

Submission ID #: 70459

Scriptwriter Name: Sri Lakshmi Priya

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Title: Assessing the Metabolic Activity of Whole Regenerating Zebrafish Ventricles *Ex Vivo* Using an Extracellular Flux Assay

Authors and Affiliations:

Konstantinos Lekkos^{1,2}, Rita Alonaizan^{1,2,3}, John Walsby-Tickle⁴, James S.O. McCullagh⁴, Lisa C. Heather¹, Mathilda T.M. Mommersteeg^{1,2}

¹Department of Physiology, Anatomy and Genetics, University of Oxford

²Institute of Developmental and Regenerative Medicine, University of Oxford

³King Faisal Specialist Hospital & Research Centre

⁴Department of Chemistry, University of Oxford

Corresponding Authors:

Konstantinos Lekkos (konstantinos.lekkos@sjc.ox.ac.uk)

Mathilda T.M. Mommersteeg (mathilda.mommersteeg@dpag.ox.ac.uk)

Email Addresses for All Authors:

Konstantinos Lekkos (konstantinos.lekkos@sjc.ox.ac.uk)

Rita Alonaizan (rita.al-onaihan@hotmail.com)

John Walsby-Tickle (john.walsby-tickle@chem.ox.ac.uk)

James S.O. McCullagh (james.mccullagh@chem.ox.ac.uk)

Lisa C. Heather (lisa.heather@dpag.ox.ac.uk)

Mathilda T.M. Mommersteeg (mathilda.mommersteeg@dpag.ox.ac.uk)

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? **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: 16

Number of Shots: 36

Interviews

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

INTRODUCTION:

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

- 1.1. **Konstantinos Lekkos:** The Seahorse assay allows for the high-throughput assessment of metabolism in intact zebrafish ventricles ex vivo.
 - 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. **Konstantinos Lekkos:** This method can be used to validate genetic perturbations of metabolic genes and characterize changes in metabolism during regeneration.
 - 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. **Konstantinos Lekkos:** Using this protocol, oxygen consumption and extracellular acidification rates of zebrafish hearts can be measured ex vivo.
 - 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. **Konstantinos Lekkos:** ECAR data reflects not only glycolysis-derived lactate but also carbon dioxide from respiration, both influencing the acidification rate.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer's NOTE: The headshots were done outside. The meeting room the authors booked was too dark and I didn't enough time to set up my light. This was because I was told two Authors had to leave for a meeting very soon. Doing the headshots outside was the quickest option I had available.

The introduction was filmed in the meeting room the Authors booked as Konstantinos was not in a rush and I had time to set up my light.

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

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

NOTE: SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi Priya) for queries regarding lab media.

2. Setting up the Extracellular Flux Analyzer and Mito Stress Test

Demonstrator: Konstantinos Lekkos

- 2.1 Turn on the system and prepare all drug solutions and media prior to beginning the experiment [1]. On the **Group definitions** page in Wave software, under the **Injection strategies** tab, assign oligomycin to port A [2], carbonyl cyanide 4 trifluoromethoxy phenylhydrazone or FCCP (*F-C-C-P*) to port B [3], and the rotenone and antimycin mix to port C from the **compound catalogue** [4].

2.1.1 WIDE: Talent sitting next to the Seahorse analyzer and prepared solutions.

Videographer's NOTE: This shot was tweaked slightly. The author told me the solutions were done already. I recorded the author doing something else (based on his guidance).

2.1.2 SCREEN: 70459_2.1.2-2.1.4_new.mp4: 00:08-00:29

2.1.3 SCREEN: 70459_2.1.2-2.1.4_new.mp4: 00:08-00:29

2.1.4 SCREEN: 70459_2.1.2-2.1.4_new.mp4: 00:08-00:29 **NOTE:** 2.1.2, 2.1.3, 2.1.4 are combined in one shot. Show the SC only once.

- 2.2 Delete any pre-existing injection strategies, once the experimental strategy is defined [1]. Then, on the **Groups** panel, define the experimental groups and assign the corresponding injection strategy [2].

2.2.1 SCREEN: 70459_2.2.1_new.mp4: 00:05-00:17

2.2.2 SCREEN: 70459_2.2.2_new.mp4: 00:07-00:56

- 2.3 On the **Plate Map** page, select the positions of the samples on the 24-well plate by clicking on each group and its corresponding wells, leaving four wells as blanks [1].

