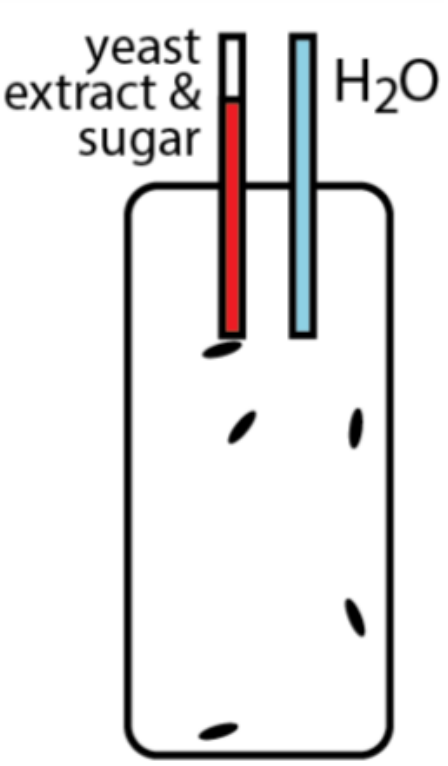

CAppillary FEeding Assay (CAFÉ)

To measure food consumption of flies

Prandiology of *Drosophila* and the CAFE assay

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Studies of feeding behavior in genetically tractable invertebrate model systems have been limited by the lack of proper methodology. We introduce the Capillary Feeder (CAFE), a method allowing precise, real-time measurement of ingestion by individual or grouped fruit flies on the scale of minutes to days. Using this technique, we conducted the first quantitative analysis of prandial behavior in *Drosophila melanogaster*. Our results allow the dissection of feeding into discrete bouts of ingestion, defining two separate parameters, meal volume and frequency, that can be uncoupled and thus are likely to be independently regulated. In addition, our long-term measurements show that flies can ingest as much as 1.7× their body mass over 24 h. Besides the study of appetite, the CAFE can be used to monitor oral drug delivery. As an illustration, we used the CAFE to test the effects of dietary supplementation with two compounds, paraquat and ethanol, on food ingestion and preference. Paraquat, a prooxidant widely used in stress tests, had a strong anorexigenic effect. In contrast, in a feeding preference assay, ethanol-laced food, but not ethanol by itself, acted as an attractant.

appetite | feeding | ingestion | preference

Understanding the physiology and regulation of appetite is an indispensable step in tackling biomedical problems such as obesity and feeding disorders. Invertebrate model systems have provided invaluable mechanistic insight into the genetic control of various biological and pathological processes, but have contributed relatively little to the understanding of the genetic underpinnings and neuronal circuitry of appetite regulation. This dearth is largely due to the limits of the available methodology. In both *Caenorhabditis elegans* and *Drosophila melanogaster*, feeding behavior is often inferred from qualitative parameters such as the amount of time spent on a given food source or the percentage of animals from a population seen eating or simply loitering on the medium at a given time (1–3). A more direct method, widely used in the nematode, is the pharyngeal pumping rate, which assumes a constant ingestion volume per pharyngeal contraction (4–6). In *Drosophila*, food can be labeled with nonabsorbable dyes (6, 7) or radioactive isotopes (8–12), but these techniques also have several limitations. Dyes progress rapidly through the digestive tract, precluding long-term measurements. Isotope labeling, on the other hand, permits long-term recordings but does not distinguish between ingestion and intestinal absorption, leading to permanent tissue incorporation. Most importantly, labeling methods require killing the flies for each measurement, making it impossible to continuously monitor the behavior of individual animals.

We describe a method allowing unambiguous recording of food ingestion in individual or groups of flies on the scale of minutes to the entire lifespan. Monitoring ingestion at short, 10-min intervals permitted the delineation of single meals. By modulating nutrient composition, we show that the parameters of meal volume and frequency are under independent control. In addition, we illustrate the usefulness of the Capillary Feeder (CAFE) for drug delivery.

Results and Discussion

Inspired by the work of Dethier with the blowfly *Phormia regina* (13, 14), we developed the CAFE, an assay allowing precise, continuous quantitation of actual ingestion in individual *Drosophila*. In the CAFE, flies consume liquid food from a graduated glass microcapillary (Fig. 1). Descent of the meniscus is clearly visible, allowing continuous, unambiguous measurement of consumption. This method obviates the need for food markers and the commonly used supportive ingredients, such as cornmeal and agar. Because the capillaries can be replaced as needed, with minimal disturbance to the animals, it is possible to monitor real-time ingestion for periods ranging from minutes to the entire lifespan.

Although much attention has been devoted to the analysis of appetite, most studies have focused on total ingestion. Prandiology, the study of specific parameters such as the size and frequency of meals, has been neglected, despite the central role played by prandial habits in the physiopathology of obesity, hypercholesterolemia, and heart disease (15). Because the sensitivity of the CAFE makes it possible to monitor ingestion on the scale of minutes, we studied the short-term feeding pattern of individual flies. This analysis revealed discrete feeding events (meals) separated by intervals of no consumption (Fig. 2*A*). With a regimen of 5% sucrose + 5% autolyzed yeast extract, we recorded an average meal volume of 0.096 ± 0.008 μl at a frequency of 0.65 ± 0.08 meal/h (Fig. 2*C*).

We next asked whether meal size and frequency can be uncoupled by manipulating food composition. Male flies feeding on a 5% sucrose solution, with no yeast extract added, showed a meal frequency similar to that of flies fed sucrose + yeast (average = 0.58 ± 0.14 meal per h, $P = 0.64$) (Fig. 2 *B* and *C*). In contrast, average meal volume increased by 56% (0.15 ± 0.02 μl, $P < 0.003$) (Fig. 2*C*). Hence, *Drosophila* feeding behavior is a function of at least two discrete, independently regulated components. It should therefore be possible to isolate mutants affecting each feeding parameter separately.

The CAFE can be used to record ingestion continuously over an extended period. We monitored individually housed male flies fed 5% sucrose + 5% autolyzed yeast extract over a 5-day period (Fig. 3*A*). Flies consumed a daily average of 1.5 ± 0.04 μl, an impressive 1.7× their body mass. This value varied between 1.3 and 2.3 μl per day per fly in different experiments. The rate of ingestion varies during the 12-h light/dark periods (data not shown). Approximately two-thirds of the daily total ingestion occurs during the light period. The linearity of the long-term accumulation patterns in Fig. 3 is due to the individual measurements being made twice daily, once during the mid-light and the other during the middark periods, and therefore does not reveal the circadian rhythm.

In *Drosophila*, social interaction can influence courtship, aggression behavior, and sleep patterns (16, 17). We compared the ingestion by flies housed individually, in pairs, or in groups

Author contributions: W.W.J. and G.B.C. contributed equally to this work; W.W.J., G.B.C., E.M.M., N.N.d.l.R., A.Y.F., and T.B. designed research; W.W.J., G.B.C., E.M.M., A.Y.F., and J.C.L. performed research; W.W.J. contributed new reagents/analytical tools; and W.W.J., G.B.C., E.M.M., N.N.d.l.R., and S.B. wrote the paper.

