FINAL SCRIPT: APPROVED FOR FILMING

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Title: Establishing In Vitro Models of Dorsal Root Ganglia Culture: Complementary Approaches for Investigating Cancer–Nerve Crosstalk

Authors and Affiliations:
Larissa C. B. Oliveira[1], Erica R. Pereira[1,2], Brandy D. Hyndman[1], Bryanna Thomson[1], Juliana M. Serpeloni[2], Lois M. Mulligan[1]

[1]Division of Cancer Biology and Genetics, Sinclair Cancer Research Institute, and Department of Pathology and Molecular Medicine, Queen's University
[2]Laboratory of Mutagenesis and Oncogenetics, Department of General Biology – Center for Biological Science, State University of Londrina

Corresponding Authors:
Lois M. Mulligan mulligal@queensu.ca
Email Addresses for All Authors:
Larissa C. B. Oliveira 22lcbo@queensu.ca
Erica R. Pereira romaoericaa@gmail.com
Brandy D. Hyndman bdh@queensu.ca
Bryanna Thomson bryanna.thomson@queensu.ca
Juliana M. Serpeloni julianaserpeloni@yahoo.com.br
Lois M. Mulligan mulligal@queensu.ca

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**

   If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

   Main lab scope – Leica MZ6 modular stereomicroscope

   Tissue culture room scope – Leica DM IL Inverted Microscope

   SCOPE: **2.7.2, 2.8.1, 2.9.1 and 2.10.1 – main lab scope**

   3.5.1 and 4.12.1 – tissue culture room scope

   Videographer: Please film all shots labeled SCOPE using 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**

   Two different rooms in the same floor. Our main lab will be used for the extraction of DRGs (Step 2.1 to 2.10) and the tissue culture room (sterile) will be used for the culturing steps (Step 3.1 to 5.3).

Current Protocol Length
Number of Steps: 29
Number of Shots: 56

Introduction

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

1.1. **Larissa Oliveira:** Our research focuses on cancer–nerve crosstalk, using 3-D Dorsal root ganglia or DRG explants and 2-D dissociated cultures to quantify neurite-guided tumor migration and test molecular strategies to limit their interaction.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.5*

What significant findings have you established in your field?

1.2. **Larissa Oliveira:** With this protocol, we have established complementary 3-D and 2-D DRG cultures, optimized neurite growth, and demonstrated an increase in cancer-cell invasion along neurite extensions.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:6.7*

What research gap are you addressing with your protocol?

1.3. **Larissa Oliveira:** We address the lack of reproducible models that capture both intricate cell-level interactions and intact nerve architecture by creating complimentary 2-D and 3-D DRG co-cultures from a single mouse.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:6.6*

What advantage does your protocol offer compared to other techniques?

1.4. **Larissa Oliveira:** Our protocol yields paired 3-D explant and 2-D neuron cultures from a single mouse, preserving ganglion architecture, enabling high-content imaging, reducing animal use, and allowing us to obtain tissue-level and single-cell data in one experiment.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:6.4*

What new scientific questions have your results paved the way for?

1.5. **Larissa Oliveira:** Our models open questions on which DRG-secreted factors drive invasion, how neuronal activity, age and genotype shape it, and whether targeting neurotrophic factor receptors could decrease neurite-guided cancer spread.

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 Care Committee at Queen's University

Protocol

2. Dissection of Mouse Spinal Column and Isolation of Dorsal Root Ganglia for In Vitro Culture

Demonstrator: Larissa Oliveira

2.1. To begin, lay a euthanized mouse on its abdomen **[1]**. Shave the fur, focusing on the area over the spinal column to expose the dorsal surface **[2]**.

2.1.1. WIDE: Talent laying the euthanized mouse in a prone position on the dissection tray. **Videographer's NOTE: 2.1.1 – take 1 WS, take 2 CU**

2.1.2. Talent using an electric razor to shave fur along the animal's back, focusing over the spinal column. **Videographer's NOTE: 2.2.1 – combined with 2.2.2**

2.2. Spray 70 percent ethanol over the animal's back **[1]**. Using paper towels, wipe from tail to skull to remove any loose fur and prevent contamination during dissection **[2]**.

2.2.1. Talent spraying ethanol evenly over the shaved dorsal area.

2.2.2. Talent wiping the back from tail to skull with paper towels to clear fur.

2.3. With a pair of sterilized scissors, make a straight incision along the dorsal midline from the tail to the skull **[1]**. Using blunt forceps, retract the skin on both sides of the incision to expose the spinal column **[2]**.

