Material

1. Equipment: Micro-Dismembrator
2. 5ml/7ml Shaking Teflon Flasks
3. 10 mm Grinding Balls (Sartorius)
4. Cleaning of equipment: Soak Teflon flasks overnight in 4 % sodium hypochlorite. Soak metal balls overnight in 4 % formaldehyde; Rinse flasks and balls with lots of hot water, RNease-free water at last and dry
5. Precool Teflon flasks and grinding balls (10 mm) in liquid nitrogen. Use a 5-ml flask for 0.2 to 0.7 gram tissue and a 7-ml flask for 0.7 to 2 grams tissue
6. Prepare Falcon-tubes and Extraction Buffer for subsquent RNA-Isolation (RLT-Buffer for Qiagen RNeasy protocol, Trizol or Phenol according to protocol)
7. Prepare a sterile syringe and 0.9mm-gauge needle for every sample

Procedure

1. Keep Tissue samples in liquid nitrogen until preparation
2. Weigh the frozen tissue samples before preparation and if necessary, breakup in smaller pieces (a hammer helps, in aluminium foil on dry ice)
3. Transfer tissue sample in precooled flask with a grinding ball, pour out remaining liquid nitrogen, close the flask and cool again 15sec in liquid nitrogen
4. Quickly screw the cooled flask on the metal holder and disrupt the tissue by shaking 10 seconds at 3000 rpm (maximum speed).
5. Transfer the flask to liquid nitrogen 15sec to cool again
6. Open flask and resuspend tissue with RNA Extraction Buffer (Buffer could freeze in flask, thaw sample before next step)
7. Transfer tissue lysat in prepared falcons and homogenize 5 times by drawing through 0.9mm gauge needle
8. Continue RNA extraction protocol with tissue lysate

Version	Tracking of changes	Name	Date