Date:

RNA Extraction Checklist

Working SOP for combined RNA extraction method for a whole blood preserved in Paxgene blood tubes
Last updated 02/04/16 by Lisa Komoroske (and formerly by Lesley Anderson)
**N.B. Throughout protocol:*

- tips and tubes used for all steps involving Trizol and chloroform or their components downstream should be put in proper container for toxic waste disposal, kept in hood
- tips and flow through of ethanol based reagents place in separate waste container in hood so as not to mix with those that need special disposal

-Store paxgenes in -80C after collection until analysis

- ❑ Take samples out of freezer to come to equilibrium at room temperature. Once at equilibrium, leave out for two hours on benchtop in tube rack (if wasn't previously).
- ❑ Mark the draw line (if not full draw).
- ❑ Take bead bag out of freezer. Place steel beads in a centrifuge tube and put tubes of beads in to freeze.
- ❑ Check that centrifuge is in refrigerator and cooled down (4C).
- ❑ While samples are thawing:
    - ❑ Make a plan in notebook (create a key)
    - ❑ Label all sample tubes, make any blank tubes as necessary
    - ❑ Check supplies for reagents, etc. (see list at end of protocol)

- ❑ After RT incubation period, invert each paxgene thoroughly 20x (can also pipette mix or lightly vortex if necessary) to get pellet suspended/sample homogenized.
- ❑ Using P1000, pipette ~***1ml*** into each 1.5ml Omni tube labeled according to sample.
  Want 8 replicates total so if full draw, will put last bit into reps roughly evenly across
- ❑ Centrifuge samples at 4C for 10 minutes at **3,500rfc** *(*in MMTD lab centrifuge this is 13.2rpm)*
- ❑ Carefully pour off supernatant in sink, dry lip with chemwipe (keep pellet)
- ❑ Homogenization of the pellet: ***This step do in hood***
    - ❑ Take beads out of frozen bead bag.
    - ❑ Quickly and ***carefully*** place one bead in each tube (use ethanol tweezers and tilt tube on its side to 'roll' it in gently.
    - ❑ Using P1000, add **500ul** of Trizol to each tube.
    - ❑ Immediately put onto Omni.
    - ❑ Use Program 2 (30 sec at 50Hz; 4m/s).
    - ❑ Check to make sure pellets have homogenized, if not can repeat Program 2 again

- ❑ Using P1000, transfer sample to clear, flat bottomed tube.
- ❑ Using P200, add **200ul/1ml trizol (=100 ul chloroform/500ul trizol)** chloroform to each sample one at a time *(or 2-4 at a time as needed -one or two in each hand)* shaking vigorously for 30-45 sec afterwards. ***This step do in hood.***
- ❑ Let stand at room temperature for **3 min**.
- ❑ Centrifuge at 13,400 rpm for **15 min at 4C**. *13.2 is highest rpm allowed.* Take freezer beads back.
- ❑ Using P200, carefully pipette off **~200-250ul** of aqueous phase into appropriately labeled 1.5ml centrifuge tube. *Be careful not to puncture DNA (white thin layer) or protein layer (on bottom), as this will contaminate your sample.*
- ❑ Using P1000, add one volume (**~200-250ul**) of 70% molecular grade ethanol to aqueous phase sample and mix

COMBINE SAMPLES AT THIS STEP

- ❑ Using P1000, pipette **700ul** of sample onto pre-labeled RNeasy mini spin column
  **N.B. for modified protocol with lower volume, this will be ~550ul, and won't need a second transfer step*
- ❑ Centrifuge for 15 sec at 10,000 rpm.
- ❑ Discard flow through and replace spin column back in original tube.
- ❑ Repeat with remaining sample if needed.
- ❑ Add multiple replicates to the same column (2-3 depending on the number of replicates)
- ❑ Using P1000, pipette **700ul** of RW1 buffer into each spin column
- ❑ Centrifuge for 15 sec.
- ❑ Discard flow through and replace spin column back in original tube.

N.B. buffer RPE and resulting flow through are simple ethanol based washes, so any tips, tubes and flow through from these steps DO NOT go into container for toxic waste-put into marked container to allow evaporation (followed by regular disposal)

- ❑ Using P1000, pipette **500ul** buffer RPE (wash buffer - yes ethanol, with date) into spin column.
- ❑ Invert ~10 times. *(to wash residual RNA off walls of column onto membrane)*
- ❑ Centrifuge for 15 sec.
- ❑ Discard flow through and replace spin column back in original tube.

REPEAT

- ❑ Using P1000, pipette **500ul** buffer RPE (wash buffer) into spin column.
- ❑ Invert ~10 times.
- ❑ Centrifuge for 15 sec. (***or 2 min***, see note below)
- ❑ Discard flow through and replace spin column back in original tube.

Optional Repeat**:** *do third wash only if having quality issues otherwise do 2 min spin above, then skip to next step below*

- ❑ *Using P1000, pipette **500ul** buffer RPE (wash buffer) into spin column.*
- ❑ *Invert ~10 times.*
- ❑ *Centrifuge for **2 min**.*

- ❑ Place each spin column in a new 2ml tube and throw away old tube.
- ❑ Centrifuge for **1 min** to remove any residual EtOH.