The authors declare no conflict of interest.

Abbreviation: CAFE, Capillary Feeder.

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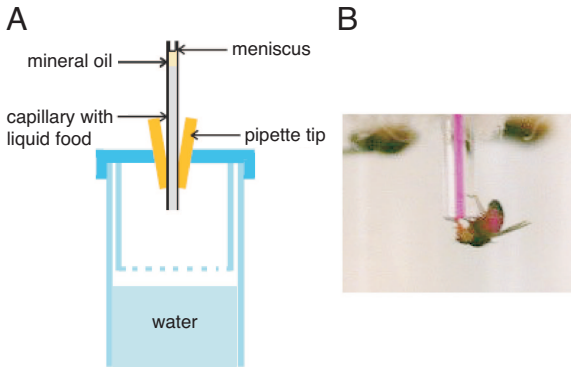

Fig. 1. The CAFE assay. (*A*) Schematic diagram. Liquid food, topped with an oil layer to minimize evaporation, is introduced via a glass capillary held in place by a pipette tip. The pierced bottom of the inner chamber provides humidity. (*B*) Fly feeding from the capillary. To facilitate visualization, a red dye has been added to the medium and can be seen in the proboscis and abdomen of the fly.

of four or eight animals per CAFE. Average ingestion per fly was identical in all groups (Fig. 3*B*), suggesting that, under the conditions used, food consumption in the CAFE is not significantly influenced by the presence of conspecifics or competition for food access. When three flies were housed per chamber, changing the number of capillaries between one and three did not influence total feeding (Fig. 3*C*), supporting the conclusion that, under these experimental conditions, the amount of and access to the food source are not limiting.

To feed in the CAFE, flies must climb onto the glass capillary and descend to reach the tip (Fig. 1*B*). Access to the medium can therefore be more strenuous than that under ordinary laboratory conditions, where flies stand on abundant solid food. We asked whether ease-of-access to the nutrient source influences ingestion volume by varying the distance between the capillary tip and the top of the chamber, on which the flies tend to accumulate and wander. For one group, the capillary opening was set immediately below the pipette tip, i.e., 4 mm below the cap (Fig. 1*A*), allowing the flies to feed without having to climb down on the capillary. In a second group, the tip was placed 6.5 mm below the cap (the default condition used in all other experiments reported here), whereas a third group had the tip placed 16.5 mm below. These variations in capillary height had no effect on ingestion rates (Fig. 3*D*). Under all conditions tested, the flies were never observed to jump or fly directly onto the capillary, instead choosing to walk from the cap onto the glass surface and treading its length to reach the opening. The conditions of the CAFE are therefore unlikely to inhibit feeding by reducing food accessibility.

Pharmacological treatments are a hallmark of behavioral and metabolic studies in *Drosophila* (18–20). The CAFE represents a significant advance for oral drug delivery, because it minimizes the amount of material required, while confirming actual ingestion and monitoring possible effects of the drug on appetite. To illustrate this application, we tested the effect of paraquat, a prooxidant drug commonly used in stress resistance tests. We compared the intake of animals offered a 5% sucrose solution with or without 20 mM paraquat. Over a 12-h period, the flies fed paraquat-laced food consumed 75% less than controls (0.23 ± 0.06 and 0.88 ± 0.16 μl, with or without paraquat; $P = 0.01$) (Fig. 4*A*). Moreover, monitoring prandial behavior during the first 6 h showed a decrease in average meal size from 0.15 ± 0.02 to 0.05 ± 0.02 μl with paraquat ($P = 0.007$) (Fig. 4*B*). Throughout the 12-h period, flies retained their climbing ability, and paraquat-induced death did not begin until 36 h (data not shown). The observed difference in intake thus suggests a bona fide anorexigenic effect of the compound, rather than nonspecific morbidity. These results stress the importance of taking into account actual ingestion upon oral administration of drugs, which are typically added to solid food.

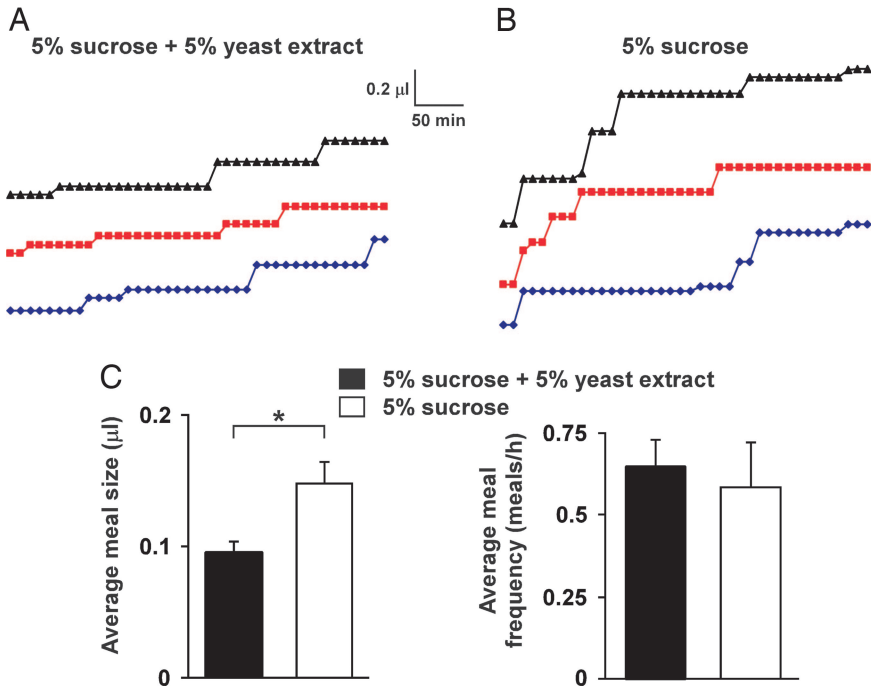

Fig. 2. Prandial behavior analyzed in the CAFE. (*A*) Intake by three individually housed male flies fed 5% sucrose + 5% yeast extract measured in 10-min intervals. A vertical rise flanked by two intervals of no intake was defined as a meal. (*B*) Intake by individual flies fed 5% sucrose. (*C*) Meal volume and frequency can be decoupled by modulating nutrient conditions. On 5% sucrose, average meal size increases, whereas meal frequency is unchanged; 5% sucrose + 5% yeast extract: $n = 10$ flies, 52 meals; 5% sucrose: $n = 4$ flies, 18 meals. All values are given as averages $\pm$ SE. *, $P \leq 0.01$, two-tailed *t* test.

