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## **Title: Pancreatic Islet Isolation and Purification from Lewis Rats Using Enzymatic Digestion and Density-Gradient Separation**

### **Authors and Affiliations:**

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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**

**Shots :** 2.2.2, 2.2.3, 2.3.1, 2.3.2, 2.4.1, 2.5.1, 2.6.1, 2.6.2, 2.6.3

*Videographer: Please capture all shots labeled SCOPE with a SCOPE kit*

**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? **Yes, 15 feet apart**

**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. **Yes**

### Current Protocol Length

Number of Steps: 28

Number of Shots: 51

# Introduction

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*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. **Henry Seaborne:** Our research focuses on developing a standardized, high-throughput Lewis rat islet isolation protocol to optimize yield, purity, viability, and functional longevity.
  - 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. **Gabrielle Rome:** Existing methods yield variable, impure, short-lived islets. This protocol standardizes digestion, purification, and pooling to improve consistency and longevity.
  - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

## CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Henry Seaborne:** This method enables studies of islet transplantation,  $\beta$ -cell function, diabetes reversal, encapsulation, and islet-immune or microenvironment interactions.
  - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.4. **Elisa Balboni**: This protocol can be applicable to preclinical transplantation, diabetes modeling, encapsulation studies, and in vitro functional assays requiring high-purity Lewis rat islets.

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

**NOTE: Moved 1.4 from Introduction to the Conclusion to stick to the JoVE video format**

*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.*

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your article address?

- 1.5. **Henry Seaborne, Research Assistant**: (authors will present their testimonial statements live)

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

What value do you expect this video article to have for other researchers working in your field?

- 1.6. **Alessandro Grattoni, Principal Investigator/Chair**: (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.

**Ethics Title Card**

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Houston Methodist Hospital

# Protocol

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## 2. Surgical Perfusion and Excision of the Rat Pancreas for Islet Isolation

**Demonstrators:** Henry Seaborne, Gabrielle Rome

- 2.1. To begin, place a euthanized rat under a microscope [1]. Using skin scissors, make a V-shaped incision starting from the pubic symphysis toward each lateral ribcage to access the abdominal cavity [2].
  - 2.1.1. WIDE: Talent placing a euthanized rat under a microscope. **TXT: Euthanasia: Isoflurane overdose**  
**AUTHORS:** Please note that JoVE requires the opening shot to be a wide, non-screen capture. This shot was added specifically to meet that requirement
  - 2.1.2. Shot of skin scissors being used to create a V-shaped incision from the pubic symphysis toward both lateral ribcages, opening the abdominal cavity.
- 2.2. Using flat forceps, retract the small and large intestines to the left side of the animal to clear the abdominal cavity workspace [1]. Then use the flat forceps and curved hemostats to unfurl the duodenum [2]. Ensure the common bile duct and hepatic portal vein are within the field of view [3].
  - 2.2.1. Shot of the small and large intestines being moved to the left side with flat forceps.
  - 2.2.2. ~~SCOPE:~~ The duodenum is being unfolded with flat forceps and curved hemostats. **NOTE: This is not a SCOPE shot**  
**Videographer: Please capture all shots labeled SCOPE with a SCOPE kit**
  - 2.2.3. SCOPE: Show the common bile duct and hepatic portal vein clearly positioned within the microscope field of view.
- 2.3. Now use the curved hemostats to retract the liver in the cephalad direction [1]. Clamp the common bile duct and hepatic vein 1 to 2 millimeters from the liver [2].
  - 2.3.1. SCOPE: Talent using curved hemostats to gently lift and retract the liver toward the head.
  - 2.3.2. SCOPE: Shot of curved hemostats clamping the common bile duct and hepatic vein approximately 1 to 2 millimeters from the liver.
- 2.4. Under the microscope view, with flat forceps and flat hemostats, gently mobilize the contents within the duodenum to clear the Sphincter of Oddi [1].
  - 2.4.1. SCOPE: Show flat forceps and flat hemostats gently manipulating the duodenal contents to expose and clear the Sphincter of Oddi.

