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Title: Application of Laser Microdissection to Uncover Regional Transcriptomics in Human Kidney Tissue

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page by the script return deadline

Videographer: Please screen capture shots for reference

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Script Length

Number of Shots: **21**

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Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Daria Barwinska**: Laser microdissection allows for the transcriptomic analysis of spatially-defined regions within kidney tissue samples, allowing the unique identification of structures of interest even after disease-induced changes **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Michael Eadon**: The main advantages of this methodology are its complementarity to other omics technologies and its remarkable tissue economy when detecting even low expressed genes **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Tarek El-Achkar**: The regional transcriptomics afforded by LMD let us identify the expression of specific genes that change during disease states and therefore offer potential therapeutic targets as part of the precision medicine approach **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

1.4. Procedures involving human subjects have been approved by the Institutional Review Board (IRB) at Indiana University.

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Protocol

2. Cryosectioning and Laser Microdissection (LMD)

2.1. After acquiring the tissue sample, use a cryostat set to minus 20 degrees Celsius to cut the specimen to a thickness of 12 micrometers **[1]** and use the slide adapter to fix the specimen to a specialized laser microdissection slide **[2-TXT]**.

2.1.1. WIDE: Talent at cryostat, cutting specimen *Videographer: Important step*

2.1.2. Specimen being attached to slide *Videographer: Important step* **TEXT: Store slides in plastic bag with desiccant cartridge at -80 °C ≤10 d**

2.2. Within 10 days of cryosectioning, mix 89.2 microliters of RNase free 10% BSA (B-S-A) in PBS with 4 microliters of FITC-Phalloidin **[1-TXT]**, 1.5 microliters of DAPI (DAP-ee) **[2]**, 2 microliters of the antibody of interest directly conjugated to Alexa Fluor 546 (five-forty-six) **[4]**, and 3.3. microliters of RNase inhibitor **[5]**.

2.2.1. Talent adding BSA in PBS to tube, with BSA + PBS and FITC-Phalloidin containers visible in frame **TEXT: BSA: bovine serum albumin**

2.2.2. Talent adding DAPI, to tube, with DAPI container visible in frame

2.2.3. Talent adding Ab to tube, with Ab container visible in frame

2.2.4. Talent adding RNase to tube, with RNase container visible in frame

2.3. Wash the slide in minus 20-degree Celsius 100% acetone for 1 minute **[1]** and place it slide in a humidity chamber **[2]**.

2.3.1. Slide being placed into acetone, with acetone container visible in frame

2.3.2. Talent placing slide into humidity chamber

2.4. Wash the top of the slide two times with fresh RNase-free PBS for 30 seconds per wash **[1]** followed by two, 30-second washes with 10% BSA in RNase-free PBS **[2]**.

2.4.1. PBS being added on top of the slide, with slide visible in frame

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2.4.2. BSA + PBS being added to slide, with slide visible in frame

2.5. After the last wash, treat the sample with the prepared antibody solution for 5 minutes at room temperature **[1]** before washing two more times with 10% BSA in RNase-free PBS **[2]**.

2.5.1. Ab mixture being added to slide, with Ab mixture container visible in frame *Videographer: Important step*

2.5.2. BSA + PBS being added to slide, with slide visible in frame

2.6. After the second wash, air dry the slide for 5 minutes in a tissue culture hood **[1]** before loading the slide onto the laser microdissection cutting platform **[2]**.

2.6.1. Slide being placed in hood

2.6.2. Talent loading slide onto device *Videographer: Important step*

2.7. Install autoclaved 500-microliter microcentrifuge collection tubes appropriate for PCR work containing 50 microliters of Extraction Buffer from an RNA isolation kit onto the device **[1]**.