2.3.1. Talent making a precise midline incision with sterilized scissors.

2.3.2. Talent using blunt forceps to peel back skin and reveal the spinal column.

2.4. Now use a fresh pair of scissors and forceps to make a horizontal incision at the tail end of the spinal column to avoid fur contamination **[1]**. Then make two parallel longitudinal incisions on either side of the spinal column to release it **[2]**. Use forceps to gently lift the spinal column and remove the surrounding connective tissue **[3]**.

2.4.1. Talent switching to a clean pair of tools and making a horizontal cut at the base of the spine.**Videographer's NOTE: 2.4.1 – combined with 2.4.2-2.4.3**

2.4.2. Talent creating two clean, parallel cuts along each side of the spine.

2.4.3. Talent lifting the spine and carefully dissecting away connective tissue.

2.5. To fully release the spinal column, make an incision at the top of the spinal column, near the skull **[1]**. Transfer the spinal column to a prechilled 10-centimeter plate containing HBSS with Penicillin-Streptomycin, kept on ice **[2]**.

2.5.1. Talent severing the spinal column at its upper end near the skull.

2.5.2. Talent placing the freed spinal column into a chilled plate with HBSS-Pen-Strep on ice.

2.6. Next, use spring scissors to remove any remaining connective tissue from the spinal column and expose the vertebrae **[1-TXT]**. ~~Place the cleaned spinal columns into a fresh, prechilled 10-centimeter plate~~ **[2]**.

2.6.1. Talent trimming off residual connective tissue with spring scissors to clearly expose vertebrae. **Videographer's NOTE: 2.6.1 – take 1: remove from plate, take 2: trim**

2.6.2. ~~Talent transferring trimmed spinal columns to a new cold plate~~. **Videographer's NOTE**: 2.6.2 – shot removed

2.7. Transfer the trimmed spinal column onto a microscope stage **[1]**. Carefully split the column along the sagittal plane using spring scissors **[2]**.
Videographer: Please film the SCOPE shots using a SCOPE kit

2.7.1. Talent positioning spinal column under the microscope. **Videographer's NOTE**: 2.7.1 – take 1 WS, take 2 MS

2.7.2. ~~SCOPE:~~ The spinal column is being split lengthwise down the middle with spring scissors. **Videographer's NOTE:** 2.7.2 – not a scope shot

2.8. Using fine-tipped forceps, remove the spinal cord from the column to reveal the dorsal root ganglia along the roots of the spinal nerves **[1]**.

2.8.1. SCOPE: The spinal cord is being removed from the column and bilateral dorsal root ganglia is being exposed. **Videographer's NOTE:** 2.8.1 – scope shot WAS audio slated

2.9. Cut the spinal nerves using spring scissors then remove any surrounding connective tissue from the dorsal root ganglia **[1]**.

2.9.1. SCOPE: Talent severing spinal nerves carefully with spring scissors and removing surrounding connective tissue. **Videographer's NOTE:** 2.9.1 – combined with 2.10.1, scope shots audio slated, use take 2

2.10. Using fine-tipped forceps, gently extract the dorsal root ganglia from the dorsal roots **[1]**. For whole mount culture, place the DRGs into a 3-centimeter plate prepared with Matrigel **[2-TXT]**.

2.10.1. SCOPE: DRGs are being pulled out with forceps.

2.10.2. Talent transferring selected DRGs to the prepared small plate. **TXT: Pool remaining DRGs into a 15 mL conical tube** **Videographer's NOTE:** 2.10.2 – audio slated

3. Whole Mount Dorsal Root Ganglion Culture

3.1. Use prechilled pipette tips to dispense 2 microliters of chilled Matrigel into each well of an 8-well glass-bottom slide placed inside a 10-centimeter plate on ice **[1]**. To prevent premature polymerization, pipette only one droplet at a time **[2]**.

3.1.1. Talent using prechilled tips to pipette Matrigel into the first well of a chilled slide.

3.1.2. CU: Talent preparing only one droplet at a time to avoid early Matrigel setting.

3.2. Next, use fine-tipped forceps to place one dorsal root ganglion on a dry plate **[1].** Swirl gently to remove excess HBSS **[2]**. Then transfer the cleaned dorsal root ganglion into the Matrigel droplet **[2]**.