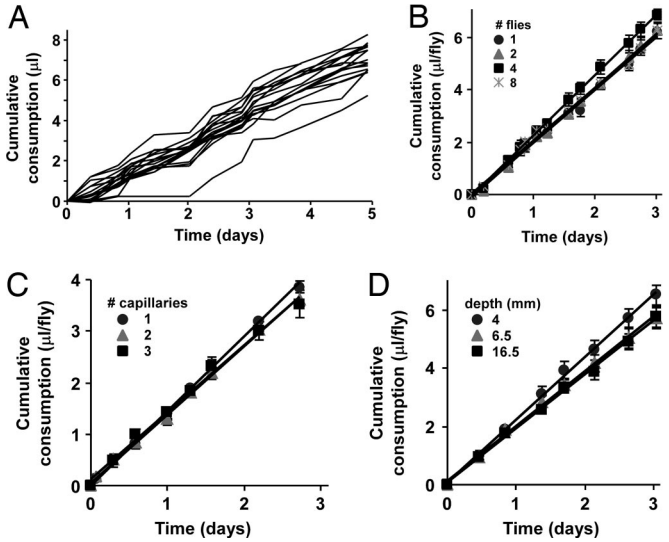

Fig. 3. Measurement of long-term food consumption in the CAFE. (*A*) Cumulative ingestion by 17 individual male flies over 5 days. Average consumption = 1.5 ± 0.04 μl per day per fly. (*B*) The number of animals per chamber does not influence individual feeding rate. One, two, four, or eight flies were housed per CAFE. Average consumption was 2.0 ± 0.02, 2.1 ± 0.1, 2.3 ± 0.1, and 2.0 ± 0.1 μl per day per fly, respectively ($R^2 > 0.98$ for each linear fit; ANOVA $P = 0.24$). (*C*) The number of capillaries per chamber does not affect food intake. One, two, or three capillaries were used per CAFE. Three flies were housed per chamber. Average consumption was 1.5 ± 0.03, 1.3 ± 0.04, and 1.3 ± 0.1 μl per day per fly, respectively ($R^2 > 0.98$; ANOVA $P = 0.25$). (*D*) Capillary depth has no effect on food ingestion. Four flies were used per CAFE, with the capillary tip placed 4 mm, 6.5 mm, or 16.5 mm below the top of the chamber, respectively (Fig. 1*A*). Average consumption was 2.2 ± 0.2, 1.9 ± 0.1, and 1.8 ± 0.1 μl per day per fly, respectively ($R^2 > 0.99$; ANOVA $P = 0.26$). In all experiments, 5% sucrose + 5% autolyzed yeast extract was served. All values are given as averages ± SE.

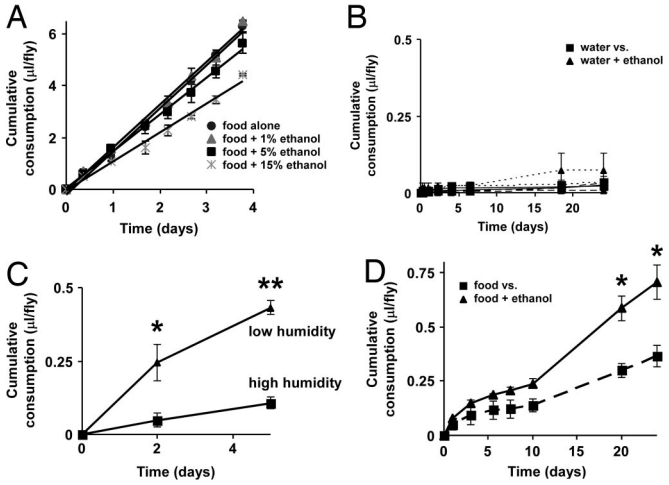

Fig. 5. Serving ethanol in the CAFE. (*A*) A dietary ethanol supplement has a modest, inhibitory effect on long-term food intake. Flies were fed 5% sucrose + 5% autolyzed yeast extract medium alone or supplemented with 1%, 5%, or 15% (vol/vol) ethanol. Average consumption was 1.7 ± 0.07, 1.7 ± 0.03, 1.4 ± 0.1, and 1.1 ± 0.01 μl per day per fly, respectively ($R^2 > 0.97$ for each linear fit; ANOVA $P = 0.018$; $n = 8$ flies per condition). (*B*) In the absence of food medium, ingestion of either plain water or ethanol is remarkably low. For 24 h, flies were offered a choice between two capillaries, one containing pure water and the other containing one of three concentrations of ethanol: 1% (dotted line), 10% (dashed line), or 50% (solid line). Maximum ingestion was <0.07 μl per day per fly with 1% ethanol ($n = 12$ animals per condition). (*C*) Desiccation stimulates water consumption. Flies were deprived of food and water for 24 h in either a humidified or nonhumidified CAFE and then provided with plain water in regular humidified conditions. (*D*) Given a choice between food (5% sucrose + 5% autolyzed yeast extract) with and without a 15% ethanol supplement, flies showed a strong preference for the ethanol-laced regimen ($n = 8$ animals per condition). All values are given as averages ± SE. *, $P < 0.05$; **, $P < 0.01$, two-tailed *t* test.

Alcoholism is a notorious health problem with major social and economic consequences. Epidemiological data indicate that 13.5% of the population in the United States suffers from alcohol abuse dependence (21). Elucidating the mechanisms of alcohol intoxication and addiction are, therefore, outstanding biomedical goals. In recent years, *Drosophila* has become a prominent model system for the study of drug physiology, with a significant number of studies centering on the effects of ethanol (18, 22, 23). However, most studies have relied on ethanol vapor, which may bear differences from oral ingestion. The CAFE readily lends itself to studies of feeding facilitation. We therefore set out to develop a protocol for oral administration of ethanol by using the CAFE. We continuously monitored the consumption of 5% sucrose + 5% autolyzed yeast extract supplemented with various concentrations of ethanol over 4 days. A 1% supplement had no effect on feeding, but adding 5 or 15% ethanol resulted, respectively, in 14% and 33% lower overall consumption (Fig. 5*A*). This effect seems relatively modest in light of the high caloric content of ethanol: The presence of 5% and 15% ethanol, respectively, doubles and quadruples the total caloric value of the medium (medium alone = 279 kcal/liter, medium + 5% ethanol = 555 kcal/liter, and medium + 15% ethanol = 1,107 kcal/liter; see *Materials and Methods*). This finding may suggest that flies only absorb and/or metabolize a fraction of the ethanol they ingest. Alternatively, caloric content may not be the main determinant of feeding rate in *Drosophila*. In any case, our work establishes a method for oral administration of ethanol to *Drosophila* over extended periods of time.

We next asked whether ethanol represents an attractive or aversive stimulus when presented acutely. In the absence of food, flies ingested a negligible amount of ethanol in any of three concentrations [1%, 10%, or 50% (vol/vol)], even when housed in the CAFE up to 24 h and therefore under considerable nutrient deprivation (Fig. 5*B*). Similarly, pure water was ingested in remarkably small amounts over the same period (Fig. 5*B*). This is attributable to the high humidity maintained in the chambers because flies starved in a nonhumidified CAFE (with no water in the outer chamber) showed significantly increased ingestion of pure water from the capillaries (Fig. 5*C*). These results demonstrate that ethanol alone does not represent a particularly attractive stimulus. Together with the long-term

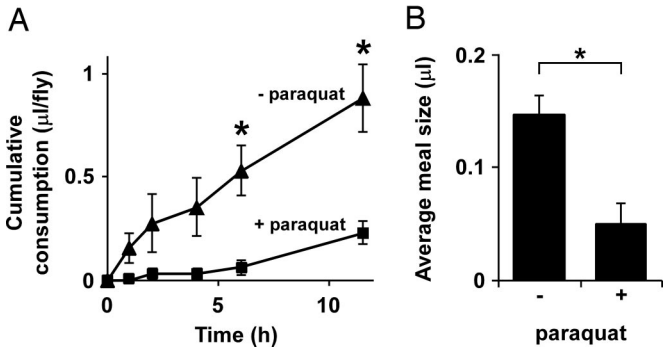

Fig. 4. Dietary paraquat inhibits food intake. (*A*) Ingestion of a 5% sucrose solution with or without 20 mM paraquat over a 12-h period ($n = 5$ flies per condition. (*B*) Paraquat inhibits meal size. Consumption was recorded every 10 min during the first 6 h of the long-term experiment shown in *A*. All values are given as averages ± SE. *, $P < 0.01$, two-tailed *t* test.

results (Fig. 5*A*), which suggest that it is not particularly aversive either, this result raised the possibility that flies are unable to detect ethanol. As a more stringent test of this scenario and of the valence of this substance, we conducted a feeding preference test in which flies were offered a choice between medium with or without a 15% ethanol supplement in two separate capillaries. Surprisingly, this test revealed a clear preference for the alcohol-containing meal (Fig. 5*D*). Together, our results indicate that ethanol constitutes an attractive stimulus in the presence of food but not by itself. A possible explanation is that ethanol itself possesses an indifferent taste but confers a metabolic advantage, such as a concentrated source of calories. Flies therefore do not ingest it when presented in isolation, but upon sampling it in their food associate that particular meal with the acquired metabolic advantage. Alternatively, the specific combination of ethanol and food may represent an attractive gustatory stimulus. More work will be required to distinguish between these possibilities and elucidate the mechanism of the ethanol preference behavior.

We have shown that the CAFE assay can reliably measure short- and long-term food ingestion of individual or groups of flies, as well as identify both inhibitory and stimulatory effects of dietary compounds on appetite. Because the CAFE requires orders of magnitude less material than the addition of drugs to solid food and because it allows simultaneous monitoring of intake, it will represent a significant advance for drug screens, in which the quantity of reagents can be a limiting factor. The CAFE should be of great value for the analysis of genetic pathways and neuronal circuits that regulate appetite in *Drosophila*, such as the insulin-like signaling pathway, hugin, and neuropeptide F (24, 25). Additionally, it should be adaptable to an automated, multi-CAFE, high-throughput format.

Materials and Methods

Preparation of the CAFE. The model used for these experiments was composed of two chambers (Fig. 1). The inner chamber, containing the flies, was prepared by paring down a 1.5-cm diameter plastic vial to 2-cm length, with the bottom pierced to allow entry of water vapor and air from the outer chamber, a 50-ml conical tube filled with 30 ml of water. Calibrated glass micropipettes (5 μl, catalog no. 53432-706; VWR, West Chester, PA) filled with liquid medium by capillary action were inserted through the cap via truncated 200-μl pipette tips. For some experiments, a mineral oil overlay ($\approx$0.1 μl) was used to minimize evaporation. Capillaries were replaced as needed. The long-term experiment in Fig. 3*A* was conducted under a 12-h-light/12-h-dark cycle in a room kept at 25°C and >70% humidity. The prandiology studies of Fig. 2 were conducted during the light period. The choice experiments in Fig. 5 were performed with two labeled capillaries, each containing a different food. Each experiment included an identical CAFE chamber without flies to determine evaporative losses (typically <10% of ingested volumes), which were subtracted from experimental readings. Average values ± SE are given.

Flies and Media. All flies tested were $\approx$1-week-old males of the Canton Special (Canton-S) strain raised on the Lewis medium used at the California Institute of Technology (26) and transferred to the CAFE from this food. Except where otherwise specified, the liquid food used in the CAFE was 5% (wt/vol) sucrose + 5% (wt/vol) autolyzed yeast extract (Bacto yeast extract; BD Diagnostic Systems, Franklin Lakes, NJ). All flies were habituated in the CAFE for 24 h, with ad libitum medium, before the measurements were started. The caloric content of the medium was calculated on the basis of the following values: 4 kcal/g (sucrose), 1.58 kcal/g (yeast extract), and 7 kcal/g (ethanol).

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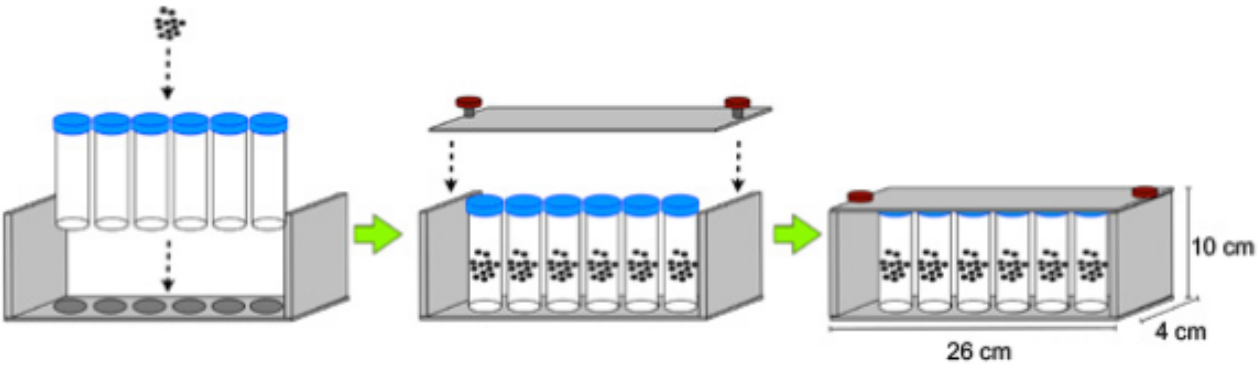
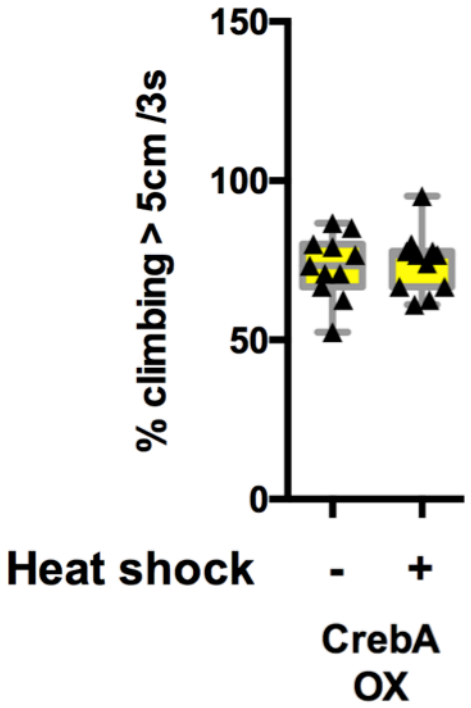


