RNA extraction with Trizol

For Whole Tissues:
1 – Isolate tissue and snap freeze in liquid nitrogen
2 – Transfer tissue to a 14-ml culture tube and add 2ml of Trizol
3 – Homogenize completely
4 – Transfer 1ml each into two fresh 1.5-ml Epp. tubes
5 – Incubate 5 min @ RT
(Recommended: Freeze in Trizol at least 1 hr @ -80°C after incubating @ RT)
*Potential indefinite stopping point @ -80°C

For Sorted Cells:
1 – Sort cells into 1.5 ml epp. tubes coated in Fetal Calf Serum (FCS) or in 500μl media with 2% FCS
(Recommended: For ≤ 100,000 cells sort directly into 500μl Trizol and proceed to step 5)
2 – Centrifuge 30 sec @ full speed, 4°C (make sure to see a pellet)
3 – Remove supernatant from each sample with a pipette tip
Leave behind a small amount of liquid so as not to disturb the pellet
4 – Add 500 μl Trizol to each sample (mix well by pipetting)
5 – Incubate 5 min @ RT
(Recommended: Freeze in Trizol at least 1 hr @ -80°C after incubating @ RT)
* Potential indefinite stopping point @ -80°C

Thaw Trizol samples at RT
6 – Add the appropriate amount of Chloroform to each sample (work in hood, do not pipette)
- For 500μl Trizol, Add 100μl Chloroform
- For 1ml Trizol, Add 200μl Chloroform

7 – Shake vigorously for approx 15 sec
8 – **Incubate 2-3 min** @ RT
9 – Centrifuge **15 min** @ 12,000 g, 4°C

10 – Transfer aqueous phase CAREFULLY to a fresh tube[1] (work on ice from this point on)
- For 500μl Trizol, Transfer ~250μl
- For 1ml Trizol, Transfer ~500μl

11 – Add 1μl Glycoblue to each sample (flick well but don't pipette to mix, quick spin, on ice)
12 – Add the appropriate amount of Isopropanol to each sample (flick well to mix, do not pipette)
- For 500μl Trizol, Add 250μl Isopropanol
- For 1ml Trizol, Add 500μl Isopropanol

13 – **Incubate 1+ hr @ -80°C**
*Potential indefinite stopping point @ -80°C

14 – Centrifuge **20 min** @ 12,000 g, 4°C
Remove supernatant with a pipette tip
15 – Wash with the appropriate amount of 75% cold ethanol (flick to loosen pellet)
- For 500μl Trizol, Wash with 500μl 75% cold ethanol
- For 1ml Trizol, Wash with 1ml 75% cold ethanol

16 – Centrifuge **15 min** @ 7,400 g, 4°C
Pipette off as much ethanol as possible, air dry 1 hr (pellet will change color)
17 – Resuspend each RNA pellet in 12.5 - 15 μl Nuclease-free water
18 – Incubate @ RT for **2-3 min**, then flick well to mix and place on ice
19 – Quantify total RNA using a Nanodrop
*Store total RNA indefinitely @ -80°C

[1] Leave behind a small amount of clear aqueous phase; do NOT pick up any pink phenol-chloroform phase; use pipette tips with a larger hole to prevent this from happening.