3.2.1. Shot of a DRG being placed on a dry plate. **Videographer's NOTE**: 3.2.1-3 – shot in 4K separately

3.2.2. Talent swirling DRG on a dry plate to remove residual buffer.

3.2.3. Talent positioning the DRG into the Matrigel droplet.

3.3. After transferring all ganglions into the droplets, incubate the glass-bottom slide in a humidified incubator at 37 degrees Celsius for 5 minutes to allow the Matrigel to polymerize **[1]**.

3.3.1. Talent placing the 8-well slide into an incubator and closing the door. **Videographer's NOTE**: 3.3.1 – shot in 4K

3.4. Next, carefully add 200 microliters of warm supplemented culture media to each well **[1]**. Incubate the slide at 37 degrees Celsius with 5 percent carbon dioxide **[2-TXT]**.

3.4.1. Talent pipetting warm culture media into each well gently.

3.4.2. Talent placing slide back in the incubator under specified conditions. **TXT: Replace media every 72 h**

3.5. Use brightfield microscopy to monitor the dorsal root ganglia daily for neuronal outgrowth and viability **[1]**.

3.5.1. SCOPE: Display image of DRG under brightfield microscope showing neuronal outgrowth. **Videographer's NOTE**: 3.5.1 – scope shot audio slated

4. Enzymatic and Mechanical Dissociation of Mouse Dorsal Root Ganglia for Primary Cell Culture

4.1. Centrifuge a 15-milliliter conical tube containing pooled dorsal root ganglia at 200 g for 5 minutes at room temperature **[1]**. After pipetting out the HBSS, rinse the ganglia with prewarmed supplemented media and gently tap **[2].**

4.1.1. Talent placing the tube in a centrifuge and starting the run.

4.1.2. Talent adding prewarmed media into the tube and tapping gently.

Videographer's NOTE: 4.1.2 – take 1 adding media, take 2 tapping the tube

4.2. Then centrifuge the tube for 5 minutes at 200 *g* to pellet the DRGs **[1]**.

4.2.1. Talent centrifuging the tube again to collect DRGs.

4.3. Replace the media with 1 milliliter of Papain solution **[1]**. Mix gently and incubate at 37 degrees Celsius in a tissue culture incubator for 20 minutes, gently agitating every 5 minutes **[2]**.

4.3.1. Talent removing media and pipetting in Papain solution carefully.

4.3.2. Talent placing the tube in an incubator and gently swirling it at regular intervals. **Videographer's NOTE:** 4.3.2 – take 1 incubator, take 2 swirling

4.4. Next, add 4.5 milliliters of supplemented media to neutralize the Papain and mix gently **[1]**. Centrifuge for 5 minutes at 400 *g* to pellet the dorsal root ganglia **[2].**

4.4.1. Talent adding media to the digested sample and gently inverting the tube to mix.

4.4.2. Talent placing the tube in a centrifuge. **Videographer's NOTE**: 4.4.2 – same shot as 4.2.1

4.5. After pipetting out the supernatant, add 1 milliliter of Collagenase IV/Dispase II *(Collagenase-Four-and-Dispase-Two)* solution and incubate again **[1]**.

4.5.1. Talent adding the enzyme solution and periodically agitating the tube during incubation. **Videographer's NOTE**: 4.5.1 – adding enzyme solution is taken here, agitating tube is same as 4.3.2

4.6. Add 4.5 milliliters of supplemented media to neutralize the enzyme solution, mix gently and centrifuge **[1-TXT]**.

4.6.1. Talent neutralizing the digestion with media and gently mixing. **TXT: Centrifugation: 500 x *g*, 5 min**

4.7. After pipetting out the supernatant, add 2 milliliters of supplemented media **[1].** Then add DNase I *(D-N-Ase-One)* to reach a final concentration of 0.2 milligrams per milliliter **[2]**. The dorsal root ganglia should now appear as a loose clump, indicating complete digestion **[3]**.

4.7.1. Talent pipetting media into the sample tube.

4.7.2. Shot of DNase I being added to the sample tube.

4.7.3. Shot of loose clump of digested DRGs in the tube. **Videographer's NOTE**: 4.7.3 – shot in 4K

4.8. For mechanical dissociation, use a 1,000-microliter filter pipette tip to triturate the ganglia solution by pipetting up and down 4 to 5 times **[1]**. Switch to a 200-microliter pipette tip and repeat trituration by pipetting 4 to 5 times **[2]**.

