Total RNA Isolation from Bulk Tissue or Isolated Cells

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1 Works for me

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EXTERNAL LINK

http://www.kpmp.org

Procedures

1 Transfer biopsy in 1.8 ml cryotube (polypropylene) in 1 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 H_2O 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.

Quality and quantity were measured using Agilent Bioanalyzer 2100

Elute RNA in 16uL

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)