Trizol RNA purification for small amounts of tissue

-Wear safety glasses and gloves for this procedure
- Homogenize tissue in about 200 ul of Trizol
- Add 1 ul LPA (Sigma)
- Adjust Trizol volume up to 1 ml
- Add 200 ul chloroform and shake for 20 seconds
- Let sit at room temperature for 5 min
- Spin 15 min 4^0C max speed
- Collect upper phase (make sure not to touch interphase or lower phase – helps not to be greedy) and transfer into a new tube. Dispose of lower phase accordingly (it has some phenol)
- Add 500 ul of isopropanol to collected upper phase and mix by inverting a few times
- Keep overnight on -20^0C (longer does not hurt)
- Spin down 30 min 4^0C max speed
- Remove supernatant
- Add 1 ml of 70% EtOH (RNase-free), invert tube a few times
- Spin 10 min 4^0C max speed
- Carefully remove supernatant (without loosing the pellet, you will probably see a small white dot = pellet)
- Quick-spin the tube to get all the droplets down
- Remove all leftovers of EtOH
- Dry pellet (if you removed all the liquid during the previous steps it should take only 10 min or so). Do not over dry the pellet – it will be harder to dissolve.
- Dissolve pellet in appropriate amount of DEPC-H_2O. Depending on amount of RNA you might need to adjust H_2O. If I don't know what to expect I add 10 ul first, let sit on ice about 10-15 min, pipet gently up and down and if RNA is still viscous I will add more water.
- Determine RNA concentration and adjust it with water if needed.