4.8.1. CU: Talent using a 1,000 microliter pipette to gently triturate the digested DRG

sample. **Videographer's NOTE**: 4.8.1 – shot in 4K

4.8.2. Talent using a smaller pipette tip to repeat gentle trituration.

4.9. Filter the resulting cloudy suspension through a 70-micrometer cell strainer into a 50-milliliter conical tube **[1]**. Then gradually rinse the strainer with 10 milliliters of media to ensure complete transfer of cells **[2]**.

4.9.1. Talent pouring cell suspension through a strainer positioned above a 50-milliliter tube.

4.9.2. Talent adding 10 mL media over the strainer.

4.10. Centrifuge the filtered cell suspension for 5 minutes at 1,000 *g* to pellet the cells **[1]**. Remove the media from the pellet **[2]** and resuspend the cell pellet in 500 microliters of supplemented media **[3]**.

4.10.1. Talent setting centrifuge parameters after placing the conical tube in the centrifuge. **Videographer's NOTE**: 4.10.1 – take 1 centrifuge, take 2 show settings

4.10.2. Talent aspirating media from the tube.

4.10.3. Talent gently resuspending the cell pellet in 500 µL of supplemented media.

4.11. Remove any residual PBS from the wells of the 8-well glass-bottom slide **[1]**. Distribute the dissociated dorsal root ganglia suspension into the collagen-coated wells and incubate **[2-TXT]**.

4.11.1. Talent aspirating leftover buffer from slide wells.

4.11.2. Talent pipetting DRG cell suspension into the collagen-coated wells. **TXT: Incubation: 37 °C in a humidified incubator with 5% CO_2; Replace media every 48 h**

4.12. Use brightfield microscopy to monitor the dorsal root ganglia-derived cells daily, assessing cell viability and neuronal outgrowth **[1]**.

4.12.1. SCOPE: Display brightfield image of healthy, growing DRG-derived cells. **Videographer's NOTE**: 4.12.1 – scope shot audio slated

5. Establishing Co-Culture Systems of Dorsal Root Ganglia and Cancer Cells

5.1. For co-culturing the whole mouse ganglia with cancer cells, first, aspirate the media from the whole mount DRG cultures on the 8-well glass-bottom slide **[1]**. Using a pipette, gently add 200 microliters of the cancer cell suspension onto the Matrigel droplet containing the dorsal root ganglia **[2]**.

5.1.1. Talent carefully aspirating culture media from the wells containing whole mount DRGs.

5.1.2. Talent pipetting the cancer cell suspension directly on top of each Matrigel droplet.

5.2. Incubate the co-culture slide in a humidified incubator at 37 degrees Celsius with 5 percent carbon dioxide **[1]**. Refresh the media daily by washing once with 200 microliters of warmed supplemented media and add 300 microliters of fresh media **[2-TXT]**.

5.2.1. Talent placing the co-culture slide into the incubator.

5.2.2. Talent adding and pipetting out 200 µL of supplemented media. **TXT: Observe cultures with a microscope every 24 h**

5.2.3. ~~Talent pipetting out 300 µL of supplemented media~~. **NOTE**: shot deleted, VO merged with the previous shot

5.3. For the co-culture of dissociated ganglia and the cancer cells, aspirate the media off the dissociated DRG cultures on the 8-well glass-bottom slide **[1]**. Then gently distribute 200 microliters of the cancer cell suspension into each well before incubating as done for whole mount cultures **[2]**.

5.3.1. Talent removing media from wells with dissociated DRG cultures. **Videographer's NOTE:** 5.3.1 – same as 5.1.1

5.3.2. Talent pipetting cancer cells into each well with dissociated DRG culture. **Videographer's NOTE**: 5.3.2 – same as 5.1.2

Results

6. Results

6.1. Establishment of DRG was less efficient in the absence of supplements and neurite extension was markedly reduced **[1-TXT]**.

6.1.1. LAB MEDIA: Figure 7. *Video editor: Highlight 7A*
TXT: DRG: Dorsal Root Ganglion

6.2. Whole-mount DRGs from older animals produced less profuse and more distinguishable neurite extension **[1]** and minimal glial cells, facilitating visualization of nerve–cancer cell interactions**[2]**.