- 2.5. Then gently clamp the duodenum approximately 5 millimeters from each side of the Sphincter of Oddi to isolate the surrounding region [1-TXT].
- 2.5.1. SCOPE: Shot of flat hemostats being placed approximately 5 millimeters on either side of the Sphincter of Oddi. **TXT: Ensure minimal compression to preserve tissue integrity**
- 2.6. Using a sterile WDM (*W-D-M*) syringe, carefully insert the needle through the duodenal wall [1-TXT]. Pass the needle through the Sphincter of Oddi and advance it into the main pancreatic duct for perfusion [2]. Perfuse 7 to 10 milliliters of WDM into the pancreas at constant pressure until the pancreas is fully distended [3-TXT].
- 2.6.1. SCOPE: Shot of the sterile WDM syringe needle penetrating the duodenal wall. **TXT: WDM: Working Dissociation Medium NOTE: 2.6.1-2.6.3 are filmed in a single shot**
- 2.6.2. SCOPE: Shot of the needle passing through the Sphincter of Oddi and advancing into the main pancreatic duct.
- ~~2.6.3.~~ SCOPE: Shot of the pancreas visibly distending as WDM is perfused. **TXT: Rate: 500 µL/s**
- 2.7. ~~Then gently dissect and free the pancreas from surrounding structures starting from the spleen, stomach, large intestine, duodenum, and mesentery [1].~~ **NOTE: This step is moved after 2.8.1**
- 2.7.1. ~~Talent sequentially dissecting the pancreas away from the spleen, stomach, large intestine, duodenum, and mesentery.~~
- 2.8. Now, remove both flat hemostats from the duodenum [1]. ~~Using flat forceps, clamp the splenopancreatic ligament and apply gentle traction [2]. Then use a pair of fine curved dissecting forceps to carefully mobilize and separate the pancreas from adjacent splenic attachments [3].~~
- 2.8.1. Talent removing both flat hemostats from the duodenum.
- 2.8.2. ~~Talent using flat forceps to clamp the splenopancreatic ligament and apply gentle traction.~~ **NOTE: 2.8.2 and 2.8.3 are not filmed**
- ~~2.8.3. Shot of fine curved dissecting forceps being used to separate the pancreas from splenic attachments.~~
- 2.9. ~~Lift the pancreatic tissue with a pair of fine forceps [1] and apply gentle counter traction with flat forceps to detach it from the stomach [2]. Apply firm traction to avulse the large intestine from the pancreas [3].~~ **NOTE: 2.9 is not filmed**
- ~~2.9.1. Talent lifting pancreatic tissue with fine curved forceps.~~

~~2.9.2. Counter traction is being applied with flat forceps to detach it from the stomach.~~

~~2.9.3. Shot of flat forceps being used to apply firm traction to separate the large intestine from the pancreas.~~

2.7. Then gently dissect and free the pancreas from surrounding structures, starting from the spleen, stomach, large intestine, duodenum, and mesentery [1].

2.7.1 Talent sequentially dissecting the pancreas away from the spleen, stomach, large intestine, duodenum, and mesentery.

2.10. ~~Next, lift the small intestine with the flat forceps [1].~~ Dissect the mesentery from the pancreas with fine curved forceps [2]. Gently lift the perfused pancreas with flat forceps [1] and use fine dissecting scissors to sever any remaining connective tissue attachments [2].

2.10.1. ~~Talent lifting the small intestine with flat forceps.~~ NOTE: 2.10.1 and 2.10.2 are not filmed

~~2.10.2. Talent dissecting the mesentery from the pancreas using fine curved forceps.~~

2.10.3. Shot of the perfused pancreas being lifted with flat forceps. NOTE: 2.10.3 and 2.10.4 are filmed in a single shot

2.10.4. Talent using fine dissecting scissors to cut remaining connective tissue.

2.11. Immediately transfer the excised pancreas into a Petri dish containing ice-cold rinse solution [1-TXT].

2.11.1. Talent placing the excised pancreas into a Petri dish filled with ice-cold rinse solution. **TXT: Harvest the next pancreas while the excised pancreas are being cleaned**

2.12. To clean the pancreases, use a pair of fine curved forceps and fine dissecting scissors to carefully remove lymph nodes, adipose tissue, mesentery, and large vasculature from the perfused pancreas if present [1].

