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# A method for isolating RNA from canine bone V.1

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**Protocol status:** Working
**We use this protocol in our group and it is working.**

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## Abstract

Extracting sufficient quantity and quality RNA from bone is essential for downstream application, such as transcriptomic sequencing, to evaluate gene expression. Isolation of RNA from bone presents a unique challenge owing to the hypocellular, brittle and mineralized matrix, which makes homogenizing the tissue difficult and provides little RNA to work with. Removal of contaminating tissue, such as bone marrow and connective tissue, is essential for isolating RNA that is unique to osteoblasts, osteoclasts and osteocytes. This protocol establishes a method to effectively isolate RNA from normal canine bone cells using the second phalanx, without contamination from other tissue types, for downstream transcriptomic analysis.
This method combines physical manipulation to remove exterior tissue, washing and centrifugation to remove cells and fat within the diaphysis, homogenization using a mortar and pestle on dry ice prior to bead dissociation, followed by acid guanidinium thiocyanate-phenol-chloroform extraction and column purification to yield sufficiant quantity and quality RNA from canine phalanges. The second phalanx was chosen due to its size small enough to fit into a 1.7 mL microfuge tube, but large enough to provide enough RNA. Mean RNA obtained using this protocol was 14.7 ug of RNA per gram of frozen bone.

## Attachments

btn-2019-0153.pdf

1.2MB

## Materials

MATERIALS

- KimWipes **Fischer Scientific**
- TRI Reagent® **Molecular Research Center, Inc.**
- RNase AWAY™ Spray Bottle, RNase in spray bottle; 475mL **Thermo Fisher Catalog #**7002
- Ceria stabilized zirconium oxide beads 0.5 mm diameter **Next Advance Catalog #**ZrOB05
- Bullet Blender **Next Advance Catalog #**Bullet Blender 24
- Bromochloropropane (BCP) **Molecular Research Center, Inc. Catalog #**BP151
- DNase I **Thermo Fisher Scientific Catalog #**18068015
- RNeasy Micro Kit **Qiagen Catalog #**74034

