Version 2

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A method for isolating RNA from canine bone V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bf9vjr66

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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.

EXTERNAL LINK

https://www.future-science.com/doi/10.2144/btn-2019-0153?fbclid=IwAR1AdUnM10wOsts2pwp5AYhi_UkxRibNLK4ZjWFpwuusnqA6_IyRxiIFeGg

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Nance R, Agarwal P, Sandey M, et al. A method for isolating RNA from canine bone. Biotechniques, 2020. Published online 17 Apr 2020.

ATTACHMENTS

btn-2019-0153.pdf

MATERIALS

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

BEFORE STARTING

Wipe all surfaces and materials with RNase Away to minimize potential degradation due to RNases. Prepare steel

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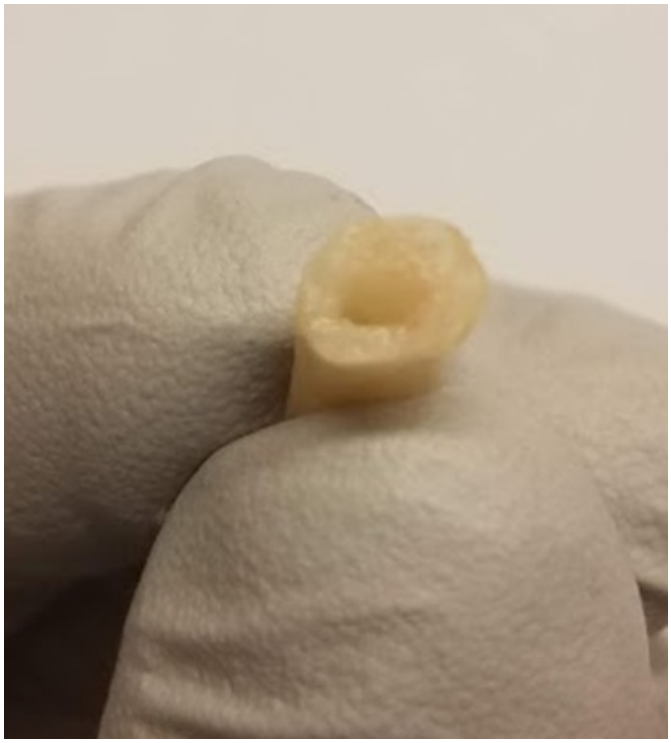
mortar and pestle RNA-free by wiping with RNase Away, wrapping in aluminum foil, and baking at **350 °F** for **06:00:00** or **Overnight** . Freeze in **-80 °C** prior to RNA extraction.

Bone Preparation

1 Remove all exterior soft tissue around disphysis using scissors/scalpel/KimWipes. Use a 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.



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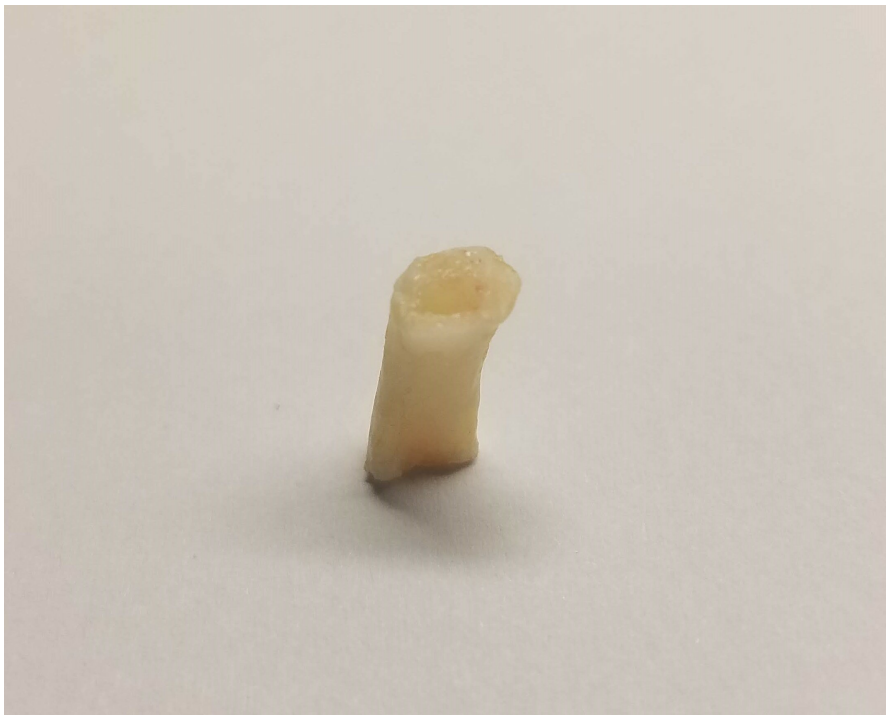

