**Supplementary file 1: Protocol of preparing standard cornmeal/agar diet for Drosophila flies**

# PROTOCOL FOR PREPARATION OF STANDARD CORNMEAL/AGAR DIET FOR FLIES

(BDSC, 2015; Hudry et al., 2019; Thurmond et al., 2019).

Ingredients:distilled water, 6.65% cornmeal, 7.15% dextrose, 5% yeast, 0.66% industrial agar, 3.4 mL/L propionic acid and 2.2% nipagin.

**(1 liter of fly food recipe for 70 vials)**

Distilled water........................................................................................... 1.03 L

Nipagin....................................................................................................... 23.3 mL

Cornmeal.................................................................................................. 70 gm

Glucose/dextrose....................................................................................... 75 gm

Yeast........................................................................................................... 15 gm

Industrial Agar.......................................................................................... 10.5 gm

Propionic acid............................................................................................ 3.7 mL

**Protocol**

Made a paste by mixing some **distilled water** with **cornmeal** and **industrial agar**, boiled for 10 minutes

Mixed **Nipagin** with **glucose** and **yeast** separately, added to the mixture above, and brought to boil while stirring thoroughly with the stirrer.

Allowed food to cool to about 60oC, added propionic acid and mixed thoroughly, allowed to cool for a while, distributed into media storage bottles (Fisher Scientific, 50-192-998, USA) and plastic fly vials (Genesee Scientific, 32-116, USA).

**NOTE:** Nipagin can only dissolve in ethanol not water, 10 gm in 100 mL (10%).

**References**

Bloomington Drosophila Stock Center (2015). Fly Food in: Drosophila Media Recipes and Methods. Indiana, USA.

Hudry, B., de Goeij, E., Mineo, A., Gaspar, P., Hadjieconomou, D., Studd, C., Mokochinski, J. B., Kramer, H. B., Plaçais, P.-Y., Preat, T., & Miguel-Aliaga, I. (2019). Sex Differences in Intestinal Carbohydrate Metabolism Promote Food Intake and Sperm Maturation. *Cell*, *178*(4), 901-918.e16. https://doi.org/10.1016/j.cell.2019.07.029

Thurmond, J., Goodman, J. L., Strelets, V. B., Attrill, H., Gramates, L. S., Marygold, S. J., Matthews, B. B., Millburn, G., Antonazzo, G., Trovisco, V., Kaufman, T. C., & Calvi, B. R. (2019). *FlyBase 2 . 0 : the next generation*. *47*(October 2018), 759–765. https://doi.org/10.1093/nar/gky1003