## Troubleshooting

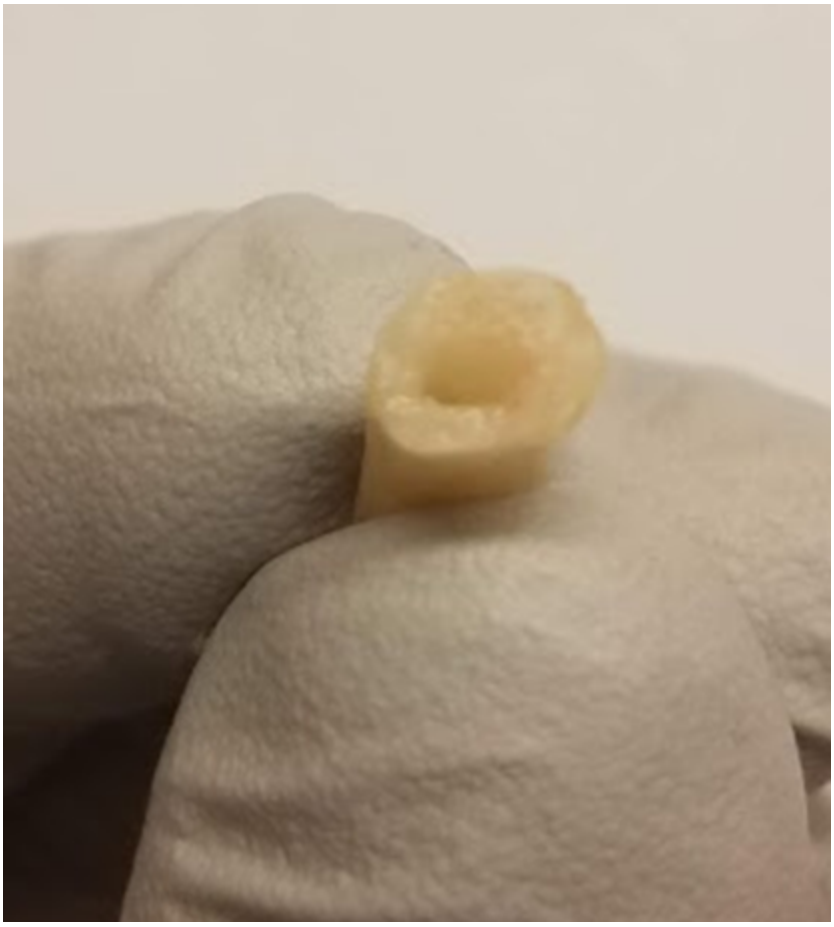
## Before start

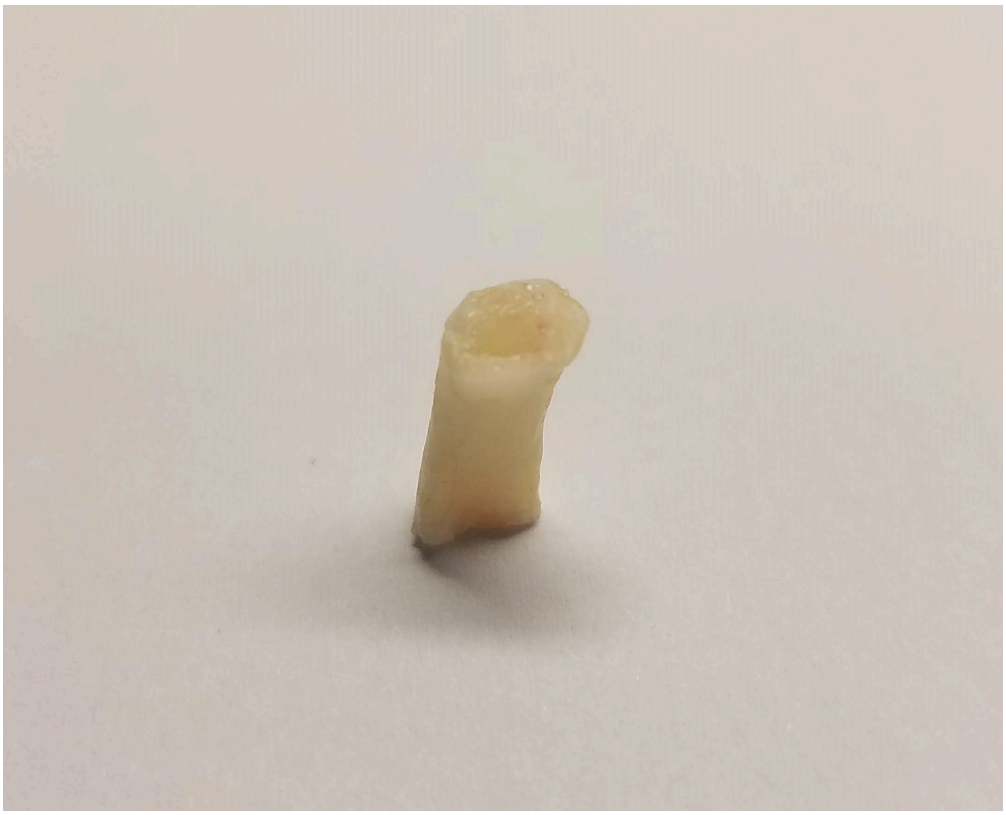
Wipe all surfaces and materials with RNase Away to minimize potential degradation due to RNases. Prepare steel mortar and pestle RNA-free by wiping with RNase Away, wrapping in aluminum foil, and baking at 350F for 6 hours or overnight. Freeze in -80C prior to RNA extraction.

