Tissue Homogenization by Dismembrator					
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## 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