2.12.1. Talent trimming away lymph nodes, adipose tissue, mesentery, and large blood vessels from the perfused pancreas.

2.13. Transfer the cleaned pancreas into a sterile 600-milliliter beaker containing 25 milliliters of cold WDM [1]. Cover the beaker with sterile aluminum foil to maintain cleanliness during transfer from the surgical workspace to the cell culture workspace [2].

2.13.1. Talent placing the cleaned pancreas into a sterile 600 milliliter beaker containing 25 milliliters of cold WDM. NOTE: 2.13.1. and 2.13.2 are filmed in a single shot

2.13.2. Shot of sterile aluminum foil covering the beaker securely.

2.14. Secure the covered beaker into the designated holders of a water bath [1]. Transfer the beaker into an orbital shaker set at 130 revolutions per minute for 10 minutes [2]. ~~Start~~

~~a 10-minute timer for digestion [3].~~ NOTE: The VO is edited for the deleted shot

2.14.1. Talent placing the covered beaker into the water bath holder. NOTE: 2.14.1. and 2.14.2 are filmed in a single shot

2.14.2. Talent placing the beaker into an orbital shaker.

~~2.14.3. Shot of a timer being set to 10 minutes~~ NOTE: Not filmed

2.15. At the 5-minute mark, manually swirl the beaker clockwise and then counterclockwise to aid tissue dissociation [1]. At the 10-minute mark, rapidly transfer the beaker and submerge it in ice to slow enzyme activity [2].

2.15.1. Talent gently swirling the beaker clockwise and then counterclockwise by hand.

2.15.2. Talent removing the beaker from the shaker and placing it into an ice container.

2.16. Then, add an equal volume of quenching buffer to the solution in the beaker to stop the digestion [1]. Using a 50-milliliter syringe fitted with a 14-gauge spinal needle, gently aspirate and expel the tissue suspension repeatedly to dissociate large tissue fragments [2].

2.16.1. Shot of quenching buffer being poured into the beaker at a 1 to 1 ratio.

2.16.2. Talent aspirating and expelling the tissue suspension through a 14 G spinal needle.

2.17. Transfer the suspension into 200 milliliter conical tubes [1]. ~~Then centrifuge the suspension at 200 g for 5 minutes at 4 degrees Celsius [2-TXT].~~ NOTE: The VO is edited for the deleted shot, and the information is added as an onscreen text in the next step

2.17.1. Talent pouring the tissue suspension into labeled 200 milliliter conical tubes.

~~2.17.2. Talent placing the 200 milliliter conical tubes into the centrifuge. TXT: Ensure appropriate bottle adapters and cushions are used for safety~~ NOTE: Not filmed

2.18. After centrifuging the suspension, carefully decant the supernatant [1-TXT] and resuspend the pellet in 100 milliliters of washing media [2].

2.18.1. Shot of supernatant being carefully poured off from the tube. TXT: Centrifugation: 200 g, 5 min, 4 °C

2.18.2. Talent gently resuspending the pellet in washing media using the 14 gauge spinal needle.

2.19. Now filter the suspension through a sterile 500-micrometer sieve placed over a sterile 600-milliliter beaker to remove large undigested tissue [1].

