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Title: A Rapid Food-Preference Assay in *Drosophila*

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **Y, Amscope SF2TRA**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **24**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Yali Zhang**: ~~Our protocol is geared toward performing phenotype screens in flies and is a powerful tool for identifying and characterizing genes of interest~~ Our protocol is designed to perform a feeding assay for the fruit fly, which is a powerful model organism enabling us to decipher the genes, cells and neuronal circuits involved in taste sensation and feeding. Like humans, flies prefer foods that are sweet, slight salty or sour, but rejects foods that are bitter, too salty or sour **[1]**. Author preferred longer version, although there are some takes using the scripted version if the longer statement takes are not usable

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Yali Zhang**: This technique's power comes from its speed and convenience – you can test many kinds of flies in a short amount of time without a complex setup **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **John Mack**: This method is an excellent assay for understanding whether flies like or dislike a particular food and how certain gene products influence taste preference **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.4. **John Mack**: Deciding whether animals prefer or reject a specific food ingredient can be challenging. Therefore, it is important to set a standard protocol for testing animal food preferences **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Starvation Vial Preparation

2.1. To prepare starvation vials for the assay, loosely compact a piece of tissue at the bottom of each empty plastic fly vial per fly genotype to be tested **[1]** such that the paper fills the space without forming a dense mass **[2]**.

2.1.1. WIDE: Talent placing tissue into vial

2.1.2. Shot of paper in bottom of vial **TEXT: Caution: Deep crevices or folds can trap flies**

2.2. Add about 3 milliliters of pure water to each vial to completely saturate the tissues without standing water **[1]**, taking care that there are no large droplets of excess water on the walls of the vials **[2]**.

2.2.1. Talent adding water to vial

2.2.2. Shot of saturated paper without droplets on vial wall(s)

2.3. Alternatively, 5 milliliters of 1% agarose without sucrose can be substituted for the soaked paper **[1]**.

2.3.1. Shot of agarose in vial

2.4. Twenty-four hours before the experiment, carbon dioxide-anesthetize 2-to-4-day-old flies **[1]** and sort the flies into groups of approximately 70 **[2]**.

2.4.1. Talent turning on CO2 Use take 2 (there is no take 1, it was unusable)

2.4.2. Talent sorting flies

2.5. Then add each group of flies to a starvation vial **[1]** and label each vial with the genotype and time of starvation initiation **[2]**.

2.5.1. Talent adding flies to vial

2.5.2. Talent label vial OR Shot of vial(s) with label(s) Use take 2

3. Reagent Preparation

3.1. Before performing an experiment, prepare a range of dilutions for each dye, using the same food with each different dye color **[1]**.

3.1.1. WIDE: Talent making dilutions, with dye and food containers visible in frame *Videographer: Important/difficult step*

3.2. Combine 0.5 grams of agarose with 50 milliliters of pure water in a microwave-safe vessel **[1]** and heat the agarose solution until it has dissolved, stirring as necessary **[2]**.

3.2.1. Talent adding agarose to water, with stock agarose container visible in frame
3.2.2. Talent placing solution into microwave

3.3. Dissolve each food component, including sucrose and any experimental compounds, in water at a 100-fold or higher concentration of the final tested concentration **[1]** and mix the agar, dye, and desired experimental compound in conical polypropylene centrifuge tubes **[2-TXT]**.

3.3.1. Talent dissolving food in water, with food container visible in frame
3.3.2. Talent mixing food, dye, and agar in tube, with food, dye, and agar containers visible in frame **TEXT: Use water instead of experimental tastant in control food samples**

3.4. After mixing, place the tubes in a 60-degree Celsius water bath until distribution **[1]** and add 1 milliliter of one color of the experimental food and dye solutions to one side of the first assay dish **[2]**. 3.4.1. and 3.4.2. are merged

3.4.1. Talent placing tube into dish *Videographer: Important step*
3.4.2. Talent adding dye to dish *Videographer: Important step*

3.5. Allow the agarose to cool until firm before adding 1 milliliter of the control food solution labeled with the second dye color to the other side of the dish **[1]**.

3.5.1. Shot of solidified red dye, then blue dye being added to other side of dish *Videographer: Important step*

3.6. Then repeat the assay dish preparation with the opposite control and experimental dye solution pair **[1]**.

3.6.1. Shot of solidified blue dye, then red dye being added to other side of dish

3.7. Use the results to identify two-dye concentrations that yield a preference index of 0 when no experimental compound is added **[1]**.

3.7.1. LAB MEDIA: Figure 1B *Video Editor: please emphasize 0 index line*

4. Two-Way Feeding Assay

4.1. To perform a two-way feeding assay, first temporarily paralyze one experimental fly vial for 3-5 minutes on ice **[1]** until no obvious motor activities, such as flying and climbing, are observed **[2]**.

4.1.1. WIDE: Talent placing vial on ice
4.1.2. Shot of immobilized flies

4.2. Once the flies have been immobilized, gently invert the vial **[1]** and tap to transfer the flies into the assay chamber **[2]**. 4.2.1. and 4.2.2. are merged

4.2.1. Talent inverting vial *Videographer: Important step* Use 6H0A3973 if possible; authors think this is cleanest take (although there are multiple takes to choose from)
4.2.2. Vial contents being tapped into assay chamber *Videographer: Important step*

4.3. Then quickly place the lid onto the chamber **[1]**. When all of groups have been transferred, move all of the assay chambers to a dark, enclosed space for 90 minutes **[2]**.