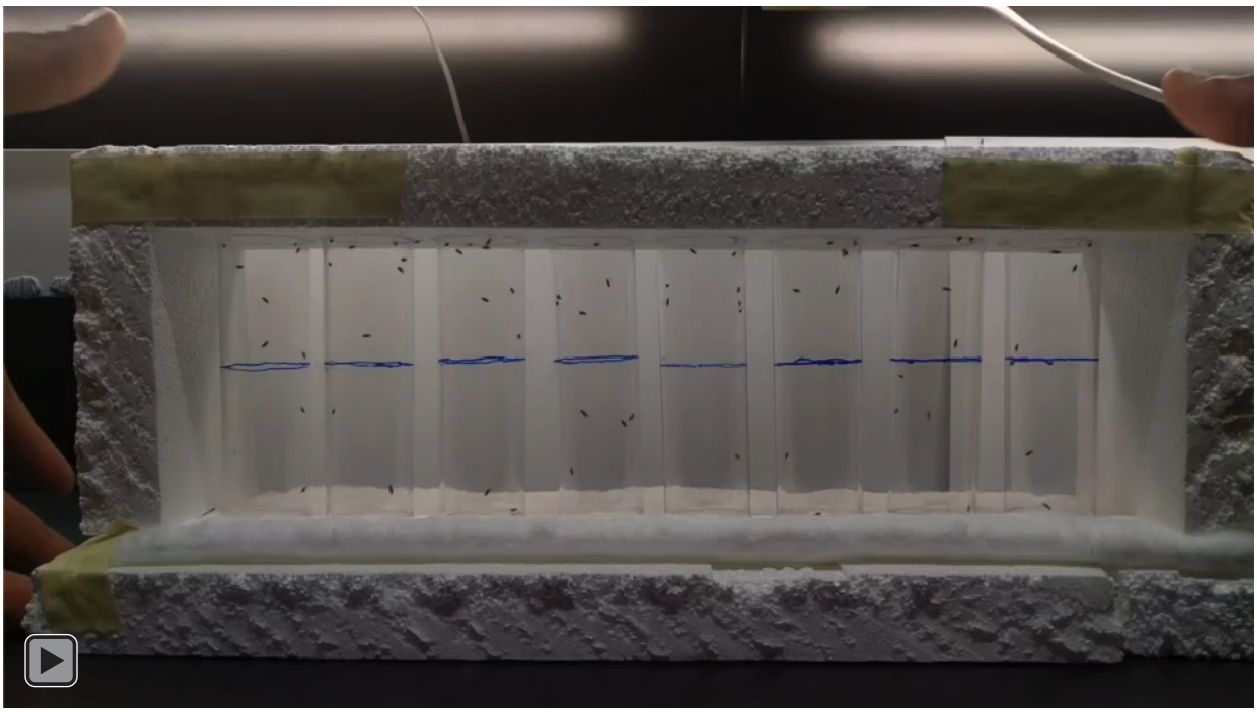
Climbing Assay

To check for fly ability to climb a surface (an indicator of fly
health)

Climbing Assay



Heat shock does not affect the ability of flies to consume food



Video Article

Methods to Assay *Drosophila* Behavior

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URL: http://www.jove.com/video/3795/
DOI: 10.3791/3795

Keywords: Neuroscience, Issue 61, *Drosophila*, locomotor dysfunction, courtship, larval crawling, RING assay, neurodegeneration

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Abstract

Drosophila melanogaster, the fruit fly, has been used to study molecular mechanisms of a wide range of human diseases such as cancer, cardiovascular disease and various neurological diseases[1]. We have optimized simple and robust behavioral assays for determining larval locomotion, adult climbing ability (RING assay), and courtship behaviors of *Drosophila.* These behavioral assays are widely applicable for studying the role of genetic and environmental factors on fly behavior. Larval crawling ability can be reliably used for determining early stage changes in the crawling abilities of *Drosophila* larvae and also for examining effect of drugs or human disease genes (in transgenic flies) on their locomotion. The larval crawling assay becomes more applicable if expression or abolition of a gene causes lethality in pupal or adult stages, as these flies do not survive to adulthood where they otherwise could be assessed. This basic assay can also be used in conjunction with bright light or stress to examine additional behavioral responses in *Drosophila* larvae. Courtship behavior has been widely used to investigate genetic basis of sexual behavior, and can also be used to examine activity and coordination, as well as learning and memory. *Drosophila* courtship behavior involves the exchange of various sensory stimuli including visual, auditory, and chemosensory signals between males and females that lead to a complex series of well characterized motor behaviors culminating in successful copulation. Traditional adult climbing assays (negative geotaxis) are tedious, labor intensive, and time consuming, with significant variation between different trials[2-4]. The rapid iterative negative geotaxis (RING) assay[5] has many advantages over more widely employed protocols, providing a reproducible, sensitive, and high throughput approach to quantify adult locomotor and negative geotaxis behaviors. In the RING assay, several genotypes or drug treatments can be tested simultaneously using large number of animals, with the high-throughput approach making it more amenable for screening experiments.

Video Link

The video component of this article can be found at http://www.jove.com/video/3795/

Protocol

1. Larval Crawling Assay

1. Larvae Collection

1. Set up an 8 ounce bottle of flies (10-15 males + 10-15 females).
2. Let flies lay eggs for 24 hours, then clear bottle of flies. (Transfer the adults into a new bottle and repeat as necessary).
3. Incubate bottle for 3-4 days, or until third instar larvae are visible.
4. Add 50 - 100 ml of 20% sucrose to the bottle with larvae and let sit for 20 minutes. Larvae will float to the top.
5. Collect larvae using a 25 ml serological pipette with the tip cut off, and place into a mesh basket.
6. Wash larvae in the mesh basket two times with deionized H_2O. Larvae are now ready for the experiments.

2. To treat larvae with drug

1. Use a brush to transport the desired number of larvae to a 5 ml beaker containing a solution 5% sucrose + drug.
2. Let larvae feed for at least 15 minutes.
3. Pour drug-treated larvae into a mesh basket and rinse. They are now ready to use.

3. Locomotor Assay (measuring total distance travelled or body wall contractions)

1. Use a brush to transport individual larva to a:
    1. 15 cm Petri dish containing 2% agarose (previously poured and allowed to harden) over graph paper with a 0.2 cm^2 grid.
        1. Count number of grid lines crossed in 1 minute.

2. Well of a glass dissection dish containing a dilute yeast paste solution.
    1. Count peristalsis contractions (full anterior to posterior movement = 1 contraction) in one minute while observing under a dissection microscope.

2. Repeat until the desired numbers of larvae have been counted.

2. Rapid Iterative Negative Geotaxis (RING) Protocol

This assay was originally described by Gargano *et al*[5].

