**gDNA extraction protocol**

* Preheat thermomixer to 56°C.
* Add 180μl ATL buffer into 2ml tube with fly in it.
* Add a stainless steel bead (**Qiagen #69989**) to the tube.
* Lyse the tissue in **Qiagen tissue lyser LT** with one round of shaking: 20 sec at 40 Hz.
* Spin down the 2ml tube with lysate – 1 min, 100g.
* Transfer the lysate, including any foam, into a 1.5 ml tube.
* Add 20μl Proteinase K; pipet up and down; invert 80x.
* Incubate the tubes for 1 hr at 56°C in thermomixer, with 15 min mixing intervals at 1000 rpm.
* Spin down the tube – 1 min, 100g.
* Add 4 μl RNase A solution (100 mg/ml); pipet up and down; invert 80x.
* Incubate the tubes for 15 min at room temperature.
* Spin down tubes in the mini-fuge
* Add 200 μl AL buffer; invert 80x; spin down
* Add 200 μl Ethanol (100%); invert 80x; spin down
* Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge 1 min, 6,100g. Discard flow-through and collection tube.
* Place DNeasy Mini spin column in a new 2 ml collection tube, add 500 μl AW1 buffer, and centrifuge for 1 min, 6,100g. Discard flow-through and collection tube.
* Place DNeasy Mini spin column in a new 2 ml collection tube, add 500 μl AW2 buffer, and centrifuge for 3 min, 20,000g. Discard flow-through only. Place spin column back onto same collection tube.
* Spin again for 3 min, 20,000g to completely dry the column.
* Place the DNeasy Mini spin column in a clean 1.5 ml micro centrifuge tube, well-labeled, and pipet 30 μl AE buffer onto the center of the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 6,000g to elute.
* Pipet the eluate back onto the DNeasy membrane and place the spin column in the same 1.5 ml tube. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 6,000g to elute.
* Discard spin column, and store the 1.5 ml tubes containing gDNA in -20°C freezer.