

Genomic DNA extraction from single fly

- 1- Set hybridization oven at 55°C
- 2- Make sure HB buffer is not precipitated. If it is - warm it up until precipitate dissolves and mix before use
- 3- Homogenize single fly with 250 ul of HB buffer (see recipe at the end of this protocol)
- 4- Add 5 ul of 10 mg/ml of Proteinase K to each tube, mix
- 5- Incubate at 55°C 2-3 hr
- 6- Add 85 ul of 5 M NaCl and vortex
- 7- Spin down pellet for 20 min at full speed
- 8- Transfer supernatant into new tubes
- 9- Add 340 ul of 100% cold EtOH, mix, put on ice for 10 min, spin for 20 min at full speed in the cold room to pellet DNA
- 10- Remove EtOH with blue tips without disturbing pellets
- 11- Add 1 ml of 70% EtOH, invert few times and spin for 15 min at full speed
- 12- Remove EtOH using blue tips
- 13- Quick spin tubes
- 14- Remove all leftovers of EtOH with yellow tips without disturbing pellets
- 15- Dry DNA until you don't see any liquid in the tube
- 16- Add 100 ul of water
- 17- Leave o/n in refrigerator
- 18- Next day: flick and spin. Freeze samples on -20 C until needed

HB buffer (olga's bench): 50 mM Tris pH 7.5

400 mM NaCl
20 mM EDTA
0.5% SDS

to make HB combine:

39 ml of water
2.5 ml of 1M Tris pH7.5
4 ml of 5M NaCl
2 ml of 0.5 M EDTA pH 8.0
2.5 ml of 10% SDS