1. Collect newly emerged adult male flies under light CO_2 anesthetization and place into a standard vial containing food (or food + drug).
2. Maintain flies at room temperature (on the bench top. ~22 °C) for 2-3 days to allow for recovery from CO_2 (and accumulation of steady-state drug levels if appropriate).
3. Transfer about 25 flies *without anesthetizing* to prepared polystyrene vials.
4. Assemble vials with flies into the RING apparatus (Figure 1).
5. Allow flies to acclimate to the environment, undisturbed, for 15-20 minutes.
6. During this time place digital camera ~1 m in front of the apparatus (on a platform if necessary to align the center of the lens at mid-height of the vials), focus and zoom the camera onto the apparatus, and set a timer to 3.0 seconds.
7. Carefully take hold of the RING apparatus with your left hand so as not to disturb the flies, and hold the timer with your right hand.
8. Sharply tap the apparatus down on the surface of the bench three times, ensuring that the tap is hard enough to knock down all the flies to the bottom of the vials.
9. Simultaneously with completion of the third tap, start the 3 second countdown timer.
10. At three seconds take a picture.
11. Reset the timer for 1 minute and start. During this time reset the camera and focus on the apparatus, and set another channel of the timer for three seconds.
12. After 1 minute, repeat steps 1.7-1.10
13. After a total of 5-6 trials, upload images onto a computer and use your favorite image viewer to open, and score the average height climbed for each vial.
14. Perform statistical analysis on your different groups comparing the mean height climbed.

3. Courtship and Mating Assay

1. First thing in the morning, clear well-producing bottles of flies to be used.
2. Over the course of the day (every 3-4 hours), collect newly emerged sexually naive males and females:
    1. Place males individually in vials or tubes with medium.
    2. Place 5-6 females together per vial/tube.
    3. Isolate collected flies at 25 °C under 12 hr light/dark for 5 days.
    4. Transfer one female into the chamber of a mating wheel.
    5. Transfer one male into the chamber of a mating wheel.
    6. Observe pair under a dissection microscope for the following behaviors:
        1. Orientation (the male orients towards the female)
        2. Tapping (the male taps the female)
        3. Wing song (the male extends and vibrates one wing)
        4. Licking (the male licks the female genitalia)
        5. Curling (the male curls its abdomen under itself)
        6. Copulation attempt (Curling activity while attempting to mount the female)

3. Observe for 10 minutes or until successful copulation, noting the time at which each behavior occurs (latency), the total time engaged in courtship behavior until copulation (to calculate the courtship index), as well as the number of pairs that successfully perform a given behavior (frequency). 100% of wild type pairs will generally mate within 5 minutes.
4. Calculate a courtship index (CI) by dividing the time spent in courtship divided by the total time until copulation. For wild type pairs this usually ranges between 0.6-0.8.

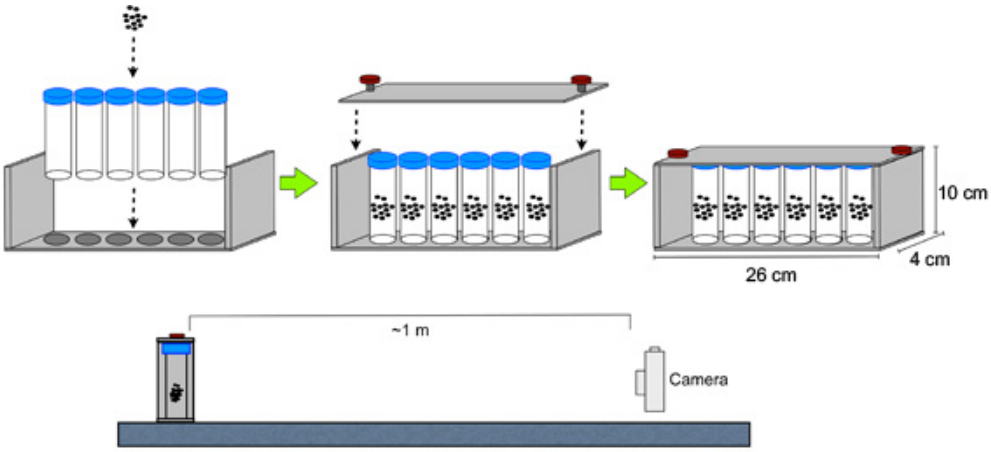
4. Representative Results

Crawling assay

Normal wild type larva will wander ~3 cm/minute, and exhibit ~40-50 body wall contractions in one minute. We have recently developed a Drosophila model of FUS/TLS-related amyotrophic lateral sclerosis that shows larval crawling defect, reduced life span and adult climbing impairment[6]. We targeted expression of wild type and mutant forms of FUS/TLS to the motor neurons (OK-371-gal4 driver) and performed a larval crawling assay. As shown below, wild type larvae crawl up to 12 cm, whereas expression of wild type FUS decreased the larval crawling ability to approximately 6 cm. Animals expressing the ALS-causing mutation R521C in FUS/TLS demonstrate a very severe impairment in their crawling movement (Figure 1), crawling only about 1 cm/minute.

Negative geotaxis RING assay

Young wild-type adult flies should have an average climbing height of ~4-5 cm in a 3-second time period (the time can be adjusted from 3 seconds to accommodate different strains or activity levels to define a given average height for a given strain/treatment). Flies that remain at the bottom are assigned a value of 0. It is not advised to use more than 25 flies per vial because it then becomes difficult to determine the location of each individual fly to measure height. No desensitization has been observed at up to the 6 consecutive trials spaced 1 minute apart that we have employed. It is critical *NOT* to reuse the polystyrene testing vials in this assay after the initial sets of data are gathered because new flies placed into used vials will not climb to the same extent as in fresh vials.



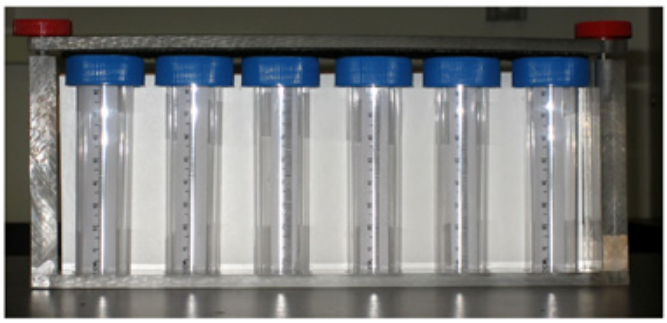


Figure 1. Setup for the RING Assay. The digital camera is placed ~1 m in front of the apparatus containing flies in the polystyrene vials; focus and zoom the camera onto the apparatus; and set a timer to 3.0 seconds.

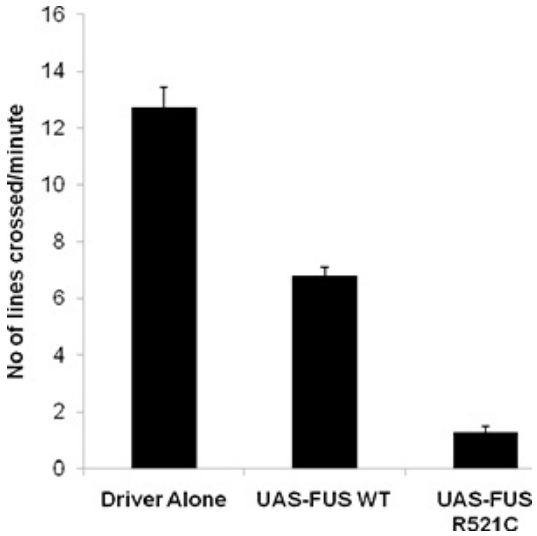


Figure 2. Representative data from the larval crawling assay using flies ectopically expressing UAS-FUS WT, and UAS-FUS R521C under the control of a motor neuron driver (OK371-gal4).

