

Extract total RNA for *Mnemiopsis leidy* (version 3, modified with Alejandro)

- 1) **Homogenize in 1ml Trizol and incubate 5 min at RT, vortex every 2 min**
 - **Start by adding 100ul of Trizol**, homogenize with a pestle
 - When the embryos have been adequately homogenized, add the remaining amount of Trizol and pipet up and down with an aerosol-free pipet tip, which further aids in homogenization. Additionally, vortex this mixture to make sure everything is mixed well.
- 2) **Add 200ul chloroform, vortex vigorously for 15 seconds, incubate 10 min at RT, vortex every 2-5 min**
- 3) **Centrifuge 20 min at 4°C at speed max. and transfer aqueous phase to fresh tube** - be very careful here not to disturb the interphase (rather leave ¼ of the aqueous phase behind, to ensure that no interphase is transferred)
- 4) **Add 500ul Isopropanol, invert to mix and incubate for 20 min at RT, vortex every 5 min**
- 5) **Centrifuge 10 min at 4°C and remove supernatant.** Please note that at this step it is often very difficult to see the pellet. For some samples we just have to assume it's there and be careful not to disturb anything at the bottom of the tube.
- 6) **Add 70% ethanol and very carefully invert once to mix and Centrifuge for 5 min at 4°C (DO NOT VORTEX)**
- 7) **Remove ethanol wash (carefully) and dry pellet 5-6 minutes (don't over-dry)**
- 8) **Dilute RNA pellet in 11ul of dH2O and take a spec reading.**

A few things to note: if you don't have many embryos/samples to start with (we usually have 100-200 embryos for particular stage), you can reduce the volume of Trizol (and every solution thereafter) to 500ul instead of 1ml. Sometimes this has helped with RNA yields, although I haven't noticed a significant difference.