

Submission ID #: 69814

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Title: Laser Capture Microdissection of Paraformaldehyde-Fixed Mouse Liver Tissue for RNA Analysis

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes, done**

SCOPE: **3.3.1**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 24

Number of Shots: 44

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Yan Wang:** Our research mainly investigates hepatocyte-related pathophysiology in the context of chronic liver diseases. This protocol enables cell isolation in liver tissue slices and RNA extraction.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Wei-Lan Zeng:** Unlike FFPE, our paraformaldehyde-fixed OCT protocol preserves RNA integrity, enabling precise microdissection and high-quality samples for downstream omics analyses.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol~~

- 1.3. **Wei-Lan Zeng:** An important consideration is preventing RNA degradation via controlled fixation, rapid staining, and strict RNase-free conditions throughout processing.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Wei-Lan Zeng:** Following this procedure, RNA can be used for real time PCR and RNA sequencing with fragmentation-optimized library kits.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Yan Wang:** Established using liver samples, this protocol enables hepatocyte subgroup profiling and supports pathology-focused hepatology research.

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

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Animal Ethics Committee at Southern Medical University

Protocol

2. Preparation of Cryosections on Polyethylene Naphthalate (PEN) Membrane Slides for Downstream Molecular Analysis

Demonstrator: Wei-Lan Zeng

- 2.1. To begin, expose PEN membrane slides to ultraviolet light for 30 minutes [1]. Coat the slides with 0.1 milligram per milliliter of poly-L-lysine and allow them to air dry [2]. Seal the edges of each slide with a neutral mounting medium [3].
 - 2.1.1. Talent placing polyethylene naphthalate membrane slides under an ultraviolet light source.
 - 2.1.2. Talent pipetting the slides with poly-L-lysine solution and leaving them to air dry.
 - 2.1.3. Talent applying neutral mounting medium along the edges of the membrane slide.
- 2.2. Using an RNase decontamination agent, wipe all accessible surfaces of the cryostat and all tools [1]. Using 75 percent ethanol, clean the brush thoroughly [2].
 - 2.2.1. Talent wiping the interior and exterior surfaces of the cryostat with ribonuclease decontamination agent.
 - 2.2.2. Talent cleaning a brush with 75 percent ethanol, and cleaning the tools (forceps) with an RNase decontamination agent.
- 2.3. Now, set the cryostat temperature to minus 20 degrees Celsius and allow it to stabilize [1].
 - 2.3.1. Shot of the cryostat display showing minus 20 degrees Celsius as the set temperature.
- 2.4. Retrieve the liver samples from minus 80 degrees Celsius storage [1]. Place the samples into the cryostat chamber for at least 15 minutes to equilibrate [2].
 - 2.4.1. Talent removing liver samples from a minus 80 degrees Celsius freezer.
 - 2.4.2. Talent placing the samples inside the cryostat chamber.
- 2.5. Apply a 2- to 3-millimeter-thick layer of OCT compound onto the specimen stage [1]. Then place the specimen stage on a quick freeze shelf or inside the cryostat chamber to pre solidify the compound layer [2].
 - 2.5.1. Talent pipetting optimal cutting temperature compound onto the specimen stage.

- 2.5.2. Talent placing the specimen stage onto the quick freeze shelf inside the cryostat.
- 2.6. Apply a small amount of OCT compound onto the pre solidified layer [1]. Place the equilibrated tissue block horizontally onto the compound [2-TXT]. Then mount the specimen stage with the attached tissue block onto the cryostat specimen head [3].
 - 2.6.1. Close up of additional optimal cutting temperature compound being applied.
 - 2.6.2. Talent positioning the tissue block horizontally and holding it in place. **TXT: Hold in place for 10 - 15 s to allow partial freezing**
 - 2.6.3. Talent attaching the specimen stage to the cryostat specimen head.
- 2.7. Now insert the blade carefully between the clamping plates and back plates from one side of the cryostat [1]. Clamp the clamping lever to secure the blade [2]. Adjust the cryostat thickness setting to 10 micrometers [3].
 - 2.7.1. Talent inserting the blade into the cryostat holder.
 - 2.7.2. Talent securing the blade using the clamping lever.
 - 2.7.3. Talent setting the thickness control to 10 micrometers.
- 2.8. Cut the tissue into sections [1] and mount them directly onto the pretreated PEN membrane slides inside the cryostat chamber maintained at minus 20 degrees Celsius [2].
 - 2.8.1. Talent sectioning tissue in the cryostat.
 - 2.8.2. Talent placing sections onto polyethylene naphthalate membrane slides.

