Submission ID #: 68294
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20829718

Title: Mouse Model of Metabolic Dysfunction-Associated Steatotic Liver Disease with Fibrosis

Authors and Affiliations:
Daniah Khoj*, Ryan Huang*, Eliza Altvater*, Zanib N Ishfaq, Xinyin Jiang, Kathleen V Axen, Jorge Matias Caviglia

Brooklyn College, City University of New York

* These authors contributed equally

Corresponding Authors:
Jorge Matias Caviglia JorgeM.Caviglia@brooklyn.cuny.edu; caviglia@outlook.com

Email Addresses for All Authors:
Daniah Khoj Daniah.Khoj38@bcmail.cuny.edu; daniah.khoj@gmail.com
Ryan Huang RHuang8@bidplymouth.org
Eliza Altvater Elza.conn@utexas.edu
Zanib N Ishfaq Zanib.Ishfaq37@bcmail.cuny.edu
Xinyin Jiang XinyinJiang@brooklyn.cuny.edu
Kathleen V Axen KAxen@brooklyn.cuny.edu
Jorge Matias Caviglia JorgeM.Caviglia@brooklyn.cuny.edu; caviglia@outlook.com

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? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

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

Current Protocol Length
Number of Steps: 12
Number of Shots: 36

Introduction

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

1.1. **Jorge Caviglia:** In our research, we focus on metabolic dysfunction-associated steatotic liver disease, or MASLD,
and specifically on the mechanisms of lipid accumulation and fibrosis.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the most recent developments in your field of research?

1.2. **Jorge Caviglia:** Some of the recent developments are
single cell and spatial transcriptomics,
the standardization of animal models of MASLD,
and the approval of semaglutide for the treatment of MASLD.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What research gap are you addressing with your protocol?

1.3. **Jorge Caviglia**: There are many animal models of MASLD,
but we found out that many did not induce liver fibrosis.
So, to address that point, we developed our mouse model of MASLD.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Jorge Caviglia:** Our mouse model induces MASLD with fibrosis
by feeding hyperphagic mice a diet similar to the average American diet.
This causes MASLD that is very similar to the disease in humans.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.1*

How will your findings advance research in your field?

1.5. **Jorge Caviglia:**
Our method will facilitate
studies on the pathophysiology of MASLD
and testing new therapies.

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

NOTE: 1.5 - slate is mislabeled as 1.4. Clip for 1.5 is B111_B109_1017XF_001

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Jorge Caviglia, Assistant Professor**: (authors will present their testimonial statements live) .

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

Protocol

2. Collagen Histochemical Staining of Mouse Liver Tissue

Demonstrator: Zanib Ishfaq

2.1. To begin, obtain a paraffin-embedded liver tissue section on a slide **[1]**. For deparaffinization, submerge the slide in xylene or citrisolv *(citri-solve)*, for 5 to 15 minutes **[2-TXT]**. Then, rehydrate the section serially in 100, 95 and 70 percent ethanol solutions, followed by distilled water, for 5 minutes in each **[2]**.

2.1.1. WIDE: Talent putting/loading the sample slides on a slide holder.

2.1.2. Talent placing slides into a glass jar containing xylene or citrisolv. **TXT: Repeat this step again with fresh xylene or citrisolv**

2.1.3. Talent taking the slide out of a staining dish labeled "100% ethanol" and dunking it in another staining dish labeled "95% ethanol". **Authors**, please place other staining jars with 95% and 70% ethanol beside the 100% ethanol jar.

2.2. Then, stain the section with Sirius red and dehydrate the slide **[1] [2]**.

2.2.1. Image of the slides in a staining dish with the staining solution which is a dark color

2.2.2. TEXT ON 2.2.1 IMAGE'S BACKGROUND:

Staining: Sirius red (direct red 80) 1 g/L in picric acid 1.3%, 1 h

Counterstaining (if required): Fast green (1 g/L) + staining solution

[Alternatively, stain nuclei with Weigert's hematoxylin]

Dehydration: 100% EtOH, 5 min, 2x

Clearing: Xylene or Citrisolv (until mounting)

2.3. Remove one slide at a time from the clearing solution **[1]** and blot it on paper towel to remove excess liquid **[2]**.

2.3.1. Talent removing a single slide from the clearing dish with forceps.

2.3.2. Talent gently blotting the slide on folded paper towels.

2.4. Add approximately 50 to 100 microliters of mounting medium to the section **[1]**. Cover the section with a coverslip and gently press it down to remove air bubbles **[2]**. Let the

slide air dry **[3]**.