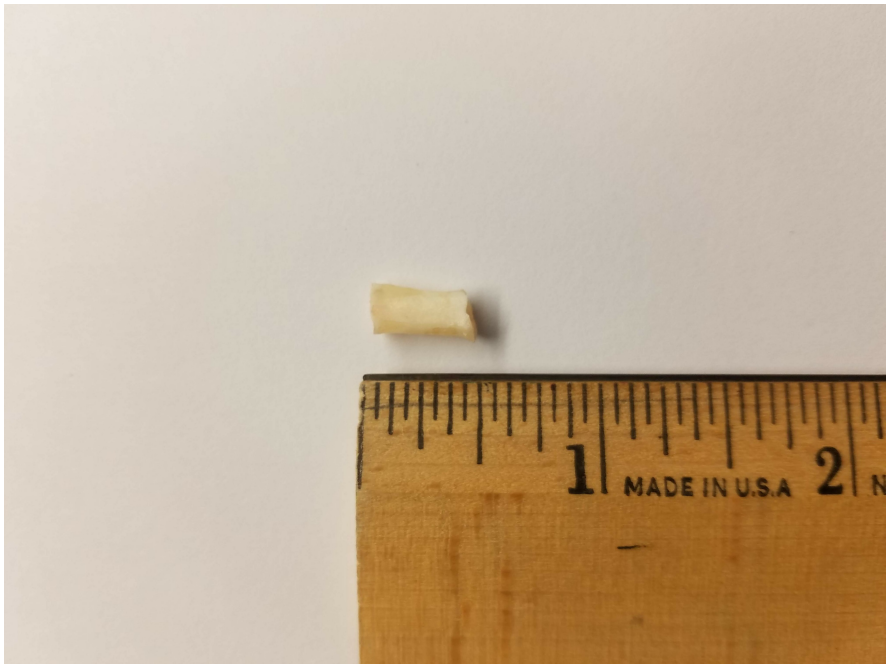

3 Place diaphysis in a 1.7 mL microfuge tube and centrifuge at **10.000 x g, Room temperature 00:10:00** . Liquid in the bottom after centrifugation is the bone marrow/fat contained within the diaphysis.

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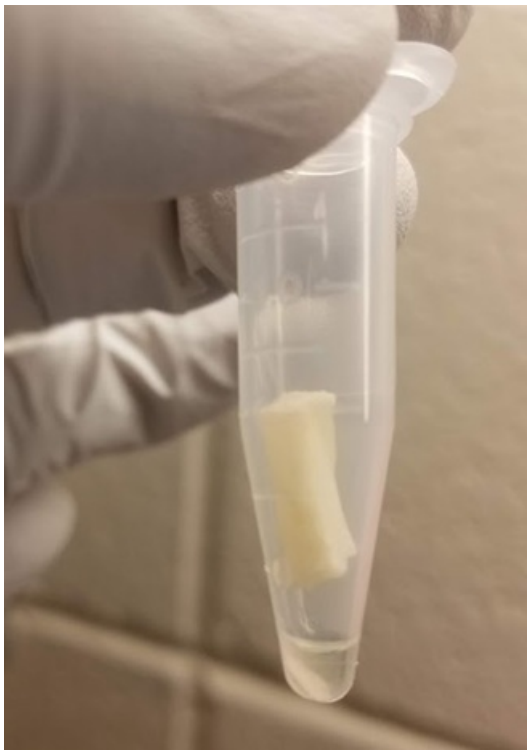

4 Transfer diaphysis to a new 1.7 mL microfuge tube. Add enough 1x PBS (Phosphate Buffered Saline) to cover the bone completely. Centrifuge at **10.000 x g, Room temperature 00:05:00** .

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, Room temperature 00:05:00** .

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6 Snap freeze bone in liquid nitrogen and store at **-80 °C** 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 **On 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 **-80 °C prior to RNA extraction.

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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 **00:00:30 spin** in Bullet Blender with a **00:01:00 incubation** **On ice** between rounds (to keep samples cool and minimize degradation).

11 Incubate samples for **00:10:00** at **Room temperature** .

12 Add **100 µl bromochloropropane (BCP)** to each tube and vortex thoroughly. Incubate samples for

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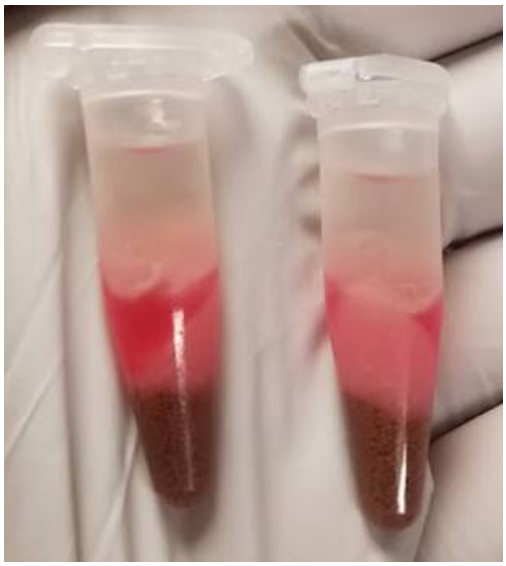
00:05:00 at Room temperature .

13 Centifuge tubes at 20.000 x g, 4°C 00:15:00 .



14 Carefully remove top aqueous layer and add 10 µl DNase I and 1/10 volume DNase I Reaction Buffer; Incubate 00:10:00 at Room temperature .

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15 Inactivate DNase by adding **10 µl of 25 mM EDTA (included)** and heat for **00:10:00** at **65 °C** .

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 **21.000 x g, Room temperature 00:01:00** .

18 After discarding eluate, add **700 µl Buffer RW1** , incubate on the column for **00:02:00** at **Room temperature** , centrifuge **21.000 x g, Room temperature 00:01:00** .

19 Discard flow through, add **500 µl Buffer RPE** , incubate on the column for **00:02:00** at **Room temperature** , and centrifuge **21.000 x g, Room temperature 00:01:00** .

20 Discard flow through, add **500 µl of 80% ethanol** , incubate on the column for **00:02:00** at **Room temperature** , and centrifuge **21.000 x g, Room temperature 00:03:00** .

21 Discard flow through and replace 2 mL collection tube and centrifuge the columns at **21.000 x g 00:05:00 , with lids open** .

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22 Replace collection tube, add **17 µl of RNase-free water** (preheated to **65 °C**), incubate for **00:10:00** on the column at **Room temperature** , and centrifuge **21.000 x g, Room temperature 00:05:00** .

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

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