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Sinai SCENT TMC - 5x5 Project Bulk RNAseq

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Cellular Senescence Net...



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Protocol status: Working

We use this protocol and it's working

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Abstract

Bulk RNA sequencing protocol

Protocols

- 1 Dissect the snap-frozen or -80°C stored tissues.
- 2 Dissected tissues are placed directly into a labeled 1.5 mL RNeasy (Qiagen) collection tube and stored at 4°C for up to 24 hours to allow the RNeasy to penetrate the tissue fully before being transferred to -20°C or lower until use.
- 3 The mRNA is extracted with the RNeasy Plus Mini kit (Qiagen) per the manufacturer's instructions.
- 4 The complementary DNA (cDNA) library is established by using the Ovation RNA-Seq v2 method (NuGen).
- 5 Samples are sequenced on an Illumina NovaSeq sequencer with 2X150 bp in the paired ends at 350M read depth per sample.
- 6 Gene and isoform expression levels will be calculated using Cufflinks.
- 7 Transcripts from mitochondrial and ribosomal RNA genes are masked and not included.
- 8 The final abundances of genes in cells are computed in FPKM (Fragments Per Kilobase of exon model per Million mapped fragments).