Isolate RNA from tissue Using TRIzol Reagent
Xiaoji Luo 01/11/08

1. Mince the fresh tissue. Place the tissue into the dish, add 1ml TRIZol reagent, then use two blade to mince it. Depending on the property of the different tissue, you need work on it from 2-10min until there are no obvious tissue blocks.
2. Transfer the tissue suspension into a 2ml tube.
3. Homogenize the tissue thoroughly by using homogenizer; keep the tube in the cooler box, which will be helpful to prevent the RNA degradation.
4. Add 270ul chloroform (i.e., 0.27ml chloroform per 1ml TRIzol reagent), vortex vigorously.
5. Spin the sample at top speed in microfuge for 15 min in cold room. Transfer the colorless upper aqueous phase into a new set of 1.5-ml tubes (Avoid touching the junks in interphase! Keep tubes on ice).
6. Add 670ul of isopropanol (i.e., about 2/3 vol. of TRIzol reagent). To maximize RNA recovery rate, you can add 3-5ul of Glycogen (**but not seeDNA as it will interfere with your A260 reading later**) to each tube. Mix well (Tubes can be kept at -80° for several days or weeks).
7. Spin at top speed for 10 min in cold room to precipitate RNA.
8. Remove the supernatant. Wash the RNA pellet with 600ul of 75% ethanol.
9. Dissolve RNA in 50ul RNase-free water. Read A260/A280 and/or check RNA integrity on RNA agarose gel. Store RNA stock at –80oC.

Note:

1. More tissue used was not supposed to mean more recovery rate and better RNA quality, the point is the suitable ratio of the certain tissue and the volume of TRIzol.
2. Always keep on ice.
3. For the tumor sample, not to use necrosis tissue.
4. For the hard tissue such as bone, you need wrap the bone in the silver paper and crush it in the liquid nitrogen. And you can place the bone in the nitrogen tank overnight in advance.