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Bulk RNA sequencing

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

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Abstract

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

Attachments

786-2002.pdf
47KB

Materials

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- PBS
- Illumina NovaSeq6000

TRIzol Reagent **Thermo Fisher Scientific** **Catalog #**15596026

Troubleshooting

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.