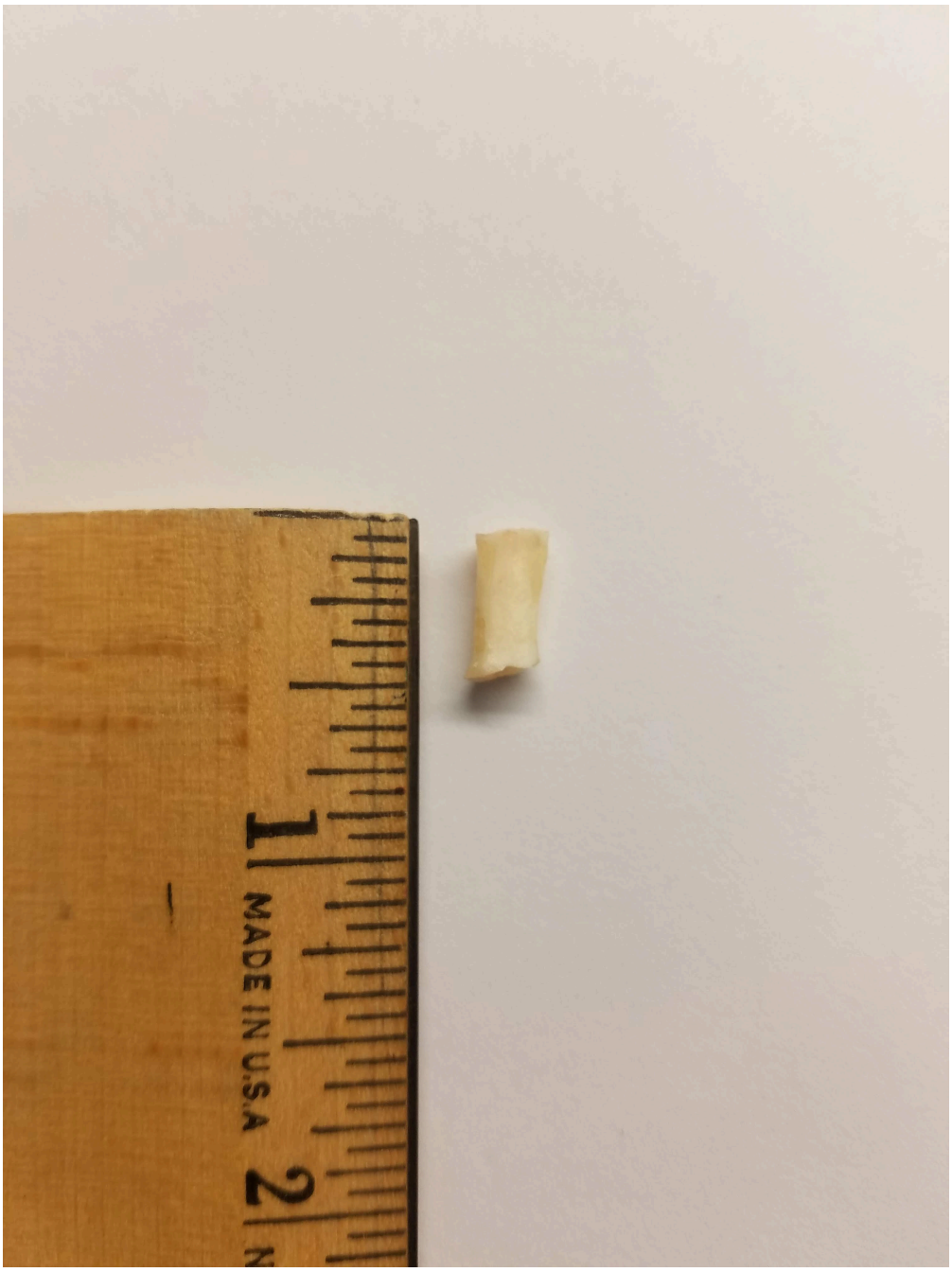
## Bone Preparation

1 Remove all exterior soft tissue around disphysis using scissors/scalpel/KimWipes. Use scalpel to scrape bone along diaphysis exterior to ensure complete removal of periosteum.
**Isolating RNA that is unique to osteoblasts, osteoclasts, and osteocytes is dependent on sufficient removal of these 'contaminating' exterior tissues.

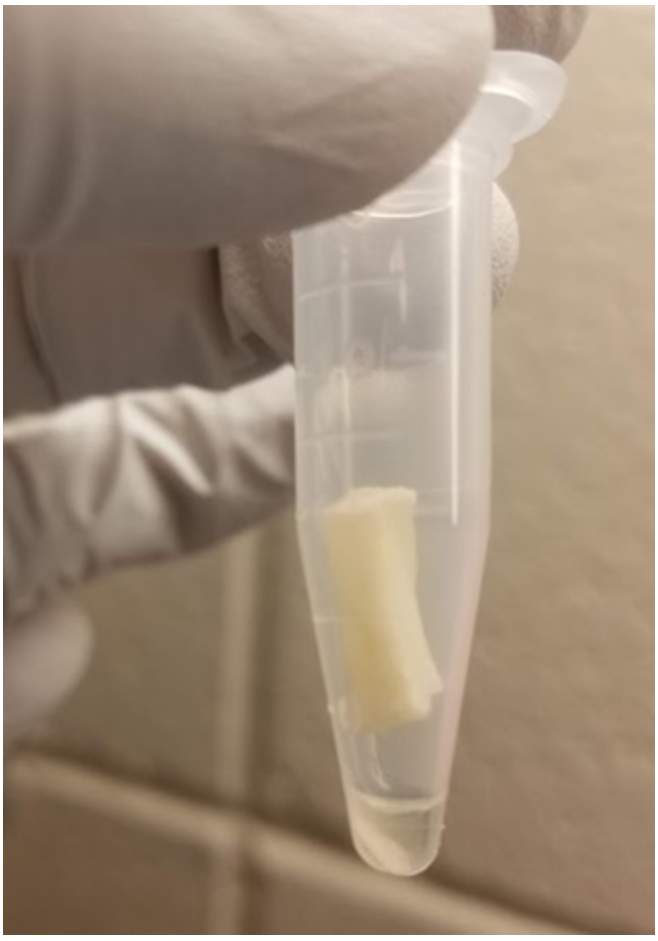
2 Cut and discard epiphyses using large bolt cutters, being careful to not disturb the tubular structure of the diaphysis.








3 Place diaphysis in a 1.7 mL microfuge tube and centrifuge at 10,000 x g for 10 min at room temp. Liquid in the bottom after centrifugation is the bone marrow/fat contained within the diaphysis.