- ❑ Place each spin column in a pre-labeled 1.5 centrifuge tube and throw away old tube.
- ❑ Using P200, add **30 ul** RNase-free water directly to membrane.
- ❑ Centrifuge for 1 min. *Use black centrifuge, place caps on right side.*

REPEAT

- ❑ Using P200, add **30 ul** RNase-free water directly to membrane.
- ❑ Centrifuge for 1 min. *Use black centrifuge, place caps on right side.*

- ❑ Throw away pink inside spin column, keep centrifuge tube with sample.
- ❑ Put sample in fridge.
- ❑ Using P10, aliquot **5 uL** into pre-labeled mini tube.

NANODROP

- ❑ Open ND1000 program on computer
- ❑ Click on Nucleic Acid
- ❑ Clean sensor with MiliQ and kimwipe
- ❑ Run with 1uL of RNase free water
- ❑ Choose sample type RNA

- ❑ Run with dilution sample: 1uL of RNase free water
- ❑ Click blank
- ❑ Flick mix and tap down sample before pipetting
- ❑ Run with 1uL of sample
- ❑ Click measure
- ❑ Record in notebook:
    - ❑ 260/280
    - ❑ 260/230
    - ❑ ng/uL
    - ❑ Describe peak

CLEANING PROCEDURE

- ❑ Put samples and QC aliquot in -80^{o} freezer
- ❑ Remove unnecessary items from hood
- ❑ Wipe down mini centrifuge lid with ethanol, put away
- ❑ Wipe down pipettes with ethanol, put away
- ❑ Dump waste from flat bottomed tubes into waste disposal, put tubes into toxic waste disposal container
- ❑ Carefully move beads into DI water in beaker #1, then-
- ❑ Dump any residual waste from omni into waste disposal, put tubes into toxic waste disposal container
- ❑ Switch/dump the ethanol tip/tube waste disposal container if appropriate (i.e., all liquid has evaporated)
- ❑ Check and wipe down large centrifuge in fridge; LEAVE ON, make sure fridge door secure with cord when closed
- ❑ Check all stock solutions and amounts of materials, make any notes for needing to re-stocking, etc.
- ❑ Clean beads (below)

CLEANING BEADS

- ❑ (First put any clean, dry beads from previous washes back into bag on lab bench if appropriate)
- ❑ Remove beads from tubes carefully into beaker with small amount of water *(gently roll beads around to ensure evenly rinsed)*
- ❑ Move beads with forceps one at a time into beaker with 10% bleach
- ❑ Leave beads for 2 min *(gently roll beads around to ensure evenly rinsed)*
- ❑ Move beads with forceps one at a time into beaker with DI water
- ❑ Leave beads for 2 min *(gently roll beads around to ensure evenly rinsed)*
- ❑ Move beads with forceps one at a time into beaker with H_2O_2
- ❑ Leave beads for 2 min *(gently roll beads around to ensure evenly rinsed)*
- ❑ Move beads into drying tip lid on chemwipe
- ❑ Leave beads overnight to dry in hood

LABELS (first 4 should just have # on lid):

1. 1.5 mL Omni tube
2. 2 mL flat bottomed centrifuge tube
3. 1.5 mL centrifuge tube
4. RNeasy spin column tube
5. 1.5 mL centrifuge tube (x3 - see below)
6. .6mL mini tube (aliquot tube with ID#, extraction date, replicate #s)

-Final labeled aliquot tubes if possible (should have 3 per sample):

- *sample ID # (top and side)*
- *location (top and side)*
- *"RNA" (on side)*
- *extraction date (on side)*

- *species (on side)*
- *collection date (on side)*
- *Replicate #*

Materials Checklist

Benchtop

- ❑ Fridge
- ❑ Omni Bead Ruptor
- ❑ Kimwipes
- ❑ Sharpie
- ❑ Large tube rack (for sample)
- ❑ Small tube rack (for centrifuge tubes)
- ❑ Pipette (P1000, P200, P10)
- ❑ Pipette tips (P1000, P200, P10)
- ❑ Steel beads
- ❑ Freezer bead bag
- ❑ Timer
- ❑ Small centrifuges (black lid)
- ❑ Large centrifuge
- ❑ Centrifuge tubes
- ❑ Flat bottom tubes
- ❑ Omni tubes
- ❑ Spin columns
- ❑ Spin column tubes
- ❑ Mini centrifuge tube (for aliquot)
- ❑ Collection Tubes

Hood set up

- ❑ Trizol/Chloroform pipette tip waste container (for eventual proper waste disposal)
- ❑ Ethanol-based waster container (for evaporation and eventual normal trash disposal)
- ❑ Ethanol cleaned tweezers
- ❑ Small beaker for aliquots
- ❑ 10% bleach beaker
- ❑ H_2O_2 beaker
- ❑ DI water beakers (2)
- ❑ Final bead drying container

Pre-made Aliquots *(need to check have enough of all these before starting)*

- ❑ 70% ethanol
- ❑ Chloroform
- ❑ Trizol
- ❑ RW1 buffer
- ❑ RPE wash buffer
- ❑ RNase-free water