2.19.1. Shot of the suspension being poured through a 500 micrometer sieve positioned over a 600 milliliter beaker.

2.20. Transfer the filtrate into new 200-milliliter conical tubes before centrifuging again [1].

2.20.1. Talent pouring the filtered suspension into clean 200 milliliter conical tubes.

### 3. Density Gradient Purification and Collection of Rat Pancreatic Islets

**Demonstrator:** Gabrielle Rome

- 3.1. For islet purification, use a pre-wetted 10 milliliter serological pipette to resuspend each tissue pellet in 15 milliliters of gradient solution [1-TXT]. Mix gently until the suspension is uniform [2].
  - 3.1.1. Talent using a pre-wetted 10 milliliter serological pipette to resuspend pellet in 15 mL gradient solution. **TXT: Gradient solution: 1.108 g/cm<sup>3</sup> gradient solution per 2 mL of pellet volume** **NOTE: 3.1.1. and 3.1.2 are filmed in a single shot**
  - 3.1.2. Talent gently pipetting up and down to achieve a uniform suspension.
- 3.2. Carefully dispense 15 milliliters of the cell suspension into the bottom of each 50-milliliter conical tube [1-TXT].
  - 3.2.1. Shot of the pipette tip positioned at the bottom of a 50 milliliter conical tube dispensing the suspension without touching the walls. **TXT: Avoid contact with tube walls to ensure clean gradients**
- 3.3. ~~Now, use preloaded gradient syringes, to sequentially overlay 10 milliliters of each gradient solution [1-TXT].~~ **NOTE: Not filmed**
  - 3.3.1. ~~Talent layering 10 mL of each gradient solution over each other.~~
- 3.4. Then use a syringe pump to dispense each gradient at a rate of 5 milliliters per minute [1] while positioning the 16-gauge needle tip against the inner wall of the tube to prevent mixing [2].
  - 3.4.1. Shot of the syringe pump delivering gradient solution at 5 milliliters per minute. **TXT: Solution 1: 1.096 g/cm<sup>3</sup>; Solution 2: 1.069 g/cm<sup>3</sup>** **NOTE: 3.4.1 and 3.4.2 are filmed in a single shot**
  - 3.4.2. Shot of the 16 gauge needle tip resting against the inner tube wall.
- 3.5. ~~Centrifuge the tubes at 700 g for 15 minutes at 22 degrees Celsius with acceleration set to 3 and deceleration set to 0 to prevent gradient mixing [1].~~ **NOTE: Not filmed, the information is added concisely in the next step**
  - 3.5.1. ~~Talent placing the gradient tubes into the centrifuge and setting acceleration to 3 and deceleration to 0 before starting the run.~~
- 3.6. After centrifuging the tubes, identify the islet layer located at the interfaces between the gradient layers [1].
  - 3.6.1. Talent pointing to the islet layer at the gradient interface.
- 3.7. Using a wetted Pasteur pipette, collect the islet layer [1]. Transfer the collected islets into a pre-chilled 200-milliliter conical tube containing 5 milliliters of washing media [2].

- 3.7.1. Talent using a wetted Pasteur pipette to aspirate the islet layer from the gradient interface. **NOTE: 3.7.1 and 3.7.2 are filmed in a single shot**
- 3.7.2. Talent dispensing the collected islets into a pre-chilled 200 milliliter conical tube containing washing media.
- 3.8. Then evenly plate the islets into non-tissue-culture-treated 100-millimeter Petri dishes **[1-TXT]**.
  - 3.8.1. Talent distributing islets evenly into non tissue culture treated 100 millimeter Petri dishes arranged on the bench. **TXT: Use 1 plate for every 5 rat pancreases processed**

## Results

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### 4. Results

- 4.1. Across the test isolation, yield was 3330 islet equivalents per pancreas with a median islet diameter in the 50–100 micrometer bin [1].
  - 4.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the tallest bar*
- 4.2. Automated sizing yielded 98,832 total objects [1], which strongly correlated with manual islet equivalents measured at Day 0 [2].
  - 4.2.1. LAB MEDIA: Figure 4B.
  - 4.2.2. LAB MEDIA: Figure 4C.
- 4.3. Object counts changed by 0.9% and islet equivalents by minus 6.11% from 2 hours to Day 1 [1].
  - 4.3.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the plot for D0 and then D1*
- 4.4. The endocrine purity was about 98.56 % based on dithizone staining at Day 1 [1].
  - 4.4.1. LAB MEDIA: Figure 5A.
- 4.5. Stimulation indices were 9.37 at Day 1 [1], 8.34 at Day 3 [2], and 6.7 at Day 7 with no significant differences between time points [3].
  - 4.5.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the bar labeled Day 1 in the stimulation index graph.*
  - 4.5.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the bar labeled Day 3 in the stimulation index graph.*
  - 4.5.3. LAB MEDIA: Figure 5B. *Video editor: Highlight the bar labeled Day 7 in the stimulation index graph.*

NOTE 032926: Step 4.6 has been deleted as the values mentioned in narration were not visible from the images.