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Title: Isolation, Purification, and Characterization of Functional Mitochondria Derived From Mouse Skeletal Muscle for Mitochondrial Transplantation

Authors and Affiliations:

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☒ Correct

☐ Incorrect

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **06/24/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 26

Number of Shots: 57

Interviews

Authors: *Answers are slightly edited in accordance with journal guidelines. 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. **Taiqiang Dai, Lu Zhao:** Our study focuses on developing a standard muscle mitochondrial isolation method verified for mitochondrial transplantation.
 - 1.1.1. 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.**~~

- 1.2. **Taiqiang Dai, Lu Zhao:** This protocol can be applied in mitochondrial transplantation and related disease research.
 - 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. **Taiqiang Dai, Lu Zhao:** This method allows researchers to detect mitochondrial purity and study mitochondrial structure, function, and cellular uptake capacity for transplantation.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.4. **Taiqiang Dai, Lu Zhao:** The greatest challenge in this protocol is isolating muscle mitochondria under low temperature, controlling digestion, and avoiding over-grinding to retain activity.
 - 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. **Taiqiang Dai, Lu Zhao:** Building on this protocol, future studies may engineer mitochondria to improve targeting, prolong functional activity, and enhance delivery for multi-organ injury repair.
 - 1.5.1. 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 Animal Ethics and Use Committee at Third Affiliated Hospital of Air Force Medical University

Protocol

2. Isolation of Skeletal Muscle Tissue

Demonstrator: Ningning Da

Authors: During filming, please point out the organs or veins before dissecting them

- 2.1. To begin, obtain a euthanized mouse [1]. In a laminar flow hood, place the mouse in a sterile Petri dish with the abdomen facing upward to fully expose the abdomen and hindlimbs [2].
 - 2.1.1. WIDE: Talent sitting with a euthanized mouse placed on the workbench. **TXT: Mice Strain: C57BL/6; Euthanasia: CO₂ inhalation**
 - 2.1.2. Talent placing the mouse into a sterile Petri dish within a laminar flow hood with the abdomen facing upward.
- 2.2. Using forceps, lift the skin of the lower abdomen near the genital region [1]. Make a small incision [2]. Perform blunt dissection to separate the skin from the underlying tissue [3]. Extend the incision laterally toward both hindlimbs to completely expose the hindlimb muscle groups [4].
 - 2.2.1. Close-up shot of the skin of the lower abdomen near the genital region being lifted with forceps.
 - 2.2.2. Close-up shot of small incision being performed.
 - 2.2.3. Close-up shot of blunt dissection being performed to separate the skin from the underlying tissue.
 - 2.2.4. Close-up shot of incision being extended laterally toward both hindlimbs to expose the hindlimb muscle groups.
- 2.3. Identify the quadriceps femoris muscle on the anterior aspect of the thigh [1]. Perform blunt dissection along the direction of the muscle fibers to carefully separate the muscle [2]. Sever the distal patellar ligament [3]. Then, sever the proximal tendon to completely isolate the muscle tissue [4].
 - 2.3.1. Show the quadriceps femoris muscle on the anterior aspect of the thigh. **Authors: Point out the quadriceps femoris muscle**
 - 2.3.2. Close-up shot of blunt dissection being performed along the muscle fibers to separate the quadriceps femoris muscle.
 - 2.3.3. Close-up shot of severing the distal patellar ligament.
 - 2.3.4. Close-up shot of severing the proximal tendon to isolate the muscle tissue.
- 2.4. Place the isolated muscle tissue immediately into pre-chilled PBS [1]. Rinse the tissue

thoroughly to remove hair and blood from the tissue surface [2].

2.4.1. Shot of placing the isolated muscle tissue into pre-chilled PBS.

2.4.2. Shot of the muscle tissue being rinsed in PBS to remove hair and blood from the tissue surface.

2.5. Using sterile gauze, gently blot excess moisture from the tissue [1]. Divide the tissue into 1.5 milliliter microcentrifuge tubes [2] and weigh the samples [3].