2.7.1. Tubes being loaded onto device *Videographer: Important step*

2.8. After loading, identify regions of interest within the sample by staining, morphology, and location **[1]**.

2.8.1. Talent at computer, identifying ROI, with monitor visible in frame *Videographer: Important step*

2.9. Use immunofluorescence to outline at least 500,000-micrometer square segments of interest **[1]**.

2.9.1. SCREEN: To be provided by Authors: ROI being outlined

2.10. Then initiate the laser microdissection to excise the region using a laser power of greater than 40 **[1]**.

2.10.1. SCREEN: To be provided by Authors: Laser cutting be initiated

2.11. Upon completion of the laser microdissection process, cap the microcentrifuge collection tubes **[1]** and flick the tubes vigorously to ensure that the contents move from the caps to the bottoms of the tubes **[2]**.

2.11.1. Talent capping tubes *Videographer: Important step*

2.11.2. Talent flicking tubes *Videographer: Important step*

2.12. After centrifugation **[1-TXT]**, incubate the tubes in a 42-degree Celsius water bath for 30 minutes **[2]** before centrifuging the tubes again **[3-TXT]**.

2.12.1. Talent placing tube(s) into centrifuge **TEXT: 30 s, 3000 x g**

2.12.2. Talent placing tube(s) into water bath

2.12.3. Talent placing tube(s) into centrifuge **TEXT: 2 min, 3000 x g**

2.13. Then transfer the supernatants to new 500-microliter tubes for minus 80-degree Celsius storage **[1]**.

2.13.1. Talent adding supernatant to tube(s), with tubes visible in the frame

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Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.1., 2.5-2.8., 2.11.

Results

3. Results: Representative Human Kidney Regional Transcriptomics Analyses

3.1. The identification of tubular sub-segments in the kidney is accomplished through antibody staining of unique tubular markers **[1]** in addition to cell morphology **[2]** and structural landmarks **[3]**.

3.1.1. LAB MEDIA: Figure 1 *Video Editor: please emphasize red signal in Figure 1B*
3.1.2. LAB MEDIA: Figure 1 *Video Editor: please emphasize yellow-outlined dissected thick ascending limb in Figure 1C*

3.2. Fluorescence labeling, in conjunction with morphological tissue features and the spatial positioning of the imaged structures, facilitates the visualization of renal sub-segments with a high degree of confidence **[1]**.

3.2.1. LAB MEDIA: Figure 2 *Video Editor: please sequentially add/emphasize images*

3.3. In this representative downstream sequencing analysis, the success rate of meeting the minimum dissection area input was >90% **[1]**, resulting in a high total mRNA quantity available for gene detection **[2]**. In this analysis, 100% of the samples met the minimum detection threshold of at least 10,000 genes **[3]**.

3.3.1. LAB MEDIA: Figure 3A
3.3.2. LAB MEDIA: Figure 3D
3.3.3. LAB MEDIA: Figure 3D *Video Editor: please emphasize green line*

3.4. After laser microdissection **[1]**, enrichment analysis of the gene expression of known markers **[2]** can be compared to those from other compartments **[3]**.

3.4.1. LAB MEDIA: Table 1
3.4.2. LAB MEDIA: Table 1 *Video Editor: please emphasize Marker column*
3.4.3. LAB MEDIA: Table 1 *Video Editor: please emphasize Fold Change column*

3.5. In this analysis, one marker for each sub-segment was identified through laser microdissection regional transcriptomics **[1]** that also yielded a specific immunohistochemical staining of the corresponding nephron sub-segment **[3]**.

3.5.1. LAB MEDIA: Figure 5
3.5.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize images*

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Conclusion

4. Conclusion Interview Statements

4.1. **Daria Barwinska**: It is important to note that laser microdissection relies heavily on the expertise of the user to precisely identify the structures of interest to be dissected within any given kidney sample **[1]**.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.9.)

4.2. **Tarek El-Achkar**: This technique allows the study of specific regional gene expression signatures that can be used to assess potential pathways involved in disease progression as well as the biology of healthy tissue **[1]**.

4.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

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