2.4.1. Talent pipetting mounting medium onto the tissue section.

2.4.2. Talent placing a coverslip on the slide and pressing it gently.

2.4.3. Talent placing the slide on a rack to air dry.

2.5. Now, observe the prepared section under a light microscope with brightfield illumination using 4x to 20x objectives **[1-TXT]**.

2.5.1. Talent placing the slide under a light microscope. **TXT: Perform polarized light microscopy and morphometric quantification**

3. Liver Hydroxyproline Quantification

3.1. Transfer approximately 500 to 1000 microliters of liver homogenate to 1.5-milliliter centrifuge tubes **[1]** and add trichloroacetic acid to reach a final concentration of 12 percent **[2]**. Vortex the tube briefly to mix the contents **[3]**.

3.1.1. Talent pipetting homogenate into labeled centrifuge tube.

3.1.2. Talent adding trichloroacetic acid into the tube under a fume hood.

3.1.3. Talent placing tubes in a vortex mixer and briefly mixing.

3.2. Incubate the tube in an ice-water bath for 30 minutes **[1]** and centrifuge it at 6,000 *g* at 4 degrees Celsius for 10 minutes **[2]**. Then, remove the supernatant with a pipette while keeping the tube on ice **[3]**.

3.2.1. Talent placing tube in an ice bath.

3.2.2. Talent transferring tube into centrifuge and closing the lid.

3.2.3. Talent using a pipette to aspirate the supernatant while tube is on ice.

3.3. Add 1 milliliter of 100% ice-cold ethanol to wash the pellet **[1]** and sonicate it to resuspend thoroughly **[2]**. After centrifuging the suspension again, aspirate the supernatant **[3-TXT]**.

3.3.1. Talent adding ice-cold ethanol to the pellet.

3.3.2. Talent placing the tube under a sonicator.

3.3.3. Talent aspirating the supernatant. **TXT: Repeat the EtOH wash step 2x more**

3.4. Following the third wash, keep the tube open to air dry the pellet for 10 minutes or longer as necessary **[1]**.

3.4.1. Talent placing tube open on a rack to air dry.

3.5. Then, add 800 microliters of 6 normal hydrochloric acid to the tube containing the protein pellet **[1]** and resuspend the pellet using a sonicator as demonstrated earlier **[2]**. Transfer the suspension to a screw-capped glass tube or vial and tightly seal the cap **[3]**. Incubate the tube in an oven at 110 degrees Celsius for about 22 hours **[4]**.

3.5.1. Talent pipetting hydrochloric acid into the tube under a fume hood.

3.5.2. Talent placing the tube under a sonicator.

3.5.3. Talent transferring content to screw-cap vial and sealing it.

3.5.4. Talent placing the vial in a temperature-controlled oven.

3.6. If needed, transfer the contents from the glass vial to a centrifuge tube before spinning **[1].** Centrifuge the hydrolyzed sample at 16,000 *g* for 10 minutes **[2]** and aspirate the supernatant containing hydroxyproline into 1.5-milliliter tube **[3]**. ~~The hydrolysates may be stored at 4 degrees Celsius at this stage **[4]**~~.

3.6.1. Talent transferring contents from glass to centrifuge tube.

3.6.2. Talent placing the sample in a centrifuge.

3.6.3. Talent aspirating and transferring clear supernatant into clean 1.5 ml tube. **TXT: The hydrolysates may be stored at 4 °C at this stage**

~~3.6.4. Talent placing the tube with hydrolysate in a 4 °C refrigerator.~~ **NOTE:** deleted, VO moved as on screen text

3.7. For hydroxyproline oxidation, add 450 microliters of chloramine solution to the tubes with sample and standards **[1]**. Mix the contents in the tubes with a vortex **[2]** and incubate them at room temperature for 25 minutes **[3]**.

3.7.1. Talent pipetting chloramine solution into tube labeled sample and standard.

3.7.2. Talent vortexing the tube briefly.

3.7.3. Talent placing tubes on bench timer during incubation.

3.8. Then, add 500 microliters of Ehrlich's reagent to each sample and standard **[1]**. After vortexing, incubate the samples at 65 degrees Celsius for 20 minutes in an incubator **[2]**. After incubation, let the tubes cool to room temperature **[3]** and observe the final

colour of the samples **[4]**. **NOTE:** deleted, VO added for the extra shot

3.8.1. Talent pipetting Ehrlich's reagent to the tubes.

3.8.2. Talent placing tubes in an incubator set at 65 degrees Celsius.

3.8.3. Talent removing tubes and leaving them on the bench.

Added shot 3.8.3 B Shot to show final color of samples

3.9. Finally, measure the absorbance of samples and standards using a spectrophotometer at a wavelength of 550 nanometers **[1]**.