2.5.1. Shot of blotting excess moisture from the tissue with sterile gauze.

2.5.2. Talent dividing and placing the tissue into 1.5 milliliter microcentrifuge tubes.

2.5.3. Talent weighing the samples.

3. Isolation and Purification of Skeletal Muscle Mitochondria

Demonstrator: Ningning Da

3.1. Place the microcentrifuge tube containing tissue on ice [1]. Using sterile scissors, mince the tissue into small fragments approximately 1 to 2 cubic millimeters in size [2].

3.1.1. Talent placing the 1.5 milliliter microcentrifuge tube containing the tissue on ice.

3.1.2. Talent mincing the tissue into small fragments with sterile scissors.

3.2. Add 8 volumes of pre-chilled 0.25 percent trypsin to the tissue fragments [1] and digest on ice for 15 minutes [2].

3.2.1. Talent adding pre-chilled 0.25 percent trypsin to the tissue fragments.

3.2.2. Talent placing the sample on ice for digestion.

3.3. Centrifuge the mixture at 1,000 g for 5 minutes at 4 degrees Celsius [1]. Discard the supernatant and retain the tissue pellet [2].

3.3.1. Talent placing the tube into the centrifuge.

3.3.2. Talent discarding the supernatant and retaining the tissue pellet.

3.4. Transfer the suspension to a glass homogenizer maintained on ice [1]. Homogenize the tissue using 10 to 12 gentle up-and-down strokes until the suspension becomes turbid [2].

3.4.1. Talent transferring the suspension to a glass homogenizer maintained on ice.

3.4.2. Talent homogenizing the tissue with gentle up-and-down strokes.

3.5. Collect the homogenate into a microcentrifuge tube [1]. Centrifuge at 1,000 g for 5 minutes at 4 degrees Celsius [2].

- 3.5.1. Talent transferring the homogenate into a 1.5 milliliter microcentrifuge tube.
- 3.5.2. Talent placing the microcentrifuge tube into the centrifuge.
- 3.6. After transferring the supernatant to a fresh 1.5 milliliter microcentrifuge tube, centrifuge at 12,000 g for 5 minutes at 4 degrees Celsius to obtain the crude mitochondrial pellet [1]. Gently resuspend the crude mitochondrial pellet in pre-chilled wash buffer [2]. Centrifuge the suspension at 1,000 g for 5 minutes at 4 degrees Celsius [3].
 - 3.6.1. Talent placing the microcentrifuge tube into the centrifuge to obtain the crude mitochondrial pellet.
 - 3.6.2. Close-up shot of gently resuspending the crude mitochondrial pellet in the pre-chilled wash buffer.
 - 3.6.3. Talent placing the suspension into the centrifuge.
- 3.7. Transfer the supernatant to a fresh centrifuge tube [1]. Centrifuge at 12,000 g for 10 minutes at 4 degrees Celsius to obtain the purified mitochondrial pellet [2].
 - 3.7.1. Talent transferring the supernatant to a fresh centrifuge tube.
 - 3.7.2. Talent placing the centrifuge tube into the centrifuge.

4. Assessment of Mitochondrial Respiratory Function

Demonstrator: Jing Wang

- 4.1. Clean the respiratory chambers, electrode compartments, and stoppers [1].
 - 4.1.1. Talent cleaning the respiratory chambers, electrode compartments, and stoppers.
- 4.2. Add 2.1 milliliters of mitochondrial respiration buffer into each respiratory chamber [1]. Ensure that no air bubbles are present in the chambers [2].
 - 4.2.1. Talent adding mitochondrial respiration buffer into each respiratory chamber.
 - 4.2.2. Shot of the respiratory chamber showing buffer without air bubbles.
- 4.3. Equilibrate the high-resolution respirometry system at 37 degrees Celsius for 30 minutes [1] and perform daily air calibration after stabilization of the oxygen baseline [2].
 - 4.3.1. Shot of the high-resolution respirometry system during equilibration at 37 degrees Celsius.
 - 4.3.2. SCREEN: 4.3.2

Videographer: Please record the computer screen for the shots labeled as SCREEN as back-up

4.4. Add freshly isolated mitochondria containing approximately 30 micrograms of protein into the respiratory chamber [1]. Seal the chamber [2].