Discussion

Drosophila behavior is tightly regulated by genetic and environmental factors. We, and others, have previously used the assays described here to gather data to examine genes related to fly behaviors and to human neurodegenerative diseases modeled in *Drosophila*[5-19]. For the crawling assay, careful selection of 3rd instar larvae is a critical step. If treating with a drug, it will take 10-15 minutes (or more depending on the type and nature of the drug) to achieve maximal effect if it has good solubility. Therefore, we routinely feed flies the drug for 15 minutes, and then wait an additional 15 minutes before testing. It is important, however, to keep drug concentrations in solution, and exposure times, between treatment groups the same for accurate comparison. CNS active drugs will usually have a maximal effect lasting ~45 minutes. The larvae should be washed well after selection (or dug feeding) to remove the fly food and allowed acclimatized for 1 minute before starting the crawling assay. The agar plate should be kept at room temperature (~22° C) for an hour, as low temperature can influence larval crawling ability. Although the larval crawling assay can provide important information regarding activity levels, it is not suitable for analysis of subtle coordination deficits. Therefore, as a screening platform it is most appropriate for a first pass examining gross activity deficits.

An adult behavior that involves fine motor coordination is courtship and mating. This behavior has been used to examine aspects of behaviors relevant to human diseases, and involves sensory processing (olfactory, visual, acoustic) in addition to fine motor control[18]. When a male notices a female, he initiates the courtship ritual that progresses in a stereotype pattern beginning with orientation behavior (turning toward and chasing the female). This is followed by wingsong, licking and tapping of the female genetalia, curling of the abdomen towards the female, and culminates in copulation, which can last several tens of minutes[20-22]. Because many of the aspects of courtship involve visual cues, dark conditions lead to a degradation of mating performance, and assays should be performed with enough light so that flies can see one another. Accordingly, white-eyed flies usually have extremely poor performance in our described assay and readers are advised against planning experiments utilizing them with this protocol. If treating the flies with a drug, put the drug into the medium the flies are isolated on (e.g. 1% agarose + 10% sucrose + drug instead of standard food when treating with drug to avoid any possible degradation of the drug by microorganisms in the food). Generally the flies are isolated into 5 ml test tubes that have 300-500 ml of food in them, plugged with cotton. Do *NOT* anesthetize the flies for transfer to the mating wheel. It is absolutely *CRITICAL* to wash the mating wheel very well between uses to remove any residual pheromones (warm water with small amount of alconox soap over night with shaking, then washed for a minimum 48 hours in deionized water with shaking, and numerous changes of water). Another consideration when performing the assay we describe is the weather. In our experience flies will not court if it is raining or looks like it will rain. They perform best on bright sunny days, regardless if the laboratory has windows or not. Our current theory is that this phenomenon is related to atmospheric pressure, but we have not investigated this.

The traditional negative geotaxis assay relies on measuring how many flies climb above a predetermined height in 10 seconds (described in http://www.jove.com/details.php?id=2504). We believe that the RING assay has certain advantages over the traditional assay. One is throughput, as six independent replicates can be measured simultaneously compared to the one of the standard assay, and the system is generally scalable. Another is sensitivity, because the average height climbed in a defined time period is quantified, rather than a pass/fail number for absolute height. Using this approach, more subtle deficits may be observed. Because of the level of throughput, the assay is more suited to screens than the more tedious traditional negative geotaxis assay. Further, Garagano *et al.* (2005) describe a computerized scoring method that if employed would further increase throughput. Primary considerations for this assay are that it is imperative that the flies are not anesthetized before testing, and that fresh vials are used after a given set of trials before a new batch of flies are tested.

Disclosures

We have nothing to disclose.

Acknowledgements

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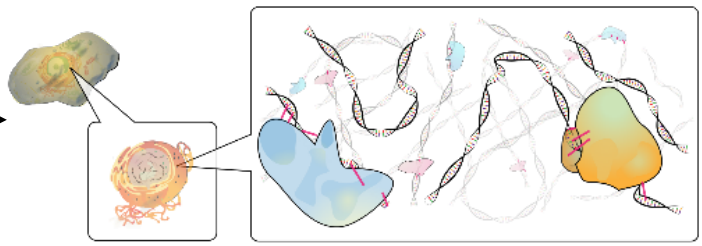
Chromatin Immunoprecipitation (ChIP)