2.3.1 SCREEN: 70459_2.3.1_new.mp4: 00:10-00:48

- 2.4 On the Protocol page, remove unnecessary **Control** or **Experimental** tabs [1] and match the software port assignments to the drug positions on the cartridge [2].

2.4.1 SCREEN: 70459_2.4.1_new.mp4: 00:09-00:15

2.4.2 Talent checking the software ports and the drug positions on the cartridge.

2.5 Set 10 cycles to baseline, 3 cycles to oligomycin, 7 cycles to FCCP, and 9 cycles to the rotenone and antimycin A mix [1]. Ensure that each cycle consists of 3 minutes of mixing, 2 minutes of waiting, and 3 minutes of measuring [2].

2.5.1 SCREEN: 70459_2.5.1_new.mp4: 00:07-00:25

2.5.2 SCREEN: 70459_2.5.2_new.mp4: 00:08-00:18

3 Loading of Sensor Cartridge

3.1 Remove the cartridge and utility plate from the humidified incubator that was set up the night before [1].

3.1.1 Talent removing the incubated cartridge and utility plate from the humidified incubator.

3.2 Next, remove the hydro booster and lid from the cartridge [1], and place the sensor cartridge onto the utility plate containing calibrant [2].

3.2.1 CU: Removing the hydro booster and lid from the cartridge. Videographer's

NOTE: 3.2.1 & 3.2.2 are combined in one shot

3.2.2 CU: Placing the sensor cartridge onto the utility plate with calibrant.

3.3 Gently pipette the inhibitors into their respective ports along the side wall and avoid tapping [1-TXT].

3.3.1 CU: Pipetting inhibitors into the ports along the side wall. **TXT: Port A: 50 μ L oligomycin; Port B: 55 μ L of FCCP; Port C: 60 μ L rotenone/antimycin A**

3.4 On the software, select **Run Assay**, name the run, and click **Start run**. Save the run with a file name and load the assay [1]. Once the tray of the analyzer opens [2], place the cartridge loaded with drugs and utility plate containing calibrant according to the plate map on the tray [3].

3.4.1 SCREEN: 70459_3.4.1_new.mp4: 00:07-01:31, 70459_3.4.1_sup_new.mp4: 00:04-00:07

3.4.2 Shot of analyzer tray opening.

3.4.3 Talent placing the cartridge and utility plate into the analyzer tray.

3.5 Press **Run assay** to start the assay [1].

3.5.1 Shot of analyzer taking in the loaded tray.

4 Loading of samples and Data Collection

4.1 After covering the islet capture microplate containing isolated hearts with a grid, click **OK** in the tray temperature alarm box and confirm all wells are successfully calibrated with the green tick on the software [1]. Then click **Open Tray** [2] to eject the utility plate [3].

4.1.1 SCREEN: 70459_4.1.1-4.1.2.mp4: 01:35-01:39

4.1.2 SCREEN: 70459_4.2.2.mp4: 00:31-00:45

4.1.3 Shot of utility plate ejecting.

4.2 Without lid, place the islet microplate into the analyzer, with A1 positioned in the top-left corner [1] and click **Load cell plate** to initiate the experiment [2].

4.2.1 Talent placing the islet microplate into the analyzer.

4.2.2 SCREEN: 70459_4.2.2.mp4: 01:00-01:10

4.3 Once the experiment is complete, click **Eject** to eject the islet microplate and cartridge [1]. Remove the ejected islet plate and cartridge from analyzer [2] and close the cartridge with lid [3]. Store the islet plate on dry ice [4]. Then click **Done** to close the tray [5].

4.3.1 SCREEN: 70459_4.3.1-4.4.1.mp4: 00:23-00:35

4.3.2 Talent removing the islet plate and cartridge from analyzer. Videographer's

NOTE: 4.3.2 & 4.3.3 are combined in one shot

4.3.3 Talent closing the cartridge with lid.

4.3.4 Talent storing the hearts in dry ice. Videographer's **NOTE: Take 2 needs to be used. The author did not like his lid being in the background in Take 1 of this shot. He has requested Take 2 of this shot to be used instead.**

4.3.5 SCREEN: 70459_4.3.1-4.4.1.mp4: 01:37-01:46

4.4 Finally, click **View results** to visualize the data [1]. Save and export the measurements from the analyzer computer [2].

