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RNA isolation for tissue V.2

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Protocol status: In development
We are still developing and optimizing this protocol

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1 Homogenization in TriZol
Add 1 mL of Trizol reagent per 30 mg of tissue and homogenize using handheld homogenizer

2 Incubate at RT for 00:05:00 to allow nucleoprotein complexes to dissociate

3 Add 1/5 the volume of Trizol (0.2 mL) of chloroform carefully, and vortex to mix well

4 Spin down at max speed in a chilled centrifuge for 00:15:00

5 Carefully remove the top aqueous phase and transfer to a new Eppendorf tube
The interphase and bottom organic phase can be saved for DNA and protein respectively

6 To the aqueous phase, add 500 µL of 100% isopropanol, mix by inversion and incubate at -20 °C for a minimum for 02:00:00

7 Spin down at max speed for 00:30:00 s to precipitate RNA

8 Remove supernatant, and add 1 mL of 75% Ethanol to wash the pellet

9 Spin down at max speed for 00:15:00 and remove supernatant

10 Resuspend pellet in appropriate volume of nuclease free water