**Materials Needed:**

* Kit: Catalog Number R2056
* Dry ice
* 200 proof ethanol
* Cleaning ethanol in squeeze bottle
* Paper towels
* Trizol (located in 4C fridge)
* Clean forceps (at least 3 or 4)
* Bead ruptor tubes (Ambion Life Technologies)
* Plate centrifuge

Homogenization

1. Turn on the bead ruptor machine and set program as below:

Program 1:

S = 5.00 M/s cycles = 02

T = 30S Dwell = 5S

1. Aliquot enough 200 proof ethanol for samples:
   1. 250ul x \_\_\_\_\_\_\_ samples = \_\_\_\_\_\_\_ul
2. Label and fill bead ruptor tubes with 250ul Trizol in the fume hood.
3. Clean forceps with ethanol and prepare ethanol squeeze bottle. Make sure to clean forceps between samples with ethanol.
4. Transfer samples from -80C freezer to dry ice in Styrofoam box.
5. Flick tube that contains sample to dislodge frozen embryos, and gently pour embryo into labeled bead ruptor tubes with 250ul Trizol.
6. Use clean forcep to puncture or crush the outer chorion of the embryo while in Trizol.
   1. It is important to work quickly so embryos don’t sit in Trizol for too long before homogenization.
7. Once all embryonic chorion have been ruptured in Trizol, place the embryos and Trizol in the bead ruptor in a balanced manner, making sure the protective metal cover locks into place before starting the homogenization program.

RNA Purification

1. WHILE IN FUME HOOD, Add one volume (250ul) of 100% ethanol directly to the sample homogenate in TRI Reagent (Trizol) and mix well by inversion.
2. Transfer mixture into **Zymo-Spin I-96 Plate** mounted on a **Collection Plate** and centrifuge for 5 minutes. Mount the plate onto a new **Collection Plate**. Discard the flow-through by emptying collection plate in sink and drying on paper towel.
3. Add 400ul Direct-zol RNA PreWash to the plate mounted on a **Collection Plate** and centrifuge for 5 minutes. Discard flow-through as described above.
4. Repeat Step 3.
5. Mount plate to a new **Collection Plate.** Add 400ul **RNA Wash Buffer** to each well of the plate and centrifuge for 5 minutes. Discard flow-through. Repeat for a total of 2 washes using RNA Wash Buffer. Centrifuge plate for additional 5 minutes for complete removal of buffer.
6. Mount **I-96 Plate** to **Elution Plate** and add 20ul/well of **DNase/RNase-Free Water** directly to the matrix. Let sit for 1 minute at room temperature. Centrifuge for 5 minutes. Repeat for a total of 2 elutions using 20ul/well.
7. Transfer samples into labeled tubes.