

Resource Summary Report

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A method for isolating RNA from canine bone

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Rebecca Nance, Payal Agarwal, Maninder Sandey, Dmytro Starenki, Jey Koehler, Abdul Mohin Sajib, Bruce F Smith 2020. A method for isolating RNA from canine bone .
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Protocol Information

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Summary: 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 sufficient 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.

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