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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



### Materials

- 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.