3.9.1. Talent placing the sample in a spectrophotometer.

Results

4. Results

4.1. Collagen staining of liver tissue with picro-sirius red, when observed with brightfield illumination, showed collagen red with a light-orange or yellow background. Collagen deposition corresponding to fibrosis was stained, as well as collagen in the blood vessel walls, the hilum, and the liver capsule **[1]**.

4.1.1. LAB MEDIA: Figure 4A *Video editor: Highlight the top row "Sirius Red (brighfield)"*

4.2. The polarized light microscopy revealed distinct birefringent collagen fibers on a dark background, enhancing visualization of fibrosis **[1]**.

4.2.1. LAB MEDIA: Figure 4A *Video editor: Highlight the second row "Sirius Red (polarized light)"*

4.3. Quantification of liver collagen-positive area in Agouti yellow mice fed with High Fat and Fructose Diet or HFFD showed significantly higher fibrosis **[1]** when compared to the wild type control mice fed with low fat and fructose diet **[2]**.

4.3.1. LAB MEDIA: Figure 4 B and C. *Video editor: Highlight the RED bars labeled "Ay-HFFD"*

4.3.2. LAB MEDIA: Figure 4 B and C. *Video editor: Highlight the BLUE bars labeled "WT-LFFD"*

4.4. Quantification of hepatic hydroxyproline content revealed a significantly higher level in HFFD mice **[1]** compared to the control mice after 16 weeks on the diet **[2]**, confirming increased collagen deposition in fibrotic livers **[3]**.

4.4.1. LAB MEDIA: Figure 5. *Video editor: Highlight the RED bar labeled "Ay-HFFD" "WT-LFFD"*

4.4.2. LAB MEDIA: Figure 5 *Video editor: Highlight the BLUE bar labeled "WT-LFFD"*

4.4.3. LAB MEDIA: Figure 5. *Video editor: Highlight the RED bar labeled "Ay-HFFD" "WT-LFFD"*

- paraffin-embedded

Pronunciation link: https://www.merriam-webster.com/dictionary/paraffin-embedded
IPA: /pəˈræfɪn ɛmˈbɛdɪd/
Phonetic Spelling: puh-RAF-in em-BED-id

- deparaffinization

Pronunciation link: https://www.merriam-webster.com/dictionary/deparaffinization
IPA: /diˌpəˌræfɪnəˈzeɪʃən/
Phonetic Spelling: dee-puh-RAF-in-uh-ZAY-shun

- xylene

Pronunciation link: https://www.merriam-webster.com/dictionary/xylene
IPA: /ˈzaɪˌliːn/
Phonetic Spelling: ZY-leen

- citrisolv

Pronunciation link: No confirmed link found
IPA: /ˌsɪtriˈsɒlv/
Phonetic Spelling: SIH-tree-SOLV

- rehydrate

Pronunciation link: https://www.merriam-webster.com/dictionary/rehydrate
IPA: /ˌriːhaɪˈdreɪt/
Phonetic Spelling: ree-HY-drate

- ethanol

Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
IPA: /ˈɛθəˌnɑːl/
Phonetic Spelling: ETH-uh-nol

- Sirius red

Pronunciation link: https://www.merriam-webster.com/dictionary/Sirius
IPA for “Sirius”: /ˈsɪriəs/
So “Sirius red” IPA: /ˈsɪriəs rɛd/
Phonetic Spelling: SEER-ee-us red

- picric acid

Pronunciation link: https://www.merriam-webster.com/dictionary/picric
IPA for “picric”: /ˈpɪkrɪk/
So “picric acid” IPA: /ˈpɪkrɪk ˈæsɪd/
Phonetic Spelling: PICK-rick AS-id

- hydroxyproline

Pronunciation link: https://www.merriam-webster.com/dictionary/hydroxyproline
IPA: /ˌhaɪdrɒksiˈproʊliːn/
Phonetic Spelling: hy-DROX-ee-PROH-leen

- birefringent

Pronunciation link: https://www.merriam-webster.com/dictionary/birefringent

IPA: /ˌbaɪrɪˈfrɪndʒənt/
Phonetic Spelling: by-ri-FRING-jent

- morphometric

Pronunciation link: No confirmed link found
IPA: /ˌmɔːrfoʊˈmɛtrɪk/
Phonetic Spelling: mor-foh-MET-rik

- spectrophotometer

Pronunciation link: https://www.merriam-webster.com/dictionary/spectrophotometer
IPA: /ˌspɛktroʊfəˈtɒmɪtər/
Phonetic Spelling: SPECK-troh-foh-TOM-uh-ter