

RNA ISOLATION with TRIZOL

and subsequent ETHANOL PRECIPITATION:

1. RNA Isolation:

- homogenize tissue samples in 500µl Trizol (usually with syringe and needle)
- vortex 15sec
- add 100µl chloroform
- vortex 15sec
- incubate 5min at room temperature
- centrifuge at 12000g (4°C) for 15min
- transfer the upper aqueous phase to a fresh tube
- add 500µl isopropanol
- centrifuge at maximum speed (4°C) for 10min
- discard the supernatant
- add 500µl 70% ethanol
- centrifuge at maximum speed (4°C) for 2min
- discard the supernatant
- air dry the pellet
- resuspend the pellet in 30µl a.dest and measure 1,5µl with the nanodrop

2. Ethanol Precipitation:

- add 1/10 volumes sodium acetat (3M, pH 5.2)
- add 2,5 volumes ice cold 100% ethanol (calculated after adding the sodium acetat)
- incubate for 1h at -80 °C (or longer)
- centrifuge at maximum speed (4°C) for 30min
- discard the supernatant
- add 500µl ice cold 80%igen ethanol
- centrifuge at maximum speed (4°C) for 30min
- discard the supernatant
- air dry the pellet
- resuspend the pellet in 10µl a.dest and use 1µl for the nanodrop