6.2.1. LAB MEDIA: Figure 8. *Video editor: Please highlight the image on the right*

6.2.2. LAB MEDIA: Figure 8. *Video editor: Show the zoomed out image*

6.3. The dissociation of DRG neurons using two enzymatic steps resulted in higher neuron recovery **[1]**.

6.3.1. LAB MEDIA: Figure 4C. Video editor: Highlight the panel showing DRG neurons dissociated with Papain, Collagenase IV/Dispase II, and DNase I.

6.4. Within 24 hours of culture, non-neuronal cells such as Schwann cells, macrophages, and fibroblasts became evident **[1]**, while neurite sprouting from DRG-derived cells was observed and further increased after 72 hours **[2]**.

6.4.1. LAB MEDIA: Figure 5. *Video editor: Highlight the area pointed at by arrows in 5B*

6.4.2. LAB MEDIA: Figure 5. *Video editor: Highlight the area pointed at by arrows in 5C*

6.5. Cancer cells seeded in Matrigel-coated transwell inserts invaded towards DRG dissociated neurons **[1]** but not towards negative controls after 24 hours of incubation **[2]**.

6.5.1. LAB MEDIA: Figure 10B

6.5.2. LAB MEDIA: Figure 10C

6.6. Whole-mount DRG cultures immersed in a Matrigel droplet allowed neurite extension outward through the matrix towards the periphery **[1]**, with careful positioning of the DRG centrally in the droplet providing maximal neurite extension without overgrowing the borders **[2]**.

6.6.1. LAB MEDIA: Figure 2C. *Video editor: Highlight areas pointed at by the arrows*

6.6.2. LAB MEDIA: Figure 3. *Video editor: Please sequentially show images A to E*

6.7. After 12 h of co-culture, cancer cells settled around the border of the Matrigel droplet

containing the DRG **[1].** After 72 hours of co-culture, cancer cells had invaded the Matrigel and moved toward the ganglion, with direct interaction between dorsal root ganglion cell extensions and cancer cells observed **[2]**.

6.7.1. LAB MEDIA: Figure 12A

6.7.2. LAB MEDIA: 11A and 12B.

6.8. Co-culture of dissociated DRG-derived cells with cancer cells allowed detailed visualization of individual cell interactions **[1]**.

6.8.1. LAB MEDIA: Figure 6C. *Video editor: Highlight areas pointed at by the arrows*

6.8.2. LAB MEDIA: Figure 13.

6.8.3. LAB MEDIA: Figure 14. *Video editor: Highlight the areas pointed at by the arrows*

Pronunciation guidance:

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1. euthanize

Pronunciation link:
https://www.merriam-webster.com/dictionary/euthanize (youglish.com, Merriam-Webster)
IPA (American): /ˈjuː.θə.ˌnaɪz/
Phonetic spelling: yoo-thuh-nighz

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2. dorsal root ganglion

Pronunciation link:
https://www.merriam-webster.com/dictionary/dorsal%20root%20ganglion (Merriam-Webster)
IPA (American): /ˌdɔːr.səl ˌruːt ˈgæŋ.gli.ən/
Phonetic spelling: dor-suhl root GANG-glee-uhn

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3. penicillin-streptomycin (as in "HBSS with Penicillin-Streptomycin")

Pronunciation links:
Penicillin: https://dictionary.cambridge.org/pronunciation/english/penicillin (Cambridge Dictionary)

Streptomycin: https://www.merriam-webster.com/dictionary/streptomycin (Merriam-Webster, How To Pronounce)

- **Penicillin IPA (American):** /ˌpen.əˈsɪl.ɪn/
  Phonetic spelling: pen-ə-SIL-in
- **Streptomycin IPA (American):** /ˌstrep-tə-ˈmī-sᵊn/
  Phonetic spelling: strep-tə-MYE-sin

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4. Matrigel (in the context "Matrigel droplet")

Pronunciation link:
https://www.howtopronounce.com/matrigel (Cambridge Dictionary, Cambridge Dictionary, Merriam-Webster, How To Pronounce)
IPA (American): /ˈmætrɪˌdʒɛl/
Phonetic spelling: MAT-ri-gel

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2. Schwann cell

Pronunciation link:

- Cambridge Dictionary shows US English pronunciation /ˈ**ʃwɑːn ˌsel/**
  Wikipedia+15Cambridge Dictionary+15How To Pronounce+15

IPA (American): /ˈʃwɑːn ˌsɛl/
Phonetic spelling: shwahn sel