RNA isolation from frozen tissue

Before you start isolating RNA, make sure your workplace is RNAse free. Use RNAseZap to clean your workplace. Wear gloves and use RNAse free eppendorf tubes and pipette tips.

1. Cut 30 sections of 30 μm (Cryo-microtome @ –20°C) and transfer to tube containing 1 ml RNAzol, shake vigorously and put on ice.
2. Homogenize samples using a Polytron Homogenizer
    - 5-10 sec homogenize
    - Shake tube
    - 5-10 sec homogenize
3. Before use and between samples clean the Polytron by rinsing with
    - RNAse free H_2O (2x)
    - RNAseZap (1x)
    - RNAse free H_2O (1x)
4. Spin samples 10 minutes at 14000 rpm and 4°C
5. Transfer supernatant (~1 ml) to fresh tube
6. Add 100 μl chloroform and mix thoroughly for 15 seconds
7. Keep samples on ice for 5-10 minutes
8. Spin samples 15 minutes at 12000rpm and 4°C
9. Transfer aqueous layer to a fresh tube
10. Add 600 μl isopropanol and mix by vortexing shortly
11. Precipitate samples at -70°C for 1 hour, or O/N at 4°C
12. Carefully pour off supernatant and add 1 ml 70% Ethanol to wash the pellet. (the pellet should come loose from the tube)
13. Spin 10 minutes at 12000 rpm and 4°C
14. Pour off ethanol and spin briefly (5 seconds), use a pipette tip to remove the remaining ethanol
15. Resuspend the RNA carefully in 200 μl RNAse free H_2O
16. Measure RNA concentration (use 10 μl RNA and 490μl H_20)
17. Run 1 μg on gel to check RNA quality
18. Add 20 μl 3M NaAc, pH 5.6 and 600 μl 100% EtOH and store at -70°C