4.4.1 SCREEN: 70459_4.3.1-4.4.1.mp4: 01:54-02:26

4.4.2 SCREEN: 70459_4.4.2.mp4: 00:02-00:16

4.5 Upload the data on the analytics interface [1] and select the oxygen consumption rate or **OCR (O-C-R)** graph from **Standard views** [2]. View the OCR and extracellular acidification rate or ECAR (**E-C-A-R**) level and correct the background using empty wells [3]. Select wells from the **Plate map** to display or hide data [4].

4.5.1 SCREEN: 70459_4.5.1.mp4: 00:14-00:29

4.5.2 SCREEN: 70459_4.5.2.mp4: 00:12-00:30

4.5.3 SCREEN: 70459_4.5.3.mp4: 00:06-00:19

4.5.4 SCREEN: 70459_4.5.4.mp4: 00:07-00:24

4.6 After quantifying the protein levels, normalize the OCR and ECAR measurements to ensure reliable comparison across samples [1].

4.6.1 SCREEN: 70459_4.6.1.mp4: 00:07-00:35

Results

5 Results

5.1 Wild-type uninjured ventricles without drug treatment [1], demonstrated stable oxygen consumption rate [2] and extracellular acidification rate [3], indicating ventricle's normal respiratory function and survival in the *ex vivo* setting [4].

5.1.1 LAB MEDIA: Figure 3A.

5.1.2 LAB MEDIA: Figure 3A *Video editor: Emphasize red bars in the graph on the left for OCR.*

5.1.3 LAB MEDIA: Figure 3A *Video editor: Emphasize red bars in the graph on the right for ECAR.*

5.1.4 LAB MEDIA: Figure 3A

5.2 A mild increase in oxygen consumption rate was observed with 10 micromolar oligomycin, 8 micromolar FCCP, and 25 micromolar rotenone and antimycin [1]. A slightly greater increase was observed with 50 micromolar oligomycin, 30 micromolar FCCP, and 45 micromolar rotenone and antimycin [2].

5.2.1 LAB MEDIA: Figure 3B. *Video editor: Please emphasize the bar for [O]10 μ M, [F]8 μ M, [RA]25 μ M*

5.2.2 LAB MEDIA: Figure 3B. *Video editor: Please emphasize the bar for [O]50 μ M, [F]30 μ M, [RA]45 μ M*

5.3 After permeabilization, the oxygen consumption rate showed a sustained reduction [1] and did not increase after FCCP injection, indicating metabolic failure of the permeabilized ventricles [2].

5.3.1 LAB MEDIA: Figure 3C. *Video editor: Please emphasize the red bar in the right graph.*

5.3.2 LAB MEDIA: Figure 3C. *Video editor: Please emphasize the red line in the left graph.*

5.4 The oligomycin treatment does not significantly alter oxygen consumption rate [1]. FCCP increased the oxygen consumption rate and extracellular acidification rate [2] with the cytosolic malate dehydrogenase 1Ab overexpressing ventricles showing the highest maximal respiratory capacity compared to control ventricles [3].

- 5.4.1 LAB MEDIA: Figure 3D. *Video editor: In left graph, please highlight the area between oligomycin and FCCP.*
- 5.4.2 LAB MEDIA: Figure 3D. *Video editor: In both graphs, please highlight the area between FCCP and rotenone/antimycin.*
- 5.4.3 LAB MEDIA: Figure 3D. *Video editor: In both graphs, please highlight the red line between FCCP and rotenone/antimycin.*

Pronunciation guides:

1. Oligomycin
Pronunciation link: <https://www.merriam-webster.com/dictionary/oligomycin>
IPA: /ˌɑːləɡoʊˈmaɪsən/
Phonetic Spelling: aa-luh-goh-my-sin
2. Carbonyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/carbonyl>
IPA: /ˈkɑːrbəˌnɪl/
Phonetic Spelling: kar-buh-nil
3. Cyanide
Pronunciation link: <https://www.merriam-webster.com/dictionary/cyanide>
IPA: /ˈsaɪəˌnaɪd/
Phonetic Spelling: sy-uh-nyde
4. Trifluoromethoxy
Pronunciation link: No confirmed link found
IPA: /traɪˌflʊroʊmɛˈθɑːksi/
Phonetic Spelling: try-floor-oh-meh-thaak-see
5. Phenylhydrazine
Pronunciation link: No confirmed link found
IPA: /ˌfɛnəlˈhaɪdrəˌzoʊn/
Phonetic Spelling: fen-uhl-hy-druh-zohn
6. Rotenone
Pronunciation link: <https://www.merriam-webster.com/dictionary/rotenone>
IPA: /ˈroʊtəˌnoʊn/
Phonetic Spelling: roh-tuh-nohn
7. Antimycin
Pronunciation link: <https://www.merriam-webster.com/dictionary/antimycin>
IPA: /ˌæntiˈmaɪsən/
Phonetic Spelling: an-tee-my-sin
8. Humidified
Pronunciation link: <https://www.merriam-webster.com/dictionary/humidified>

