

Rearing of *Drosophila* on Holidic Media (HM) for feeding behavior assays

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Abstract

This protocol 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.](#)

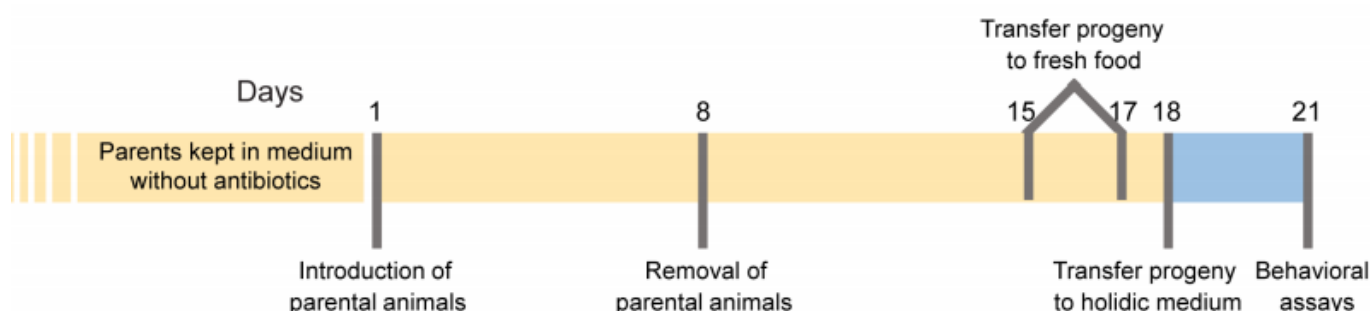
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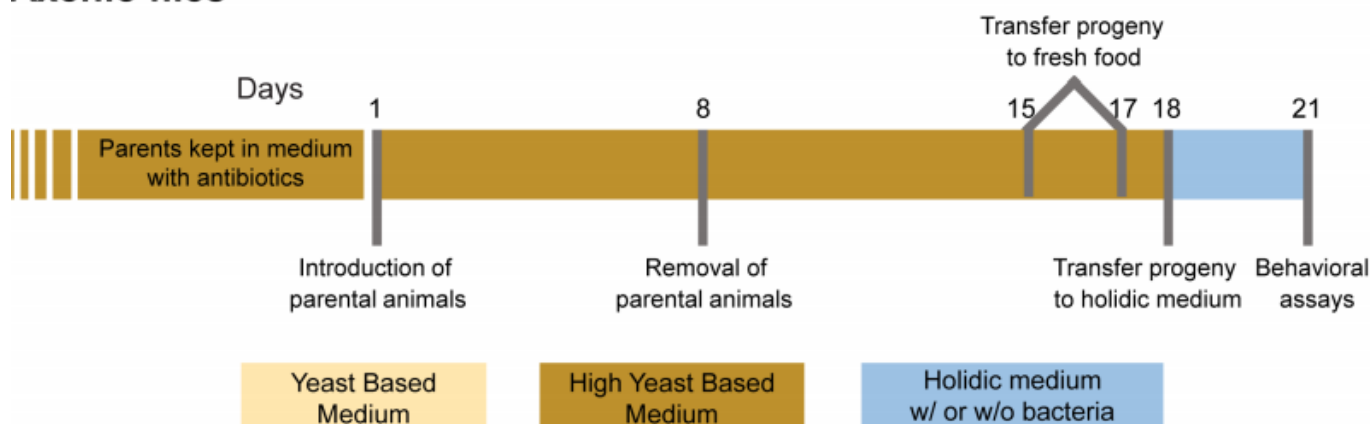
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Guidelines

Non-axenic flies



Axenic flies



Prepare yeast-based medium (YBM) as follows:

- mix 8 g agar, 80 g barley malt syrup, 22 g sugar beet syrup, 80 g corn flour, 10 g soya flour, 18 g instant yeast, 8 ml propionic acid, and 12 ml nipagin (15% in 96% ethanol) and fill up to 1000 ml using milliQ filtered water
- autoclave before pouring into polypropylene fly culture vials (VWR, #734-2261)
- after solidifying, the food is supplemented with instant yeast granules on the surface

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 and bacterial media.

Protocol

Step 1.

Prepare yeast-based medium (YBM) according to the [Guidelines](#).

📌 NOTES

Carlos Ribeiro 11 Apr 2017

YBM can be stored at 18°C up to 3 days before use.

Fly culture

Step 2.

Set up fly cultures using 5 females and 4 males per YBM vial to ensure a homogenous density of offspring among experiments.

Fly culture

Step 3.

Keep the crosses in a dedicated incubator at 25°C, 70% relative humidity, and a 12-hr-light-dark cycle. Remove parental flies after 3 to 7 days and wait 14 days (since the day the crosses were set up) to obtain adult flies.

If you are using temperature sensitive alleles adjust rearing temperature accordingly and plan your experiments to account for the delay in the development.

Preparing adult flies to be tested for feeding behavior

Step 4.

Sort the progeny according to the desired genotype, and collect 16 females into fresh YBM. Add 5 wild-type males to ensure that the females are mated. When testing males collect 20 males into fresh YBM.

Preparing adult flies to be tested for feeding behavior

Step 5.

If you wish to manipulate the flies' microbiome start the liquid bacterial cultures following the protocol [Growing Drosophila gut bacteria](#).

Preparing adult flies to be tested for feeding behavior

Step 6.

To ensure a well-fed state and minimize the microbial load, transfer the flies to fresh YBM after 48 hours.

Preparing adult flies to be tested for feeding behavior

Step 7.

After 24 hours on fresh YBM transfer the flies to the different HM. For this, prepare all the different HM needed according to the [Holidic media \(HM\) preparation](#) protocol. If required, inoculate HM with the commensal bacteria following the [Inoculation of Holidic Media \(HM\) with bacteria](#) protocol.

Preparing adult flies to be tested for feeding behavior

Step 8.

Keep the flies on HM (with or without commensal bacteria) for 72 hours, and immediately test for feeding behavior.

📌 NOTES

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Flies treated using this protocol usually have a very low internal bacterial load.