Methods for co-Isolation of DNA and RNA from Frozen Tissue
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DNA & RNA were extracted via the AllPrep DNA/RNA Universal Kit from Qiagen. Biological samples were cut in 20-25mg pieces on a dry ice batch, then placed in tubes with a steel beat for mechanical homogenization and buffer for lysis. Samples were then lysed and homogenized in a highly denaturing guanidine isothiocyanate-containing buffer, which immediately inactivates DNases and RNases to ensure isolation of intact DNA and RNA. The lysate was then passed through an AllPrep DNA Mini spin column. This column, in combination with the high-salt buffer, allows selective and efficient binding of genomic DNA. On-column Proteinase K digestion in optimized buffer conditions allows purification of high DNA yields from all sample types. The column was then washed and DNA was eluted in TE buffer. Flow-through from the AllPrep DNA Mini spin column was digested by Proteinase K in the presence of ethanol. This optimized digestion, together with the subsequent addition of further ethanol, allowed for appropriate binding of total RNA, including miRNA, to the RNeasy Mini spin column. Samples were then digested with DNase I to ensure high-yields of DNA-free RNA. Contaminants were efficiently washed away and RNA was eluted in water.