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Bench and Bedside - Bench

RNA sequencing of the nephron transcriptome: a technical note

Jae Wook Lee [1, 2, *]

[1] Epithelial Systems Biology Laboratory, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA
[2] Kidney Research Institute, Seoul National University, Seoul, Korea

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A B S T R A C T

To understand the functions of the kidney, the transcriptome of each part of the nephron needs to be profiled using a highly sensitive and unbiased tool. RNA sequencing (RNA-seq) has revolutionized transcriptomic research, enabling researchers to define transcription activity and functions of genomic elements with unprecedented sensitivity and precision. Recently, RNA-seq for polyadenylated messenger RNAs [poly(A)′-mRNAs] and classical microdissection were successfully combined to investigate the transcriptome of glomeruli and 14 different renal tubule segments. A rat kidney is perfused with and incubated in collagenase solution, and the digested kidney was manually dissected under a stereomicroscope. Individual glomeruli and renal tubule segments are identified by their anatomical and morphological characteristics and collected in phosphate-buffered saline. Poly(A)′-tailed mRNAs are released from cell lysate, captured by oligo-dT primers, and made into complementary DNAs (cDNAs) using a highly sensitive reverse transcription method. These cDNAs are sheared by sonication and prepared into adapter-ligated cDNA libraries for Illumina sequencing. Nucleotide sequences reported from the sequencing reaction are mapped to the rat reference genome for gene expression analysis. These RNA-seq transcriptomic data were highly consistent with prior knowledge of gene expression along the nephron. The gene expression data obtained in this work are available as a public Web page (https://helixweb.nih.gov/ESBL/Database/NephronRNAseq/) and can be used to explore the transcriptomic landscape of the nephron.

Introduction

Profiling all the transcripts expressed in the glomerulus and each renal tubule segment will greatly advance our understanding of the functions and pathophysiology of the kidney. This task requires a precise, unbiased, and high-throughput transcriptomic method that enables researchers to create a catalog of all the RNA species and accurately measure their quantities in a cell.

However, gene expression profiling methods that have been used in renal transcriptomics such as microarrays [1,2] or Sanger sequencing of complementary DNAs (cDNAs) [3–5] suffer from low sensitivity and high false positivity. The utility of microarrays is limited by the requirement of prior knowledge of genes expressed in a cell and by a narrow range of dynamic expression due to signal saturation. Sanger sequencing of cDNAs is low throughput and not sensitive enough to detect lowly expressed transcripts.

* Corresponding author. Center for Medical Innovation, Biomedical Research Institute, Seoul National University Hospital, 28 Yongon-dong, Jongno-gu, Seoul, Korea.
E-mail address: jwleemd@gmail.com (JW Lee).

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RNA sequencing (RNA-seq) uses next-generation sequencing (NGS) technologies to profile the whole transcriptome in a massive parallel manner [6]. In this method, RNAs of interest [i.e., messenger RNAs (mRNAs), microRNAs, or other noncoding RNAs] are converted into adapter-ligated cDNAs and sequenced in a parallel manner, generating massive amount of short DNA sequences (typically 35–100 base pairs) [6]. These nucleotide sequences (commonly called reads) are either mapped to a reference genome or assembled to generate a *de novo* transcriptome. Reads mapped to the reference genome can be visualized on a genome browser to explore transcriptional activity across the genome or can be counted to quantify the expression level of each transcript. Compared with microarrays or Sanger sequencing, RNA-seq has many advantages, including higher sensitivity (requiring lower amount of RNAs), low false positivity (no background signals originating from cross-hybridization), unlimited range of dynamic expression (no signal saturation), and capability to process many samples in high-throughput settings (many samples can be multiplexed and sequenced in parallel).

Recently, RNA-seq transcriptomic data for glomeruli and 14 different renal tubule segments collected from rat kidneys have been published [7]. This review discusses the technical aspects of RNA-seq profiling of the nephron, focusing on how RNA-seq and classical microdissection can be combined to profile the transcriptomes of the rat nephron. This review does not intend to provide an in-depth review of the NGS technologies. Readers are referred to excellent reviews on the principles of NGS [8,9]. For more general information on RNA-seq, the author would like to recommend a well-curated online Web site available at http://rnaseq.uoregon.edu/.

