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RNA Extraction, Library Preparation, Bulk RNA Sequencing, and Analysis of Mouse Proximal Large Intestine

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We use this protocol and it's working

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Abstract

This protocol details the procedure for the RNA extraction, library preparation, bulk RNA sequencing, and analysis used to assess differentially expressed genes in the mouse proximal large intestine.

Materials

- TRIzol Reagent (Zymo Research)
- Direct-zol RNA MiniPrep Plus Kit (Zymo Research)
- DNase I (included with kit)
- NanoDrop spectrophotometer
- Agilent 2100 Bioanalyzer (Agilent Technologies)
- ABclonal FAST RNA-seq Library Prep Kit
- Illumina NovaSeq X sequencing platform
- STAR aligner v2.7.1
- Mus musculus GRCm36 Primary Assembly reference genome (GENCODE Release M36)
- featureCounts
- R v4.3.0
- DESeq2 R package
- clusterProfiler R package

Tissue collection

1 Dissect approximately 1 cm of proximal large intestine from each mouse.

2 Immediately place tissue into TRIzol reagent (Zymo Research) and flash-freeze on dry ice.

3 Store samples at –80 °C until RNA extraction.

Total RNA Extraction

4 Thaw samples on ice.

5 Extract total RNA using the Direct-zol RNA MiniPrep Plus Kit (Zymo Research) according to the manufacturer's protocol.

5.1 Perform on-column DNase I treatment as specified by the kit to remove genomic DNA.

6 Measure RNA concentration and purity using a NanoDrop spectrophotometer.

7 Assess RNA integrity using an Agilent 2100 Bioanalyzer.

Library Preparation and Sequencing

8 Prepare RNA-seq libraries using the ABclonal FAST RNA-seq Library Prep Kit according to the manufacturer's instructions.

9 Perform sequencing using 50 bp paired-end reads on an Illumina NovaSeq X platform with a target a sequencing depth of ~30 million reads per sample.

Bioinformatics Analysis

10 Align raw sequencing reads to the Mus musculus GRCm36 Primary Assembly reference genome (GENCODE Release M36) using STAR aligner v2.7.1 with default parameters.

11 Quantify gene-level read counts using featureCounts. Retain only protein-coding genes with a minimum total count ≥ 10 across all samples.

12 Import count data into R (v4.3.0). Normalize counts and perform differential expression analysis using the DESeq2 package.

12.1 Identify differentially expressed genes by an adjusted p-value < 0.05 and Log2 fold change threshold as defined per comparison.

13 Perform gene ontology and pathway enrichment analyses using the clusterProfiler package. Using the following criteria: Adjusted p-value < 0.1, log2 fold change > 0.2