3. Hematoxylin-Staining of Cryosections and Laser Capture Microdissection for RNA Isolation

- 3.1. Thaw the slides at room temperature for 20 minutes [1]. Wash the slides 3 times with RNase-free PBS for 10 seconds each wash [2].
 - 3.1.1. Talent placing slides on a laboratory bench at room temperature.
 - 3.1.2. Talent gently rinsing slides in ribonuclease free PBS.
- 3.2. Immerse the slides in filtered Mayer's hematoxylin solution for 5 to 10 seconds [1]. Then wash the slides 3 times with ribonuclease free water for 10 seconds each wash to remove residual hematoxylin [2].
 - 3.2.1. Talent dipping the slides into a container of filtered Mayer's hematoxylin solution.
 - 3.2.2. Talent rinsing slides with ribonuclease free water.
- 3.3. Assess the staining quality under a microscope [1]. Using absorbent paper, blot the slides dry [2-TXT].

- 3.3.1. SCOPE: 69814_screenshot_1 00:10-00:22
- 3.3.2. Talent gently blotting the edges of the slides with absorbent paper. **TXT: Let slides air dry completely**

- 3.4. Next, wipe the laboratory bench surface with RNase decontamination agent [1]. Turn on the controller and allow the microscope to complete initialization [2].
 - 3.4.1. Talent cleaning the bench surface with ribonuclease decontamination agent.
 - 3.4.2. Talent switching on the controller and waiting as the system initializes.
- 3.5. Rotate the key clockwise from the **Off** position to the **On** position to initiate the laser system [1].
 - 3.5.1. Talent turning the laser system key from Off to On.
- 3.6. Turn on the computer and launch the laser capture microdissection software, then wait for initialization to complete [1].
 - 3.6.1. SCREEN: 69814_screenshot_2 00:00-00:27
- 3.7. Now insert a sterile 0.2-milliliter PCR tube into the collection device [1]. Using a pipette, add 10 microliters of proteinase K digestion buffer to the cap of the PCR tube [2].
 - 3.7.1. Talent placing a sterile 0.2 milliliter PCR tube into the collection device.
 - 3.7.2. Talent pipetting 10 microliters of proteinase K digestion buffer into the tube cap.
- 3.8. Load the collection device into the microscope specimen holder [1].
 - 3.8.1. Talent positioning the collection device into the microscope holder.
- 3.9. In the **Change Collector Device** window, go to adjust Reference Point. Adjust the camera focus to clearly view the reference point and use the arrow controls to center it if necessary [2].
 - 3.9.1. SCREEN: 69814_screenshot_3 00:15-00:44
- 3.10. Load a tissue section into the specimen holder with the tissue facing down and the label positioned to the right [1].
 - 3.10.1. Talent placing the slide into the specimen holder with correct orientation.
- 3.11. Select the 10X objective lens and identify regions of interest with specific pathological features [1]. Navigate to the **Laser** menu, click **Calibrate**, select **Yes**, and complete calibration [2].
 - 3.11.1. SCREEN: (69814_screenshot_4 00:00-00:22)
 - 3.11.2. SCREEN: (69814_screenshot_4 00:23-00:45)
- 3.12. In the laser control panel, set **Laser Power** to **50**, **Aperture** to **5**, and **Speed** to **9** [1].

3.12.1. SCREEN: (69814_screenshot_5 00:00-00:18)

3.13. Then use the graphic toolbar to draw a circle around the target area [1]. Select **Single Shape**, press **Start Cut** to perform laser capture microdissection, and position the collection device to collect the dissected tissue [2].

