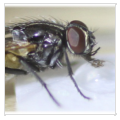

Feeding bacteria to house flies for microbe fate and gene expression analysis.

Version 2

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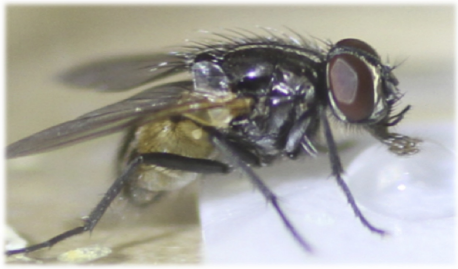
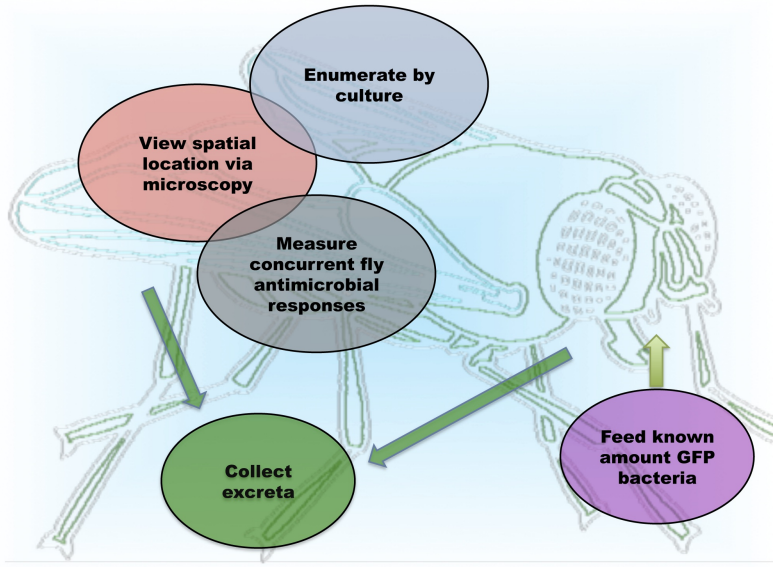
ABSTRACT

Objective: To feed individual house flies a specific amount of bacteria in order to determine bacteria “fate” (persistence, via enumeration; spatiotemporal location, via microscopy) and house fly immune response (whole fly or tissue-specific, via downstream mRNA or protein expression analyses)





PROTOCOL STATUS

Working

working protocol used in our laboratory

SAFETY WARNINGS

BEFORE STARTING

Note: The bacteria culture conditions (e.g. media including antibiotics) will vary based on the bacteria species and plasmid, if any.

Preparing House Flies for Bacterial Feeding

1 Collect pupae from colony and surface sanitize for 2 minutes each with gentle swirling in 10% bleach, sterile H_2O, 100% ethanol, sterile H_2O.

> NOTE
> NOTE: surface sanitization is optional, and may delay eclosion.

Store pupae in a sterile container (e.g. petri dish, secured with rubber band or tape) or individually-house in 15 ml conical tubes until eclosion.

Hold in incubator at 28°C, or if extra time is needed you can hold at room temperature which may slow development by a few days.

Separate emerged flies from puparia after eclosion to reduce contamination by trans-stadial bacteria.

2 Prepare fly containers: Use either small petri dishes, 50 ml beakers covered with foil, or small plastic containers withlids (make air holes with pin).

Autoclave or sanitize containers by washing (sterile petri dishes are ready to use). In each container, place a small square of parafilm (~15 x 15 mm) in the bottom; this is where you will place the food droplet (image below in step 7).

3

Before adding flies to the containers, prepare sterile meal of 10% fly food solution or 10% sucrose and pipet 5-10 µl onto the parafilm squares in the containers.

Knock down flies with CO_2 or put in the freezer (~3 min) and transfer each fly to the container using soft forceps, then close lid tightly.

The fly will wake up and eventually will eat the droplet, which serves to (a) flush any remaining bacteria from the fly gut and (b) provide basic sustenance before fasting.

> NOTE
> For experiments, it's best to use newly eclosed, 2-3 day old flies

4 Hold flies at room temperature and either fast for 12 hours and proceed to step 5 or fast and flush again with a sterile 10% sucrose meal, then fast 12 h a second time.

To add a second meal, briefly anesthetize with CO_2 or chilling first, then add droplet by pipettor.

Preparing bacterial culture

5 Maintain stock cultures of bacteria and refer to protocols for bacterial growth and growth curves for the time to grow.

Seed culture: inoculate large volume (e.g. 100-250 ml) with a single colony of bacteria of interest and grow overnight (8-12 h). Agai, follow bacterial growth protocols which should outline temperature, RPM, media, etc.

> NOTE
> Prepare seed cultures the day before fly feeding, so that these steps are concurrent with fly preparation and fasting (above).
>
> Start seed culture and grow overnight (the day before fly feeding) but do not incubate longer than 8-12 hours, otherwise most of the bacteria will die, negating the purpose of the seed culture.
>
> Use appropriately sized flasks for overnight culture; e.g. if gowing in 250 ml, use a 500 ml flask, if growing in 100 ml, use a 250 ml flask.

6 Take 100 µl of overnight seed culture and add to sterile culture broth (volume determined for each particular bacteria species, but usually 100 ml), incubate (usually at 37°C rotating at 200 RPM.)

Grow to desired OD_{600} (again, this will vary for each species).

NOTE

Growth times, OD vs. CFU relationships will vary widely across bacteria species and spectrophotometers, so it is advised you run through a growth curve before fly feeding.

The goal is to grow to the OD that corresponds to the CFU/ml that gives you the CFU you want in the 2 µl droplet. When doing immune stimulation analysis, usually feed more than 10^5 CFU/fly; so 10^8 CFU/ml is the range you want to get minimum 2 x 10^5 CFU in 2 µl

Feeding House Flies on Culture Droplets

7 After 12 hour fasting, place the flies in the incubator at 37°C for ~15 min and then remove back to room temperature carefully place 2 µl droplet of bacterial culture onto the parafilm square.

NOTE

It may be necessary to anesthetize flies in order to place the droplet without having the fly escape.

Be sure to check whether the entire 2 µl has been dispensed from the pipetter.

8 Observe flies for ingestion of the droplet. Mark lids containers after the flies ingested the entire 2 µl droplet of bacteria, and include only those that have ingested the droplet within 30 minutes of placement.

All others must be discarded (including partial or late ingestion) as this introduces variability.

NOTE

If feeding BL2 bacteria: Infected flies must be stored in their containers (primary container) then within a secondary container in case of breach. Secondary container can be large bins with lids, cages, etc. Just make sure both primary and secondary containers allow for sufficient air circulation.

Droplet enumeration

9 After all flies consume the 2 µl droplet, serially dilute the stock culture (from which the fed aliquot came) in sterile media and plate on selective agar to enumerate the CFU of bacteria fed to the flies.

E.g., you would take 100 µl of stock culture, serially dilute in 900 µl each of media with antibiotics (or sterile PBS) and plate the appropriate dilutions on solid media with antibiotics*.

Incubate plates at 37°C for 24 hours.

Count colonies on UV lightbox, to be sure you only count the GFP colonies*.

NOTE

* Most of the microbes fed to flies for downstream assays are genetically modified, containing plasmids with antibiotic resistance markers and usually a flourescent tag (e.g. GFP). This allows for selection on media and differentiation of the microbe of interest (which will be expressing GFP and will grow in the presence of the particular antibiotics) and other "flora" which may still be present in the gut.