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Title: A High Throughput Microplate Feeder Assay for Quantification of Consumption in Drosophila

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No.**

Please, integrate Supplementary video S1 in the production.

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

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 videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits. **All participants have been vaccinated**

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 17
Number of Shots: 46

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Robert Anholt:** The microplate feeder assay provides a simple high throughput, economical method to measure Drosophila feeding behavior and it offers multiple advantages over other more elaborate methods.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.1.3.*

1.2. **Jeffrey Hatfield:** Quantifying consumption by measuring absorbance with a plate reader eliminates manual measurements and obviates manual data entry. Data are also amenable to programmatic extraction and processing.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.7.2.*

OPTIONAL:

1.3. **Trudy Mackay:** With this high throughput assay we can measure the consumption of water-soluble nutrients, drugs, pharmaceuticals, or toxins, and the system can be modified for applications to a variety of insect species.

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

Protocol

2. Starvation Plate Preparation, Fly Sorting, and Starvation

2.1. Begin by pouring the molten agarose into a reagent trough **[1]** and dispense 80 microliters of molten agarose into each well of a 96-well microplate using a multichannel pipette **[2].** Refrigerate the leftover agarose for up to a week in a sealed bag and re-melt it for making additional plates **[3]**.

2.1.1. WIDE: Talent pouring molten agarose into a reagent trough.
2.1.2. Talent dispensing the molten agarose into the 96-well plate.
2.1.3. Talent placing the leftover agarose in the refrigerator.

Videographer's NOTE: Shots 2.1.1 and 2.1.2 were merged.

2.2. If the barrier strips are too loose, coil them around the finger to give them curvature to hold them in the channels **[1].** Insert the barrier strips into the barrier strip channels to prepare the couplers **[2]**.

2.2.1. Talent coiling the barrier strips around their finger.
2.2.2. Talent inserting the barrier strips into the channels.

2.3. Affix the coupler to a starvation plate, making sure not to use the coupler to manipulate the plate as the coupler may slip off **[1]**. Ensure that the angled corner of the coupler matches the angled corner of the microplate to maintain correct orientation **[2]**. *Videographer: This step is important!*

2.3.1. Talent affixing the coupler to the starvation plate.
2.3.2. Talent ensuring correct orientation of the plate and the coupler.

Videographer's NOTE: Shots 2.3.1 and 2.3.2 were merged.

2.4. Under CO_2 anesthesia, sort 3 to 5-day old flies **[1]**. Load individual flies by column into the starvation plate **[2]**. Close each column as it fills by adjusting its barrier strip to the closed position **[3]**. *Videographer: This step is important!*

2.4.1. Talent sorting the flies.
2.4.2. Talent loading the flies into the starvation plate.
2.4.3. Talent closing the column.

Videographer's NOTE: Shots 2.4.1 and 2.4.2 were merged.

2.5. Carefully record the sample layout within the microplate **[1]**. Once the starvation plate is filled, allow the flies to recover spontaneously after removing the CO_2 and starve them for 6 hours starting from their initial anesthetization time **[2]**.

2.5.1. Talent recording the sample layout.
2.5.2. ECU: A shot of the flies in the starvation plate.

3. Liquid Food and Feeder Plate Preparation

3.1. Prepare 10 milliliters of liquid food in a 15-milliliter conical tube by dissolving 0.4 grams of sucrose and 0.1 gram of yeast extract in 10 milliliters of distilled water **[1-TXT]**. Vortex the tube until the solids fully dissolve **[2]**. Add 40 microliters of dye stock solution **[3]** and transfer the liquid food into a 10-milliliter syringe tipped with a 0.45-micrometer filter **[4]**. NOTE: Step description has been edited as two new shots were added during shoot.

3.1.1. Talent preparing the liquid food. **TEXT: 4% sucrose, 1% yeast extract, 40 µg/mL of FD&C Blue #1**
3.1.1/B Talent vortexing the tube.
3.1.1/C Talent adding dye in the tube.
Videographer's NOTE: Two new shots were added after 3.1.1
3.1.2. Talent transferring the liquid food into the syringe.

3.2. Filter approximately 1.5 milliliters of the solution at a time into a 1.7-milliliter microcentrifuge tube **[1]**. Set the syringe containing the solution aside and filter the additional solution as needed during feeder plate preparation **[2]**.

3.2.1. Talent filtering the solution into the centrifuge tube.
3.2.2. Talent placing the syringe aside.

Videographer's NOTE: Shots 3.2.1 and 3.2.2 were merged.

3.3. Prepare a feeder plate by sealing the bottom of a 1536-well microplate with a sealing film **[1]**. Use a sealing paddle to adhere to the film thoroughly **[2]**, then trim excess film off the left and right edges with a razor blade **[3]**.

3.3.1. Talent sealing the bottom of the 1536-well plate.
3.3.2. Talent using a sealing paddle to adhere the film thoroughly.
3.3.3. Talent trimming the excess film off.

Videographer's NOTE: Shots 3.3.1, 3.3.2, and 3.3.3 were merged.

3.4. Dispense 10 microliters of the filtered liquid food column-wise into the upper-left-hand well for each cluster of four wells of the 1536-well microplate **[1]**. Once all the wells are filled, apply a sealing film to the top of the plate, following the same steps used to seal the bottom of the microplate. Repeat for the desired number of plates **[2]**. *Videographer: This step is important!*

3.4.1. Talent dispensing the liquid food into the wells.
3.4.2. Talent sealing the top of the plate.

3.5. Centrifuge the plates at 200 x *g* for 10 seconds to settle the fluid **[1]**. Do not allow the plate to be chilled since this can cause condensation to build up in the wells, obscuring absorbance readings **[2]**.