3.13.1. SCREEN: 69814_screenshot_6 00:00-00:12

3.13.2. SCREEN: (69814_screenshot_6 00:13-00:45)

3.14. To capture documentation images, select **File** and choose **Save Image As** to save before and after images in the preferred format [1].

3.14.1. SCREEN: 69814_screenshot_7 00:00-00:29)

3.15. After collecting the desired cells, click **Unload** to remove the collector from the stage [1]. Verify that 10 microliters of proteinase K digestion buffer remains in the cap [2-TXT].

3.15.1. SCREEN: 69814_screenshot_8 00:00-00:18)

3.15.2. Talent checking buffer volume in the tube cap, and closing the cap. **TXT: Replenish buffer if necessary and close the cap promptly**

3.16. Vortex the collection tubes thoroughly [1]. Spin the collection tubes briefly in a centrifuge [2]. Store the tubes at minus 80 degrees Celsius until RNA extraction [3].

3.16.1. Talent placing the tubes on a vortex mixer.

3.16.2. Talent placing tubes into a centrifuge.

3.16.3. Talent placing the tubes into a minus 80 degrees Celsius freezer for storage.

Results

4. Results

- 4.1. The hematoxylin-stained liver section on the PEN slide was visualized under the microscope before laser capture microdissection [1] and after laser capture microdissection [2].
 - 4.1.1. LAB MEDIA: Figure 2A.
 - 4.1.2. LAB MEDIA: Figure 2B.
- 4.2. Regions of interest exhibiting microvesicular steatosis were precisely collected into the cap of a PCR tube, preserving intact cellular morphology [1]
 - 4.2.1. LAB MEDIA: Figure 2C.
- 4.3. Total ribonucleic acid extracted from approximately 1,000 and 2,000 laser capture microdissection-captured cells yielded 110.7 nanograms and 213.3 nanograms, respectively [1].
 - 4.3.1. LAB MEDIA: Figure 3A. *Video editor: Sequentially highlight the bar labeled “1000 cells” and the bar labeled “2000 cells”.*
- 4.4. All samples showed high purity, with absorbance 260 by 280 nanometer ratios between 1.9 and 2.1 [1]. Gel electrophoresis revealed distinct 28S and 18S ribosomal ribonucleic acid bands [2].
 - 4.4.1. LAB MEDIA: Figure 3B.
 - 4.4.2. LAB MEDIA: Figure 3C. *Video editor: Point to the bands labeled “28S” and “18S” on the gel image.*
- 4.5. Bioanalyzer profiles of ribonucleic acid from as few as 1,000 cells gave a ribonucleic acid integrity number of 6.7 [1].
 - 4.5.1. LAB MEDIA: Figure 3D and E. *Video editor: Highlight the bar labeled “1000 cells” in E*
- 4.6. qRT-PCR for the housekeeping gene β -actin yielded cycle threshold values ranging from 17 to 19 [1]. Bioanalyzer assessment of sequencing libraries constructed from 50 nanogram of total ribonucleic acid showed a characteristic peak between 300 and 500 base pairs [2].
 - 4.6.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the amplification curves crossing the threshold between cycle 17 and cycle 19 on the horizontal axis.*
 - 4.6.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the main peak located between 300 and 500 base pairs on the horizontal axis.*

4.7. RNA sequencing of these libraries yielded high-quality data, with both Q20 and Q30 scores exceeding 90% and a low error rate across replicates [1].