4.3.1. Talent placing lid onto dish *Videographer: Important step* Some takes merge 4.3.1. and 4.2.2. are merged
4.3.2. Talent placing dish into dark *Videographer: Important step*

4.4. At the end of the assay, invert the chambers in a minus 20-degree Celsius freezer for about an hour **[1]** before placing each chamber under a stereo microscope **[2]** to examine the abdominal color of the flies in each experimental group **[3]**.

4.4.0. Added shot: Dishes being flipped
4.4.1. Talent placing inverting chamber into freezer
4.4.2. Talent placing dish under microscope
4.4.3. ~~SCOPE: Shot of flies in dish~~ LAB MEDIA: 4.4.3.psd

4.5. Count a fly if the abdomen is more than 50% colored, indicating a robust feeding **[1]**.

4.5.1. ~~SCOPE: Shot of fl(ies) with >50% colored abdomen(s)~~ LAB MEDIA: 4.5.1

4.6. Discard any flies in which the abdomen contains only a tiny food spot, which is indicative of poor eating **[1]**.

4.6.1. ~~SCOPE: Shot of fl(ies) with only tiny food spot~~ LAB MEDIA: 4.6.1.

4.7. When all of the files have been counted, use the equation to quantify the food preference index for each group **[1-TXT]**.

4.7.1. BLACK TEXT WHITE BACKGROUND:

$$\frac{(Number\ of\ flies\ eating\ experimental\ food) - (Number\ of\ flies\ eating\ control\ food)}{(Number\ of\ flies\ eating\ experimental\ food) + (Number\ of\ flies\ eating\ control\ food) + (Number\ of\ flies\ eating\ both)}$$

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
3.1., 3.5., 3.6., 4.2., 4.3.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.1. ensuring that the dye concentrations used do not influence food preference is paramount.

Results

5. Results: Representative *Drosophila* Food Preference Assessment by Rapid-Food Preference Assay

5.1. In this assay, a 35-millimeter dish **[1]** was divided into two equal feeding compartments containing agarose food coupled with red or blue dye **[2]**.

5.1.1. LAB MEDIA: Figure 1A
5.1.2. LAB MEDIA: Figure 1A *Video Editor: please sequentially emphasize red and blue compartments*

5.2. To exclude dye bias **[1]**, the blue and red dye concentrations were carefully refined to yield an approximate "0" preference when only these two dyes were added **[2]**.

5.2.1. LAB MEDIA: Figure 1
5.2.2. LAB MEDIA: Figure 1 *Video Editor: please emphasize preference index line at 0*

5.3. Ninety minutes after wet-starved adult fly loading **[1]**, fly abdominal colors appeared as blue **[2]** or red if the animal predominantly consumed blue or red food, respectively **[3]**. If a fly consumed both blue and red, the abdomen appeared purple **[4]**.

5.3.1. LAB MEDIA: Figure 2A
5.3.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize blue/green fly abdomen*
5.3.3. LAB MEDIA: Figure 2A *Video Editor: please emphasize red fly abdomen*
5.3.4. LAB MEDIA: Figure 2A *Video Editor: please emphasize purple fly abdomen*

5.4. Only flies that ingested a considerable amount of food were scored **[1]**, while flies with an insufficient food intake were not counted **[2]**.

5.4.1. LAB MEDIA: Figures 2B and 2C *Video Editor: please emphasize Figure 2B fly*
5.4.2. LAB MEDIA: Figures 2B and 2C *Video Editor: please emphasize Figure 2C fly*

5.5. Comparing this Petri-dish-based assay to the multiwell-plate-based assay **[1]** revealed that the two feeding methods gave essentially the same assaying feeding results in response to sweet **[2]** … bitter **[3]** … and salty food in wild-type flies **[4]**.

5.5.1. LAB MEDIA: Figures 3A-3C
5.5.2. LAB MEDIA: Figures 3A-3C *Video Editor: please emphasize Figure 3A*
5.5.3. LAB MEDIA: Figures 3A-3C *Video Editor: please emphasize Figure 3B*
5.5.4. LAB MEDIA: Figures 3A-3C *Video Editor: please emphasize Figure 3C*

5.6. Notably **[1]**, however, it is much faster to prepare and distribute food in a Petri dish **[2]** than in a 60-well multiwell plate **[3]**.

5.6.1. LAB MEDIA: Figure 3D
5.6.2. LAB MEDIA: Figure 3D *Video Editor: please emphasize Petri dish data bar*
5.6.3. LAB MEDIA: Figure 3D *Video Editor: please emphasize Multiwell plate data bar*

Conclusion

6. Conclusion Interview Statements

6.1. **John Mack**: Be sure to always to identify what dye concentrations to use, especially after making new dye stocks. The concentrations can even be different between fly lines **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.1., 3.4.)

6.2. **Yali Zhang**: Our Petri dish-based, two-choice assay is a powerful tool for investigating how flies perceive external and internal nutrients to elicit appropriate feeding behaviors **[1]**.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera