

Oct 04, 2024

Sinai SCENT TMC - 5x5 Project Bulk RNAseq

DOI

dx.doi.org/10.17504/protocols.io.x54v926w4l3e/v1

HIMC at Mount Sinai¹

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Cellular Senescence Net...



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DOI: **dx.doi.org/10.17504/protocols.io.x54v926w4l3e/v1**

Protocol Citation: HIMC at Mount Sinai 2024. Sinai SCENT TMC - 5x5 Project Bulk RNAseq. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.x54v926w4l3e/v1>

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Protocol status: Working

We use this protocol and it's working

Created: October 04, 2024

Last Modified: October 04, 2024

Protocol Integer ID: 109194

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).