4.4.1. Talent adding freshly isolated mitochondria into the respiratory chamber.

4.4.2. Talent sealing the respiratory chamber.

4.5. After equilibrating the chamber for 5 to 8 minutes, record basal oxygen consumption [1].

4.5.1. SCREEN: 4.5.1

4.6. In the respiratory chamber [1], add the reagents sequentially [2]. Stabilize the reaction for 5 minutes, then record complex I or CI (*C-one*) leak respiration [3].

4.6.1. Shot of the respiratory chamber.

4.6.2. TEXT ON A PLAIN BACKGROUND:

CI substrates

2 M pyruvate - 5 μ L

2 M glutamate - 10 μ L

400 mM malate - 10 μ L

4.6.3. SCREEN: 4.6.3

4.7. Add 10 microliters of 500 millimolar adenosine diphosphate or ADP (*A-D-P*) to induce oxidative phosphorylation [1] and record CI oxidative phosphorylation or OXPHOS (*Ox-phos*) respiration [2]. Add 10 microliters of 0.3 molar magnesium chloride [3].

4.7.1. Talent adding adenosine diphosphate to the respiratory chamber.

4.7.2. SCREEN: 4.7.2

4.7.3. Talent adding magnesium chloride to the respiratory chamber.

4.8. Introduce 20 microliters of 1 molar succinate [1]. Record CI and CII OXPHOS after stabilization [2].

4.8.1. Talent adding succinate to the respiratory chamber.

4.8.2. SCREEN: 4.8.2

4.9. Add 2 microliters of 0.01 millimolar adenosine triphosphate or ATP (*A-T-P*) synthase inhibitor [1]. Record proton leak respiration [2].

4.9.1. Talent adding adenosine triphosphate synthase inhibitor to the respiratory chamber.

4.9.2. SCREEN: 4.9.2

4.10. Titrate 1 millimolar carbonyl cyanide m-chlorophenyl hydrazone or CCCP (*C-C-C-P*) in three incremental additions of 2 microliters at 2-minute intervals until oxygen consumption reaches a plateau [1]. Record maximal electron transfer system capacity [2].

4.10.1. Talent adding CCCP incrementally to the respiratory chamber.

4.10.2. SCREEN: 4.10.2

4.11. Add 1 microliter of 1 millimolar rotenone to inhibit CI activity [1].

4.11.1. Talent adding rotenone to the respiratory chamber.

4.12. Then, add 1 microliter of 5 millimolar antimycin A to inhibit CIII activity [1]. Record residual oxygen consumption [2].

4.12.1. Talent adding antimycin A to the respiratory chamber.

4.12.2. SCREEN: 4.12.2

4.13. Introduce 5 microliters of 800 millimolar ascorbate and 5 microliters of 200 millimolar N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride or TMPD (*T-M-P-D*) [1]. Record maximal CIV respiratory activity after stabilization [2].

4.13.1. Talent adding ascorbate and TMPD to the respiratory chamber.

4.13.2. SCREEN: 4.13.2

4.14. After obtaining the mitochondrial respiration parameters, calculate the respiratory control ratio [1].

4.14.1. SCREEN: 4.14.1

NOTE: Please contact the writer and PS integrator after video assembly and uploading on frame io. We need to add a comment for the authors to highlight the areas in the graphs of screen recordings

Results

5. Results

- 5.1. Mitochondria isolated using both trypsin digestion or TD (*T-D*) and normal extraction or NE (*N-E*) methods retained functional integrity [1]. The NE method exhibited respiratory control ratio or RCR (*R-C-R*) values of approximately 8 to 9 [2], whereas the TD method exhibited RCR values of approximately 9 to 10, indicating better preservation of mitochondrial respiratory function [3].

