Consumption Assay

1. Make sure all your food has the same percentage of FD&C blue dye #1 in it (2.5% works well). Different hours of the day, genotypes, ages, sexes, and diets produce different feeding rates so these should all be controlled. A light-dark cycling incubator will ensure feeding occurs around the same time each day.

2. Before starting, identify the time in which flies eat the most: Maintain flies on their respective diets and flip them onto the FD&C blue dye #1 version of that food for 2-hour intervals. Collect flies that have fed from 8-10, 10-12, 12-2, 2-4, etc. Each group of flies will only be used for one 2 hour period. Assay as below and choose the 2-hour interval with the highest amount of food consumed for your experiment.

3. Collect and freeze flies in groups of 4 after 2 hours in 1.5 ml tubes at -20°C. Do not keep flies that look sticky or are covered in blue food, as this will interfere with results.

   Also collect and freeze flies who have not eaten blue food in groups of 4 as controls.

4. Homogenize flies in 200 µl of PBS using a pellet pestle and centrifuge for 1 minute at at least 13,000 rpm. You will quantify this supernatant, so be careful not to pick up fly debris.

5. The amount of blue dye in the samples is calculated from a standard curve made by serial dilution of blue dye in PBS along with homogenate from age-matched flies fed non-dyed food, in order to correct for the absorbance of homogenate alone.
   a. Plate blank: 150 µl PBS & 50 µl of non-blue fly homogenate
   b. Standards: 148 µl PBS, 50 µl non-blue fly homogenate, & 2 µl standard
      i. Make Stock Standards= 31.25, 62.5, 125, 250, 500, & 1000 µg/ml FD&C blue #1
      ii. Final Concentrations= .3125, .625, 1.25, 2.50, 5.00, & 10.0 µg/ml FD&C blue #1
   c. Samples: 150 µl PBS & 50 µl experimental blue fly homogenate

6. Read the absorbance at 630 nm using a microplate spectrophotometer.

7. Calculating volume of food consumed from how much dye is found in each sample (assuming 2.5% blue dye in food):

$$\frac{\mu g\ blue\ dye}{ml} \times \frac{.2\ ml}{.5\ flies} \times \frac{100\ \mu l\ food}{2500\ \mu g\ blue\ dye} \times \frac{1}{2\ hours} = \frac{\mu l\ food}{hour}/fly$$