4.7.1. LAB MEDIA: Table 1. *Video editor: Highlight the column with Q20 and Q30 values*

1. polyethylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyethylene>
IPA: /ˌpɑːliˈeθəˌliːn/
Phonetic Spelling: pah·lee·eth·uh·leen
2. naphthalate
Pronunciation link: <https://www.howtopronounce.com/naphthalate>
IPA: /ˈnæfθəˌleɪt/
Phonetic Spelling: naf·thuh·layt
3. ultraviolet
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>
IPA: /ˌʌltrəˈvaɪələt/
Phonetic Spelling: ul·truh·vy·uh·luht
4. poly-L-lysine
Pronunciation link: <https://www.howtopronounce.com/poly-l-lysine>
IPA: /ˌpɑːliˌɛlˈlaɪsiːn/
Phonetic Spelling: pah·lee el ly·seen
5. ribonuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonuclease>
IPA: /ˌraɪboʊˈnuːkliːˌeɪs/
Phonetic Spelling: rye·boh·noo·klee·ays
6. cryostat
Pronunciation link: <https://www.merriam-webster.com/dictionary/cryostat>
IPA: /ˈkraɪəˌstæt/
Phonetic Spelling: kry·uh·stat
7. equilibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
IPA: /ɪˈkwɪlɪˌbreɪt/
Phonetic Spelling: ih·kwil·uh·brayt
8. specimen
Pronunciation link: <https://www.merriam-webster.com/dictionary/specimen>
IPA: /ˈspɛsəˌmɛn/
Phonetic Spelling: spes·uh·muhn
9. hematoxylin
Pronunciation link: <https://www.merriam-webster.com/dictionary/hematoxylin>

- IPA: /ˌhi:məˈtɑ:ksəlɪn/
Phonetic Spelling: hee·muh·tok·suh·lin
10. microdissection
Pronunciation link: <https://www.merriam-webster.com/dictionary/microdissection>
IPA: /ˌmaɪkroʊdɪˈseɪʃən/
Phonetic Spelling: my·kroh·duh·sek·shuhn
11. proteinase
Pronunciation link: <https://www.howtopronounce.com/proteinase>
IPA: /ˈproʊtiˌneɪs/
Phonetic Spelling: proh·tee·nays
12. digestion
Pronunciation link: <https://www.merriam-webster.com/dictionary/digestion>
IPA: /daɪˈdʒɛstʃən/
Phonetic Spelling: dye·jes·chuhn
13. centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsentrəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
14. microvesicular
Pronunciation link: <https://www.howtopronounce.com/microvesicular>
IPA: /ˌmaɪkroʊvɪˈsɪkjələr/
Phonetic Spelling: my·kroh·vih·sik·yuh·ler
15. steatosis
Pronunciation link: <https://www.howtopronounce.com/steatosis>
IPA: /ˌstiːəˈtoʊsɪs/
Phonetic Spelling: stee·uh·toh·suhs
16. morphology
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>
IPA: /mɔːrˈfɑːlədʒi/
Phonetic Spelling: mor·fol·uh·jee
17. ribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic>
IPA: /ˌraɪboʊnuːˈkliːɪk/
Phonetic Spelling: rye·boh·noo·klee·ik
18. nanogram
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanogram>
IPA: /ˈnænəˌɡræm/
Phonetic Spelling: nan·uh·gram
19. electrophoresis
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>
IPA: /ɪˌlektroʊfəˈriːsɪs/
Phonetic Spelling: ih·lek·troh·fuh·ree·suhs
20. ribosomal
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribosomal>

IPA: /raɪˈboʊsəməl/

Phonetic Spelling: rye·boh·suh·muhl

21. bioanalyzer

Pronunciation link: <https://www.howtopronounce.com/bioanalyzer>

IPA: /ˌbaɪoʊˈænəˌlaɪzər/

Phonetic Spelling: bye·oh·an·uh·ly·zer

22. integrity

Pronunciation link: <https://www.merriam-webster.com/dictionary/integrity>

IPA: /ɪnˈteɡrəti/

Phonetic Spelling: in·teg·ruh·tee

23. sequencing

Pronunciation link: <https://www.merriam-webster.com/dictionary/sequencing>

IPA: /ˈsiːkwənsɪŋ/

Phonetic Spelling: see·kwuhn·sing

24. replicate

Pronunciation link: <https://www.merriam-webster.com/dictionary/replicate>

IPA: /ˈreplɪkət/ , /ˈreplɪˌkeɪt/

Phonetic Spelling: rep·lih·kuht / rep·lih·kayt