5.1.1. LAB MEDIA: Figure 5A.

5.1.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the light orange bar labelled 'NE' in the graph titled 'RCR'.*

5.1.3. LAB MEDIA: Figure 5A. *Video editor: Highlight the black bar labelled 'TD' in the graph titled 'RCR'.*

- 5.2. Both TD [1] and NE groups exhibited biochemical coupling efficiencies approaching 1 [2], indicating that mitochondrial bioenergetic function was preserved following isolation [3].

5.2.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the black bar labelled 'TD' in the graph titled 'ATP'.*

5.2.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the light orange bar labelled 'NE' in the graph titled 'ATP'.*

5.2.3. LAB MEDIA: Figure 5B.

1. Euthanized

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

IPA: /'ju:θəˌnaɪzd/

Phonetic Spelling: YOO·thuh·nyzd

2. Laminar

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

IPA: /'læməˌnær/

Phonetic Spelling: LAM·uh·ner

3. Blunt dissection
Pronunciation link: No confirmed link found
IPA: /blʌnt dɪ'sekʃən/
Phonetic Spelling: blunt dih·SEK·shuhn
4. Quadriceps
Pronunciation link: <https://www.merriam-webster.com/dictionary/quadriceps>
IPA: /'kwɑːdrəˌseps/
Phonetic Spelling: KWAH·druh·seps
5. Femoris
Pronunciation link: No confirmed link found
IPA: /fi'mɔːrɪs/
Phonetic Spelling: fih·MOR·iss
6. Patellar
Pronunciation link: <https://www.merriam-webster.com/dictionary/patellar>
IPA: /pə'telər/
Phonetic Spelling: puh·TEL·er
7. Microcentrifuge
Pronunciation link: No confirmed link found
IPA: /ˌmaɪkroʊ'sentrɪˌfjuːdʒ/
Phonetic Spelling: my·kroh·SEN·trih·fyooj
8. Mitochondria
Pronunciation link: <https://www.merriam-webster.com/dictionary/mitochondria>
IPA: /ˌmaɪtə'kɑːndriə/
Phonetic Spelling: my·tuh·KAHN·dree·uh
9. Mitochondrial
Pronunciation link: <https://www.merriam-webster.com/dictionary/mitochondrial>
IPA: /ˌmaɪtə'kɑːndriəl/
Phonetic Spelling: my·tuh·KAHN·dree·uhl
10. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /'trɪpsɪn/
Phonetic Spelling: TRIP·sin
11. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuːpər'neɪtənt/
Phonetic Spelling: soo·per·NAY·tuht
12. Homogenizer
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenizer>
IPA: /hə'mɑːdʒəˌnaɪzər/
Phonetic Spelling: huh·MAH·juh·ny·zer
13. Homogenate
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenate>
IPA: /hə'mɑːdʒəˌneɪt/
Phonetic Spelling: huh·MAH·juh·nayt

