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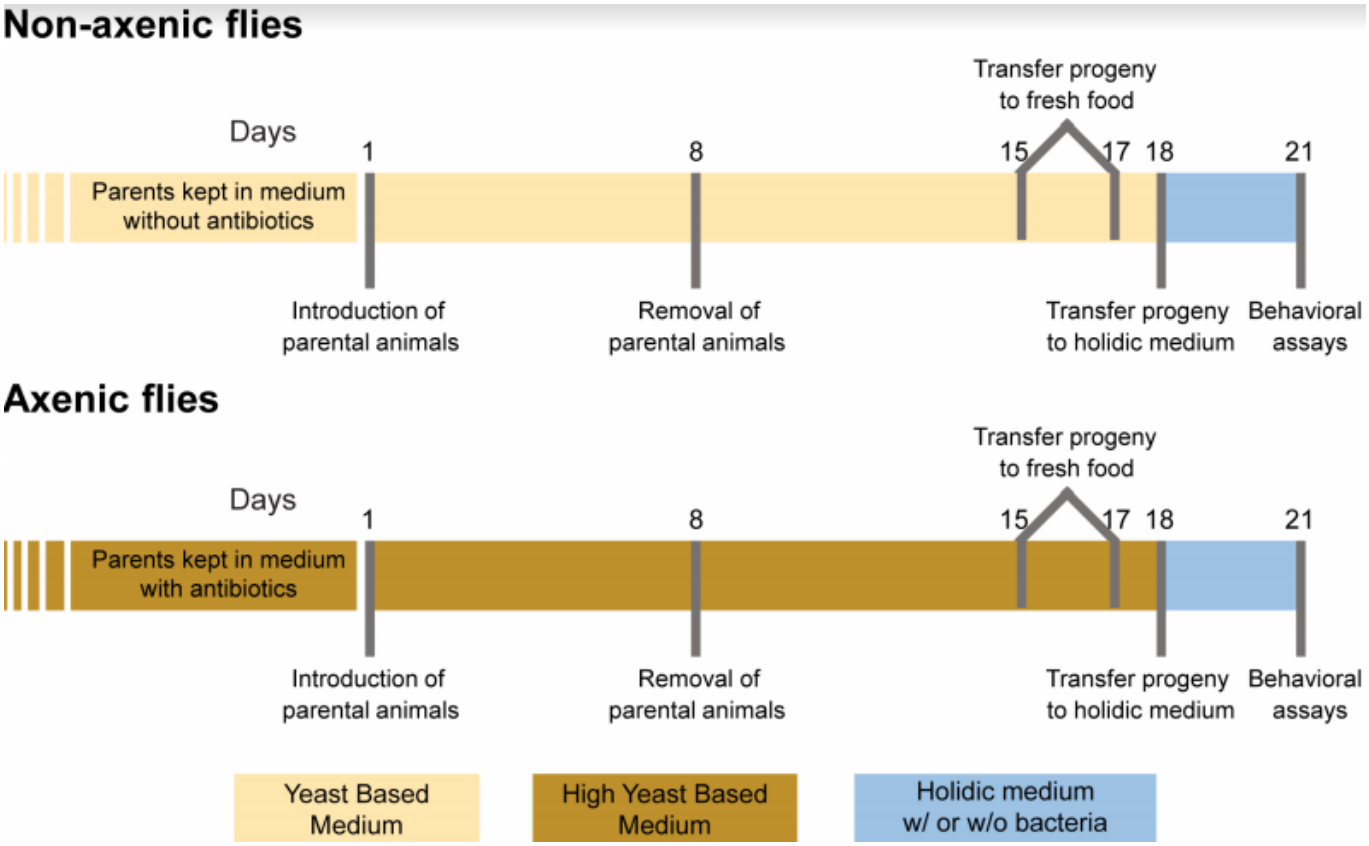
Guidelines



Prepare high yeast-based medium (HYBM) as follows:

- add 8 g agar, 80 g barley malt syrup, 22 g sugar beet syrup, 80 g corn flour, 10 g soya flour, **41.67 g instant yeast**, 8 ml propionic acid, 12 ml nipagin (15% in 96% ethanol), **416.7 µg/ml tetracycline (high dose), 41.67 µg/ml chloramphenicol, 41.67 µg/ml ampicillin, and 8.333 µg/ml erythromycin** and make up to 1000 ml of milliQ filtered water
- autoclave before pouring into polypropylene fly culture vials (VWR, #734-2261)
- **DO NOT supplement the food with instant yeast granules on the surface**

The high yeast concentration is to compensate for the developmental delay observed in axenic larvae (Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F. Lactobacillus plantarum Promotes Drosophila Systemic Growth by Modulating Hormonal Signals through TOR-Dependent Nutrient Sensing. Cell Metab. 2011;14: 403–414. doi:10.1016/j.cmet.2011.07.012).

Perform all fly rearing, maintenance, and behavioral testing at 25°C in climate-controlled chambers at 70% relative humidity in a 12-hr-light-dark cycle (Aralab, FitoClima 60000EH).

Before start

Prepare the required fly media.

Protocol

Step 1.

Prepare high yeast-based medium (HYBM) according to Guidelines.

NOTES
Carlos Ribeiro 11 Apr 2017

HYBM can be stored at 18ºC up to 3 days before use.

Step 2.

Put females and males to lay eggs in cages containing apple juice plates with yeast paste for 3 to 5 h at 25ºC. Avoid longer time periods to prevent the presence of larvae in the protocol.

DURATION
04:00:00

Step 3.

Collect eggs by rinsing the apple juice plates with deonized water and transfering the liquid with eggs to nitex baskets. Ensure that no larvae contaminate the egg collection!

Step 4.

Immerse the nitex baskets containing the embryos into a petri dish containing 2.5% active chlorine (50% bleach) for 2 min until the chorion is removed. Confirm that the eggs are dechorionated using a stereo microscope.

DURATION
00:02:00

Step 5.

Immerse the nitex baskets containing the dechorionated eggs into a petri dish containing 70% ethanol for 2 min.

DURATION
00:02:00

Step 6.

Move to the laminar flow hood maintaining the dechorionated eggs in the ethanol solution.

To avoid contaminations, the next steps of this protocol are performed in a laminar flow hood.

Step 7.

Immerse the nitex baskets containing the dechorionated eggs into a petri dish containing autoclaved distilled water for 2 min.

DURATION
00:02:00

Step 8.

Transfer the embryos using a sterilized brush from the basket into sterile HYBM (autoclaved before pouring it into sterile culture vials) containing antibiotics.

NOTES
Carlos Ribeiro 22 Mar 2017

The high yeast concentration is to compensate for the developmental delay observed in axenic larvae.

(see: Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F. Lactobacillus plantarum Promotes Drosophila Systemic Growth by Modulating Hormonal Signals through TOR-Dependent Nutrient Sensing. Cell Metab. 2011;14: 403–414. doi:10.1016/j.cmet.2011.07.012).

Step 9.

Assess the absence of bacteria according to the protocol to calculate the internal bacterial load of the flies.

Step 10.

Transfer axenic flies regularly into vials containing freshly prepared antibiotic-supplemented sterile HYBM.

ANNOTATIONS
Margarida Anjos 10 Apr 2017

If axenic flies are kept at room temperature flip to new food every 25 days.

Step 11.

Regularly assess the absence of bacteria.