Microdissection of renal tubule segments

Collagenase-assisted manual microdissection of renal tubule segments, first reported by Burg et al in 1966 [10], has been successfully used in renal physiology for more than 4 decades. This method expanded the scope of renal research to glomeruli and tubule segments that had not been accessible by micropuncture. To collect glomeruli and renal tubule segments for RNA-seq profiling, a protocol previously published in the article by Wright et al [11] was used with minor modifications. A male Sprague Dawley rat weighing 150–200 g is killed by decapitation (Animal Study Protocol No. H-0110R2, approved by the Animal Care and Use Committee, National Heart, Lung, and Blood Institute). After a midline incision of the abdominal wall, the left renal artery is selected by introducing a ligature in the aorta between the left and renal arteries. Then, a thin plastic catheter is introduced through a slit made on the wall of the aorta below the level of the left renal artery, and through this catheter, the left kidney is perfused with 10 mL of ice-cold, bicarbonate-free dissecting solution (NaCl 135 mmol/L; Na_2HPO_4 1 mmol/L; Na_2SO_4 1.2 mmol/L; $MgSO_4$ 1.2 mmol/L; KCl 5 mmol/L; $CaCl_2$ 2 mmol/L; glucose 5.5 mmol/L; and HEPES 5 mmol/L, adjusted to pH 7.4), followed by 10 mL of collagenase solution [1 mg/mL of collagenase B (purified from *Clostridium histolyticum*, Roche Diagnostics, Indianapolis, IN, USA) and 1 mg/mL of bovine serum albumin (MP Biomedicals, Santa Ana, CA, USA) in the dissecting solution] warmed to 37°C. Before use, the dissecting solution in which collagenase and bovine serum albumin are to be dissolved is usually bubbled with 100% O_2 for 10 minutes to mitigate hypoxia. It is crucial that the blood in the left kidney is completely removed by the initial perfusion with the dissecting solution because protease inhibitors in the plasma prevent collagenase from acting on the kidney. Although some renal tubule segments (e.g., the cortical collecting duct) can be dissected without collagenase digestion, most parts of the nephron cannot be dissected if the kidney is not properly digested. To facilitate the perfusion process, the wall of the inferior vena cava needs to be cut so that the blood and solution returning from the left kidney via the left renal vein can easily exit the circulation. After the perfusion with the collagenase solution, the left kidney is removed and cut into ~1 mm^3 cubes, put into the same collagenase solution, and incubated in a chamber filled with O_2 at 37°C for 30–90 minutes. The concentration of collagenase and the duration of incubation need to be adjusted, depending on which tissue compartment is going to be dissected. The cortex can be digested in 1% collagenase for 30 minutes. The outer and inner medullas require a higher collagenase concentration and longer duration (1% and 45 minutes for the outer medulla; 3% and up to 90 minutes for the inner medulla), along with hyaluronidase of the same concentration as collagenase. Even with a higher concentration of collagenase, digestion of the inner medulla was successful only once in every 3 or 4 experiments. A thorough pretreatment of the dissecting solution and other apparatuses to inactivate ribonuclease is generally not needed, although general precautions used in RNA works (i.e., wearing gloves, using ribonuclease-inactivating products) need to be followed. This is probably because the content of ribonuclease is not high in the kidney compared with other organs such as the spleen or the pancreas.

After digestion, the tissue chunks are taken out of the collagenase solution, washed twice in ice-cold dissecting solution to end digestion process, and put in a glass dish containing ice-cold dissecting solution. This dish is then brought under a stereomicroscope for microdissection. To minimize tissue degradation, the digested kidney tissue needs to be maintained at a cool temperature, ideally at 4°C. It is generally recommended to use a stereomicroscope that has a coolant-circulating system attached to the bottom of the stage of the microscope. If the glass dish containing the digested tissue is maintained at 4°C, a microdissection session can be extended up to 4 hours with minimal tissue degradation. For optimal identification of tubule segments, it is better to have the light source for the stereomicroscope below the object stage.

The digested kidney tissue is examined using Dumont No. 5 forceps (https://www.dumonttweezers.com/Tweezer/Tweezer/469). Before use, the tips of the tweezers need to be sharpened and polished with a piece of sandpaper so that the tweezers can easily grab and manipulate tubule segments and a dissected tubule segment or other irrelevant tissue does not stick to the surface of the tweezer tips. The degree of tissue digestion can be assessed by trying to grab and separate tissue chunk using tweezers. If it is too difficult to separate tubules from surrounding tissue, the tissue chunks can be transferred back to the collagenase solution for more digestion (up to 5–10 minutes).

Identifying individual renal tubule segments requires working knowledge of renal anatomy. Through practice, the dissector becomes more and more familiar with the morphology and locations of individual renal tubule segments. The anatomical and morphological characteristics of renal tubule segments were described in detail in the article by Wright