IPA: /hju:'mɪdəˌfaɪd/

Phonetic Spelling: hyoo·mid·uh·fyd

9. Calibrant

Pronunciation link: No confirmed link found

IPA: /'kælɪbrənt/

Phonetic Spelling: kal·uh·bruhnt

10. Pipette

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

IPA: /paɪ'pet/

Phonetic Spelling: py·pet

11. Inhibitors

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

IPA: /ɪn'hɪbətərz/

Phonetic Spelling: in·hib·uh·terz

12. Islet

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

IPA: /'aɪlət/

Phonetic Spelling: eye·luht

13. Microplate

Pronunciation link: No confirmed link found

IPA: /'maɪkroʊˌpleɪt/

Phonetic Spelling: my·kroh·playt

14. Extracellular

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

IPA: /ˌɛkstrə'sɛljələr/

Phonetic Spelling: ek·struh·sel·yuh·ler

15. Acidification

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

IPA: /əˌsɪdəfə'keɪʃən/

Phonetic Spelling: uh·sid·uh·fuh·kay·shuhn

16. Quantifying

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

IPA: /'kwɑːntəˌfaɪŋ/

Phonetic Spelling: kwahn·tuh·fy·ing

17. Normalize

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

IPA: /'nɔːrməˌlaɪz/

Phonetic Spelling: nor·muh·lyze

18. Ventricles

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

IPA: /'ventrɪkəlz/

Phonetic Spelling: ven·trih·kuhlz

19. Ventricle
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ventricle>
 IPA: /'ventrɪkəl/
 Phonetic Spelling: ven·trih·kuhl
20. Respiratory
 Pronunciation link: <https://www.merriam-webster.com/dictionary/respiratory>
 IPA: /'rɛspərəˌtɔːri/
 Phonetic Spelling: res·puh·ruh·tor·ee
21. Ex vivo
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ex%20vivo>
 IPA: /ˌɛks ˈviːvʊv/
 Phonetic Spelling: eks·vee·voh
22. Micromolar
 Pronunciation link: <https://www.merriam-webster.com/dictionary/micromolar>
 IPA: /ˌmaɪkroʊˈmoʊlər/
 Phonetic Spelling: my·kroh·moh·ler
23. Permeabilization
 Pronunciation link: No confirmed link found
 IPA: /ˌpɜːmiəbələˈzeɪʃən/
 Phonetic Spelling: per·mee·uh·buh·luh·zay·shuhn
24. Permeabilized
 Pronunciation link: No confirmed link found
 IPA: /ˌpɜːmiəˌbələɪzd/
 Phonetic Spelling: per·mee·uh·buh·lyzd
25. Metabolic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/metabolic>
 IPA: /ˌmɛtəˈbɑːlɪk/
 Phonetic Spelling: met·uh·baal·ik
26. Cytosolic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cytosol>
 IPA: /ˌsaɪtəˈsɔːlɪk/
 Phonetic Spelling: sy·tuh·saal·ik
27. Malate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/malate>
 IPA: /'mæleɪt/
 Phonetic Spelling: mal·ayt
28. Dehydrogenase
 Pronunciation link: <https://www.merriam-webster.com/dictionary/dehydrogenase>
 IPA: /ˌdiːhaɪˈdrɑːdʒəˌneɪs/
 Phonetic Spelling: dee·hy·draa·juh·nays
29. Overexpressing
 Pronunciation link: No confirmed link found

IPA: /ˌoʊvərɪkˈspresɪŋ/

Phonetic Spelling: oh·ver·ik·spret·ing

30. Maximal

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

IPA: /ˈmæksəməl/

Phonetic Spelling: mak·suh·muhl