Fly heads
Normally fed flies

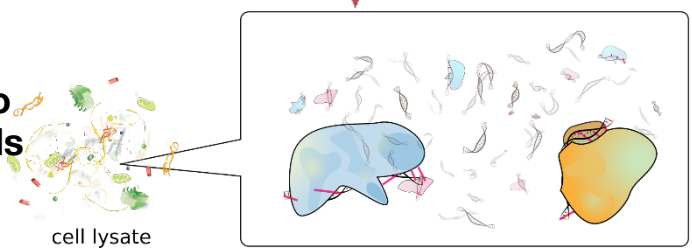
Extract nuclei



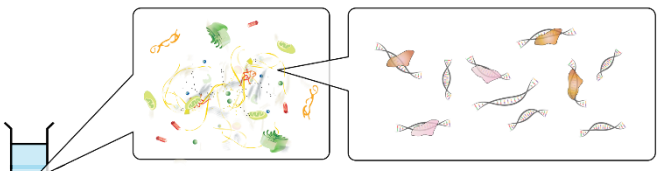
Shear DNA strands by sonicating

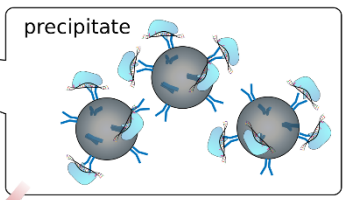
~200bp

CrebA antibody coupled to Protein-A Sepharose beads

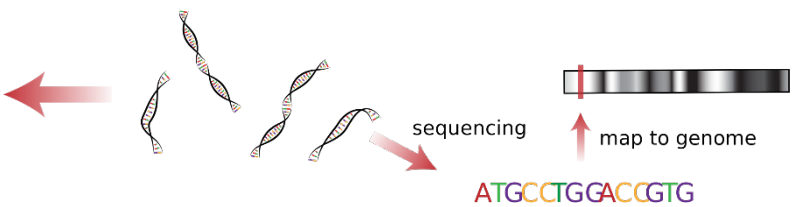


Add bead-attached antibodies to immunoprecipitate target protein

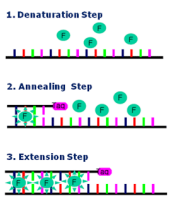




unlink protein; purify DNA



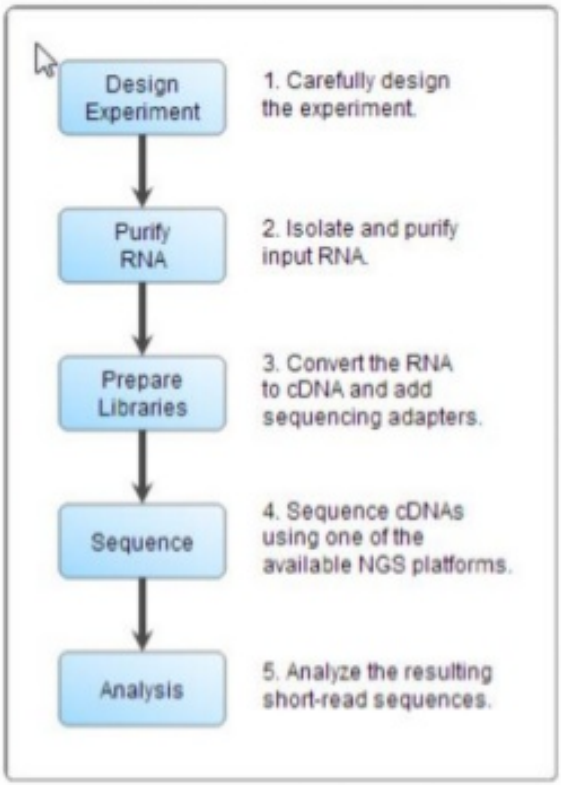
Target Validation

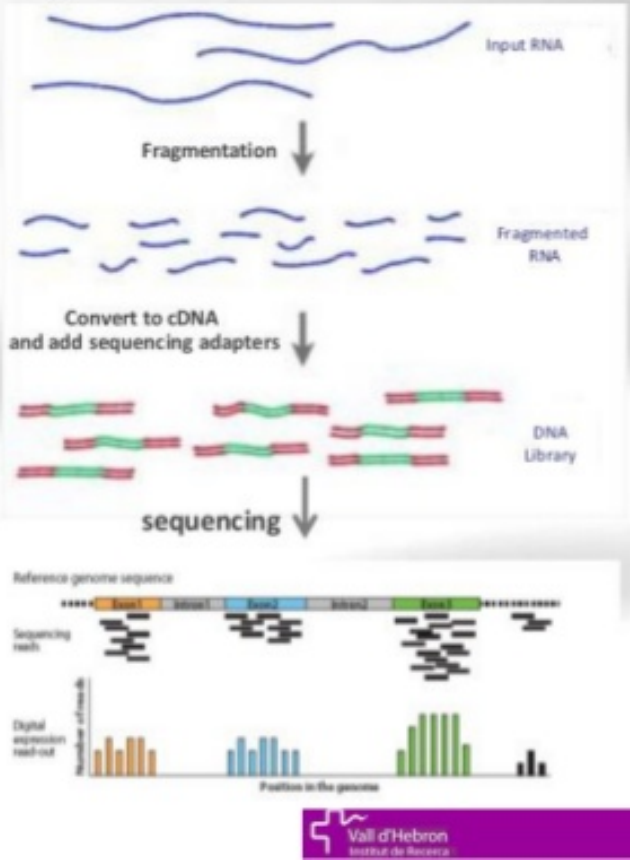


Secretory Pathway genes ➔ Sec61B

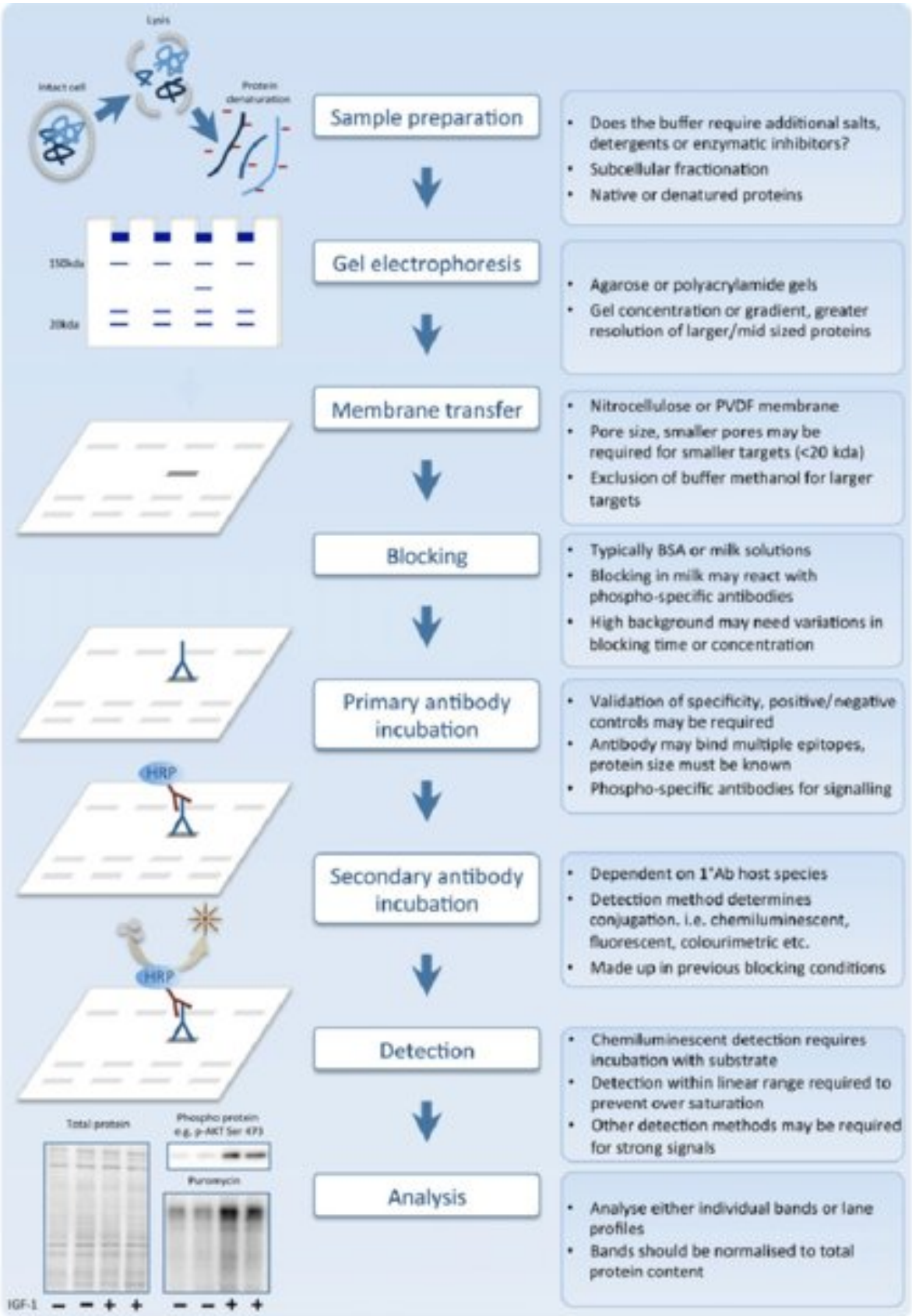
mRNA-Seq

RNA-seq analysis workflow





Western Blot Pipeline



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