4 Transfer diaphysis to a new 1.7 mL microfuge tube. Add enough 1x PBS (Phosphate Buffered Saline) to cover the bone completely. Centrifuge at 10,000 x g for 5 min at room temp.

5 Transfer diaphysis to a new 1.7 mL microfuge tube. Add enough 1x PBS to cover the bone completely and centrifuge again at 10,000 x g for 5 min at room temp.

6 Snap freeze bone in liquid nitrogen and store at -80C for up to 8 months until RNA extraction.

## Bone Homogenization/RNA Extraction

7 Prepare two 1.7 mL Eppendorf Safe Lock** microfuge tubes, each containing 1 mL Tri-Reagent and approximately 0.5 g of zirconium oxide beads (0.5 mm diameter) and place tubes in ice.
**Eppendorf Safe Lock tubes prevent sample leakage during subsequent Bullet Blender step.

8 Grind bone into a fine powder using a frozen RNase-free steel mortar and pestle** and hammer on top of a bed of dry ice, working quickly to avoid RNase degradation.
**Mortar and Pestle can be prepared RNase-free by wiping with RNase Away, wrapping in aluminum foil, and baking at 350F for 6 hours or overnight. Freeze in -80C prior to RNA extraction.

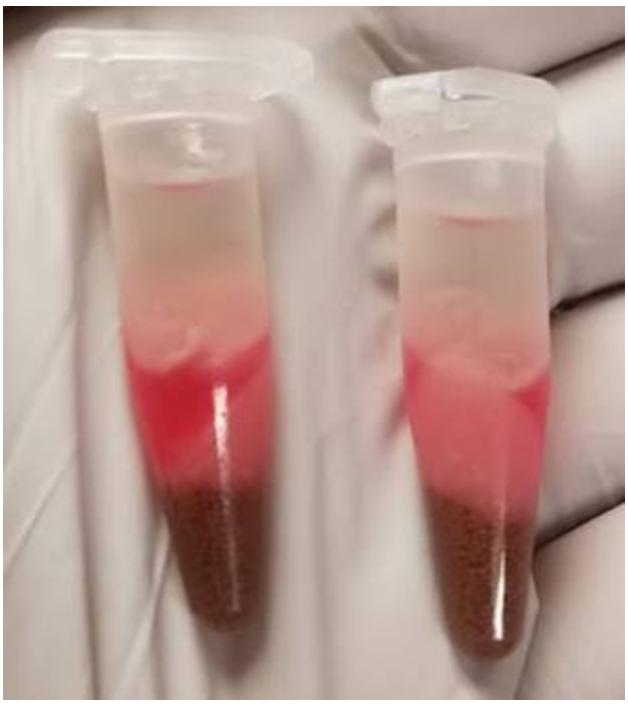
9 Divide the bone powder into the two previously prepared microfuge tubes containing 1 mL Tri-Reagent and 0.5 g zirconium beads.

10 Using a Bullet Blender at speed "5.5", subject samples to 4 rounds of a 30 second spin in Bullet Blender with a 1 minute incubation on ice between rounds (to keep samples cool and minimize degradation).

11 Incubate samples for 10 min at room temp.

12 Add 100 uL bromochloropropane (BCP) to each tube and vortex thoroughly. Incubate samples for 5 min at room temp.

13 Centifuge tubes at 20,000 x g for 15 min at 4C.



14 Carefully remove top aqueous layer and add 10 uL DNase I and 1/10 volume DNase I Reaction Buffer; Incubate 10 min at room temp.

15 Inactivate DNase by adding 10 uL of 25 mM EDTA (included) and heat for 10 min at 65C.

16 Add equal volume 70% ethanol and mix well by pipetting.

17 Apply sample to RNA column from RNeasy Micro Kit placed in a 2 mL collection tube and centrifuge 1 min at 21,000 x g at room temp.

18 After discarding eluate, add 700uLBuffer RW1, incubate on the column for 2 min at room temp, centrifuge 1 min at 21,000 x g at room temp.

19 Discard flow through, add 500uLBuffer RPE, incubate on the column for 2 min at room temp, and centrifuge 1 min at 21,000 x g at room temp.

20 Discard flow through, add 500uLof 80% ethanol, incubate on the column for 2 min at room temp, and centrifuge for 3 min at 21,000 x g at room temp.

21 Discard flow through and replace 2 mL collection tube and centrifuge the columns at 21,000 x g for 5 min with lids open.

22 Replace collection tube, add 17uLof RNase-free water (preheated to 65C), incubate for 10 min on the column at room temp, and centrifuge for 5 min at 21,000 x g at room temp.

23 Concentration and purity can then be determined using a Nanodrop spectrophotometer.