14. Respirometry
 Pronunciation link: No confirmed link found
 IPA: /ˌrɛspəˈrɑːmətri/
 Phonetic Spelling: res·puh·RAH·muh·tree
15. Equilibrate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
 IPA: /ɪˈkwɪləˌbreɪt/
 Phonetic Spelling: ih·KWIL·uh·brayt
16. Pyruvate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/pyruvate>
 IPA: /paɪˈruːˌveɪt/
 Phonetic Spelling: py·ROO·vayt
17. Glutamate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/glutamate>
 IPA: /ˈgluːtəˌmeɪt/
 Phonetic Spelling: GLOO·tuh·mayt
18. Malate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/malate>
 IPA: /ˈmæləˌteɪt/, /ˈmeɪləˌteɪt/
 Phonetic Spelling: MAL·uh·tayt / MAY·luh·tayt
19. Adenosine
 Pronunciation link: <https://www.merriam-webster.com/dictionary/adenosine>
 IPA: /əˈdɛnəˌsiːn/, /əˈdɛnəsən/
 Phonetic Spelling: uh·DEN·uh·seen / uh·DEN·uh·suhn
20. Adenosine diphosphate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/adenosine%20diphosphate>
 IPA: /əˈdɛnəˌsiːn daɪˈfəːsˌfeɪt/
 Phonetic Spelling: uh·DEN·uh·seen dy·FOSS·fayt
21. Oxidative phosphorylation
 Pronunciation link: No confirmed link found
 IPA: /ˈɑːksəˌdeɪtɪv ˌfɑːsfərɪˈleɪʃən/
 Phonetic Spelling: OK·suh·day·tiv foss·fer·uh·LAY·shuhn
22. Succinate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/succinate>
 IPA: /ˈsʌksəˌneɪt/
 Phonetic Spelling: SUK·suh·nayt
23. Adenosine triphosphate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/adenosine%20triphosphate>
 IPA: /əˈdɛnəˌsiːn traɪˈfəːsˌfeɪt/
 Phonetic Spelling: uh·DEN·uh·seen try·FOSS·fayt
24. Synthase
 Pronunciation link: <https://www.merriam-webster.com/dictionary/synthase>

- IPA: /'sɪn θeɪs/
Phonetic Spelling: SIN·thays
25. Carbonyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/carbonyl>
IPA: /'kɑːrbəˌnɪl/
Phonetic Spelling: KAR·buh·nɪl
26. Cyanide
Pronunciation link: <https://www.merriam-webster.com/dictionary/cyanide>
IPA: /'saɪəˌnaɪd/
Phonetic Spelling: SY·uh·nyd
27. m-Chlorophenyl
Pronunciation link: No confirmed link found
IPA: /ˌmɛtə ˌklɔːroʊˈfɛnəl/
Phonetic Spelling: MET·uh klor·oh·FEN·uhl
28. Hydrazone
Pronunciation link: No confirmed link found
IPA: /'haɪdrəˌzoʊn/
Phonetic Spelling: HY·druh·zohn
29. Rotenone
Pronunciation link: <https://www.merriam-webster.com/dictionary/rotenone>
IPA: /'roʊtəˌnoʊn/
Phonetic Spelling: ROH·tuh·nohn
30. Antimycin
Pronunciation link: No confirmed link found
IPA: /ˌæntɪˈmaɪsɪn/
Phonetic Spelling: an·tih·MY·sin
31. Ascorbate
Pronunciation link: <https://www.merriam-webster.com/dictionary/ascorbate>
IPA: /əˈskɔːrˌbeɪt/
Phonetic Spelling: uh·SKOR·bayt
32. N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride
Pronunciation link: No confirmed link found
IPA: /ˌtɛtrəˈmɛθəl ˌpɛrə ˌfɛnələnˈdaɪəmiːn ˌdaɪhaɪdrəˈklɔːraɪd/
Phonetic Spelling: tet·ruh·METH·uhl pair·uh fen·uh·leen·DY·uh·meen
dy·hy·druh·KLOR·yd
33. Biochemical
Pronunciation link: <https://www.merriam-webster.com/dictionary/biochemical>
IPA: /ˌbaɪoʊˈkɛmɪkəl/
Phonetic Spelling: by·oh·KEM·ih·kuhl
34. Bioenergetic
Pronunciation link: <https://www.merriam-webster.com/dictionary/bioenergetic>
IPA: /ˌbaɪoʊˌɛnərˈdʒɛtɪk/
Phonetic Spelling: by·oh·en·er·JET·ik