JUL 16, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.rm7vzx362gx1/v1

Protocol Citation: Connor Monahan 2023. Bulk RNA sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzx362gx1/v1

Protocol status: Working
We use this protocol and it's working

Created: Jul 12, 2023

Last Modified: Jul 16, 2023

PROTOCOL integer ID: 84902

Keywords: ileum, bulk RNA sequencing, gut, small intestine

Bulk RNA sequencing

Connor Monahan[1]

[1]Columbia University

Connor Monahan
Columbia University

ABSTRACT

This protocol details bulk RNA sequencing from the mouse small intestine.

ATTACHMENTS

786-2002.pdf

MATERIALS

Materials

- PBS
- Illumina NovaSeq6000

TRIzol Reagent Thermo Fisher Scientific Catalog #15596026

Procedure

1 Collect the ileum from a PBS-perfused mouse.

2 Thoroughly flush the ileum sample with cold 1x PBS.

3 Incubate samples in TRIzol reagent (Thermo Fisher Scientific, Cat #15596026; Waltham, MA) and store at -80 °C until ready for bulk RNA sequencing.

4 Extract RNA and construct library using Illumina TruSeq chemistry.

5 Sequence the libraries using an Illumina NovaSeq6000.

6 Samples are multiplexed in each lane, which yielded targeted number of paired-end, 100bp reads for each sample. RTA (Illumina) for base calling and bcl2fastq2 (version 2.19) are used for converting BCL to fastq format, coupled with adaptor trimming.

7 Perform a pseudoalignment to a kallisto index created from transcriptomes (GRCm38) using kallisto (0.44.0).

8 Test differentially expressed genes under various conditions using DESeq2R packages designed to test differential expression between two experimental groups from RNA-seq counts data.

9 Genes are considered differentially expressed if they had an adjusted p-value <0.05 and a log2fold change below or above 0.5. Normalize the differential expression for each gene.