3.5.1. Talent centrifuging the plates.
3.5.2. A shot of the 1536-well microplate.

4. Exposure

4.1. Perforate the wells on the top surface of the plate with the needle probe tool equipped with a 0.25-millimeter diameter needle using the same order to perforate as was used when dispensing the solutions **[1]**. Wipe off the needle between solutions to prevent cross-contamination **[2]**. Flip the plate and perforate the wells on the bottom **[3-TXT].** *Videographer: This step is important!*

4.1.1. Talent perforating the wells on the top surface of the plate.
4.1.2. Talent wiping the needle.
4.1.3. Talent flipping the plate and perforating the wells on the bottom. **TEXT: Do not touch the perforations**

Videographer's NOTE: Shots 4.1.1 and 4.1.2 were merged.

4.2. Read the plate's absorbance at 630 nanometers without a lid **[1]**. Place an internal lid on the top sealing film to ensure that the condensation rings encircle the perforated wells **[2]**, then place the external lid on the plate **[3]**.

4.2.1. Talent taking the plate's absorbance.
4.2.2. Talent placing the internal lid on the top sealing film.
4.2.3. Talent placing the external lid on the plate.

Videographer's NOTE: Shots 4.2.2 and 4.2.3 were merged.

4.3. Place the feeder plate face-up on the coupler such that the guides align the appropriate holes of the feeder plate and starvation plate **[1]**. Ensure the coupler and plates are correctly oriented **[2]**. *Videographer: This step is important!*

4.3.1. Talent placing the feeder plate on the coupler.
4.3.2. Talent checking the orientation.

4.4. Once all the feeder plates are loaded onto the couplers, open the wells for the plates by adjusting the barrier strips on the coupler **[1]**. Place the coupler and plate assemblies in the secondary container **[2]**. Place the lower half of a pipette box containing soaked paper towels into each secondary container to provide humidity **[3]**. *Videographer: This step is important!*

4.4.1. Talent opening the wells for the plates.
4.4.2. Talent placing the coupler and plate assemblies in the secondary container.
4.4.3. Talent placing the lower half of a pipette box containing soaked paper towels into the secondary container.

4.5. Close the lid of the secondary container **[1]** and transfer it to a controlled environment **[2-TXT]**. Allow the flies to consume for 22 hours **[3]**. After the 22 hours of exposure, check each plate for dead flies and update the plate layout accordingly **[4]**.

4.5.1. Talent closing the lid of the secondary container.
4.5.2. Talent transferring the containers to a controlled environment. **TEXT: 25 °C, humidity controlled, 12 h light: dark cycles**
4.5.3. SCREEN: JoVE Review - Supplementary Video S.1.mp4. 00:05 – 00:10
4.5.4. Talent updating the plate layout.

Videographer's NOTE: 4.5.4 should be the beginning of 4.6 but it can work on the end of 4.5. Ask the authors if it should be moved.

4.6. After all the plates are checked, anesthetize the flies *en masse* by pumping CO_2 inside the secondary container **[1]**. After approximately 60 seconds, ensure that all the flies are immobilized **[2]**. Gently tamp the flies into the starvation plate **[3]** and replace the plastic barrier strips **[4]**.

4.6.1. Talent anesthetizing the flies.
4.6.2. Talent checking if flies are immobilized.
4.6.3. Talent tamping the flies into the starvation plate.
4.6.4. Talent replacing the plastic barrier strips.

Videographer's NOTE: Shots 4.6.2, 4.6.3, and 4.6.4 were merged.

4.7. Remove the feeder plates for reading **[1]**. Re-read the plate's absorbance at 630 nanometers. Repeat the process until all plates have been read **[2]**.

4.7.1. Talent removing the feeder plates.
4.7.2. Talent reading the plate's absorbance.

Results

5. Results: Quantification of Consumption in Drosophila

5.1. Evaporation was quantified for every well and was found to determine whether any correlations exist among the wells of individual plates **[1]**.

5.1.1. LAB MEDIA: Figure 6A.

5.2. Pearson correlation coefficients for evaporation versus rows **[1]** and evaporation versus columns were calculated to evaluate trends between evaporation and well locations **[2]**.

5.2.1. LAB MEDIA: Figure 6B.

5.2.2. LAB MEDIA: Figure 6C.

5.3. Consumption for 3-to-5-day old *Canton-S B* flies was quantified to establish the validity of the protocol **[1]**.

5.3.1. LAB MEDIA: Figure 7B.

5.4. Flies were given a choice between a 4 percent sucrose solution with 1 percent yeast extract and a 4 percent sucrose solution supplemented with 15 percent ethanol and 1 percent yeast extract. Both males and females showed an overwhelming preference for the solution with ethanol and yeast extract **[1]**.

5.4.1. LAB MEDIA: Figure 8. *Video editor emphasize the columns labeled 'EtOH.'*

Conclusion

6. Conclusion Interview Statements

6.1. **Jeffrey Hatfield:** It is essential to maintain consistency when constructing the feeder plates, ensuring that each fly is presented with an identical consumption scenario in regard to food volume, evaporation, and access.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll : 3.4.1. , 4.1.1.*

6.2. **Trudy Mackay:** This technique will allow researchers in the Drosophila field to perform large-scale screens of consumption and preference behaviors with higher throughput and at lower cost compared to conventional methods.

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