

### **Technical considerations and common pitfalls**

1. It is key to have a large starting population (about 500  $\mu$ l) as this will allow to work quickly without being concerned about losing worms along the way.
2. Use large orifice tips when handling worms, using normal tips will cause stress and/or damage them.
3. The used chemicals are volatile, therefore, make sure to keep the lids closed as much as possible and parafilm your plates during the conditioning period.
4. Do not pipet up and down before pulling up the M9 buffer/worm mixture. Instead, go to the middle of the pellet and pull up the M9 buffer/worm mixture.
5. When washing worms off HGM plate, make sure to gently apply the M9 to the plate and gently swirl it in order to avoid dislodging bacteria off the plate.
6. In between the different steps of the assay, plates (conditioning plates and hold plates) can be kept in a closed container in order to avoid varying environmental conditions to affect the worm's behavior
7. When testing the mock and conditioned worms simultaneously, make sure to keep both conditions far apart from one another.
8. Worm should be let to settle by gravity, do not centrifuge.
9. Do not pipette worms against the side of the tube, as this can damage the worms and interfere with the assays
10. Try to remove as much M9 liquid as possible, so that worms can quickly get to the OP50 food source when transferred to the conditioning plates. Worms will stick to the inside of the pipette tip and can be conserved by pipetting smaller volumes.
11. All washing steps should be performed as quick as possible (but with consideration of potential stressful manipulations) in order to avoid starvation while sitting in the M9 buffer.
12. Working quickly is also important during the chemotaxis assay. Additionally, it is recommended to test not more than three genotype at the same time. It is however possible to work in parallel, but six genotypes should be the limit.
13. When studying cognitive decline, washes should be reduced to a minimum. Ideally, late L4's are washed off HGM plates and distributed over fresh plates while ensuring that worms which will be tested at day 3 of day 5 of adulthood will have enough food for 3 or 5 days, respectively.