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Total RNA Isolation from Bulk Tissue or Isolated Cells

DOI

<https://dx.doi.org/10.17504/protocols.io.8dehs3e>

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Protocol status: Working

We use this protocol and it's working

Created: October 17, 2019

Last Modified: May 05, 2020

Protocol Integer ID: 28806

Keywords: total rna isolation, isolated cell, rna, bulk tissue, cell, isolation, tissue

Procedures

- 1 Transfer biopsy in  1.8 mL cryotube (polypropylene) in  1.0 mL RNAlater
- 2 Use color coding cap inserts to indicate biopsy origin
- 3 Store at  -20 °C until RNA extraction
- 4 Perform microdissection of glomeruli / tubulointerstitium
- 5 Store dissected tissue portion in less than  50 µL RNAlater solution and store at  -20 °C until total RNA extraction
- 6 For total RNA/DNA extraction use "AllPrep DNA/RNA Micro Kit" (# 80284) from Qiagen following respective protocol. Use optional Beta-ME for glomerular compartment.

Note: Elute RNA in  16 µL H₂O in nucleotide low-binding tubes (Eppendorf #022431021) and store at  -80 °C .

Note: Keep first flow-through (< 80 bases) for future analysis of small RNAs.

Note

Quality and quantity were measured using Agilent Bioanalyzer 2100

Elute RNA in 16µL

cDNA Generation

- 7 Use NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (rRNA depletion workflow)1. Use NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (rRNA depletion workflow)
- 8 1.Store cDNA at  -20 °C



NGS Library Preparation

- 9 Use NovaSeq 6000 to sequence. (The University of Michigan Advanced Genomics Core)¹. Use NovaSeq 6000 to sequence. (The University of Michigan Advanced Genomics Core)