RNA Isolation by Combined Trizol-RNeasy method

1 Phase Separation
- 1.1 Homogenize sample as per TRIzol/QIAzol instructions
- 1.2 Incubate lysate at room temp for 5 minutes
- 1.3 Transfer sample to a clean microcentrifuge tube
  - 1.3.1 If isolating from tissue, centrifuge at >12K x g for 15 min at 4C
  - 1.3.2 Transfer supernatant to a clean microcentrifuge tube
- 1.4 Add 200uL chloroform per 1mL TRIzol/QIAzol used
- 1.5 Shake sample vigorously for 15 sec
- 1.6 Incubate at room temp for 3 min
- 1.7 Centrifuge at >12K x g for 15 min at 4C
- 1.8 Carefully transfer the upper, aqueous layer to a clean microcentrifuge tube

2 Binding to RNeasy column
- 2.1 Add an equal volume of 70% EtOH and vortex to mix thoroughly
- 2.2 Transfer up to 700uL of sample mixture to RNeasy column
- 2.3 Centrifuge at >16K x g for 15 sec at room temp
- 2.4 Place column on a clean 2mL collection tube
- 2.5 Repeat steps 2.2 - 2.4 until entire sample has been loaded onto column

3 On-column DNase
- 3.1 Add 350uL Buffer RW1 to the column
- 3.2 Centrifuge at >16K x g for 15 sec at room temp
- 3.3 Place column on a clean 2mL collection tube
- 3.4 Make master mix with 10uL DNase I stock soln for every 70uL Buffer RDD
- 3.5 Apply 80uL DNase I working soln directly to column & incubate 15 min at room temp
- 3.6 Add 350uL Buffer RW1 to the column
- 3.7 Centrifuge at >16K x g for 15 sec at room temp
- 3.8 Place column on a clean 2mL collection tube

4 Column washes & RNA elution
- 4.1 Add 500uL Buffer RPE to the column
- 4.2 Centrifuge at >16K x g for 15 sec at room temp
- 4.3 Place column on a clean 2mL collection tube
- 4.4 Add 500uL Buffer RPE to the column
- 4.5 Centrifuge at >16K x g for 2 min at room temp
- 4.6 Place column on a clean 2mL collection tube
- 4.7 Centrifuge at >16K x g for 1 min at room temp
- 4.8 Place column on a clean 1.5mL microcentrifuge tube for elution
- 4.9 Add 30uL RNase-free H2O to column & incubate 1 - 5 min
- 4.10 Centrifuge at >16K x g for 1 min at room temp
- 4.11 Repeat elution by applying eluate to column & incubating 1 -5 min
- 4.12 Centrifuge at >16K x g for 1 min at room temp
- 4.13 Assess quantity/purity by NanoDrop
- 4.14 Assess quality by Agilent Bioanalyzer