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# Title: Modeling Persistent *Pseudomonas aeruginosa* Infection in Wounded Zebrafish Larvae

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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, done 2.2.1 - 2.3.1 - 2.3.2 - 2.3.3**

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: 15
Number of Shots: 30 (4 Scope)

# Introduction

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

1.1. **Flore Nilly:** Chronic infections caused by the bacterium *Pseudomonas aeruginosa* are tolerant to antibiotics and very difficult to treat. Our objective is to develop an in vivo model that will accelerate the discovery of efficient therapies.

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 current experimental challenges?

1.2. **Flore Nilly:** Antibacterial drugs are mostly screened in vitro and testing drugs in chronically infected mice is a complex challenge. To fill the gap between these two approaches, we propose an alternative pre-clinical model using wounded zebrafish larvae.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 1*

What advantage does your protocol offer compared to other techniques?

1.3. **Flore Nilly:** Our model of persistent infection in zebrafish, based on the use of clinical strains of *Pseudomonas aeruginosa*, reproduces antibiotic tolerance. Whereas microinjection is commonly used to infect zebrafish larvae, our wounding method reflects a natural infection mode and is well-suited for screening of therapeutic compounds.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

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

**Testimonial Questions (OPTIONAL):**

***Videographer: Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.***

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

1.4. **Flore Nilly, CNRS engineer**: I see research as a space for sharing. Publishing a JoVE video offers clarity and precision rarely achieved with written protocols, enhancing reproducibility and accessibility. This visibility will strengthen the impact of our work and supports knowledge transfer within the scientific community.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

Authors: Could you please also deliver the above statements in French?

Je considère la recherche comme un espace de partage. La publication d'une vidéo JoVE offre une clarté et une précision rarement atteintes avec des protocoles écrits, améliorant ainsi la reproductibilité et l'accessibilité. Cette visibilité renforcera l'impact de nos travaux et favorisera le transfert de connaissances au sein de la communauté scientifique.

*Videographer: Please film the testimonials in both English and French*

# Protocol

**2. Embryo Injury and Infection of Wounded Embryos**
**Demonstrator:** Flore Nilly

2.1. To begin, place 25-gauge needles on top of two chopsticks to facilitate handling **[1]**.
2.1.1. WIDE: Talent aligning and securing 25-gauge needles across the tops of two parallel chopsticks on a workstation. **TXT: Anesthetize embryos with 0.02% Tricaine**

2.2. Using a stereo microscope, identify and remove embryos that show abnormal development or are non-viable **[1]**. Move the dish in a circular motion to gather the remaining embryos at the center **[2]**.
2.2.1. SCOPE: 2.2.1.
2.2.2. Talent gently rotating the petri dish in a circular motion.

2.3. Now, use two vertically oriented needles to isolate each embryo, positioning the left needle at the tail to keep the body straight **[1]**. With the right needle, make a single cut at the edge of the notochord to remove the fin **[2]**. Complete each cut swiftly, ensuring all embryos are immersed in bacterial solution within 10 minutes **[3]**.
2.3.1. SCOPE: 2.3.1.
2.3.2. SCOPE: 2.3.2 00:30-00:39.
2.3.3. SCOPE: 2.3.3-obj-10X.mp4.

2.4. For the infection procedure, vortex the *Pseudomonas aeruginosa* solution under a type 2 biological safety cabinet **[1]** and add it at approximately $1 \times 10^7$ colony-forming units per milliliter to a 6-well plate **[2]**.
2.4.1. Talent vortexing a tube containing *P. aeruginosa* solution.
2.4.2. Talent transferring the solution to a 6-well plate inside a biosafety cabinet.

2.5. Use a disposable glass Pasteur pipette to collect the wounded embryos **[1]** and transfer them into the bacterial solution **[2]**. Incubate the 6-well plate at 28 degrees Celsius for 1.5 hours **[3]**.
2.5.1. Talent picking up injured embryos using a glass Pasteur pipette.
2.5.2. Talent adding the picked embryos into the wells containing bacterial solution.
2.5.3. Talent placing the 6-well plate into an incubator set to 28 degrees Celsius.

2.6. After incubation, retrieve the infected embryos and place them under the microbiological safety for washing **[1]**.
2.6.1. Talent placing the dish under a safety cabinet.

2.7. Now, transfer the embryos with a glass pipette into 10 milliliters of fish water without methylene blue, minimizing the transferred volume **[1]** and incubate for 30 minutes at room temperature **[2]**.
  2.7.1. Talent transferring embryos using a glass pipette into a container with 10 milliliters of clean fish water.
  2.7.2. Talent setting the dish aside for incubation.

2.8. Then, transfer the embryos again with a glass pipette into 4 milliliters of fish water without methylene blue and incubate briefly **[1]**.
  2.8.1. Talent performing a second transfer of embryos into fresh fish water using a pipette.

2.9. Next, with the pipette, transfer infected embryos individually into a 24-well plate, adding 1 milliliter of fish water without methylene blue to each well **[1]**. Place the multi-well plate inside a plastic box and transfer it into an incubator set at 28 degrees Celsius **[2]**.
  2.9.1. Talent pipetting individual embryos into separate wells of a multi-well plate containing 1 milliliter of fish water each.
  2.9.2. Talent placing the plate in the incubator.

## 3. Counting Bacterial Burden in Infected Embryos

3.1. Prepare 1.5-milliliter microcentrifuge tubes with 95 microliters of 1X PBS for each infected larva **[1]**.
  3.1.1. Talent adding PBS to multiple 1.5 milliliter microcentrifuge tubes.

3.2. Transfer the larvae into a 6-well plate containing 4 milliliters of fish water without methylene blue to wash and remove planktonic bacteria **[1]**.
  3.2.1. Talent using a pipette to move larvae into the wells filled with clean fish water.

3.3. Place each washed embryo into a microcentrifuge tube with PBS, transferring as little liquid as possible **[1]**.
  3.3.1. Talent gently transferring each larva into a separate microcentrifuge tube using minimal liquid.

3.4. Now, use a pestle to crush each embryo against the side of the microcentrifuge tube, leaving the pestle inside the tube afterward **[1]**.
  3.4.1. Talent pressing a plastic pestle against the side of each microcentrifuge tube to crush individual embryos, then leaving the pestle inside.

3.5. Then, lift the pestle and add 100 microliters of 2 percent PBS-Triton to rinse residual bacteria from the pestle, achieving a final concentration of 1 percent **[1]**. Vortex the tube **[2]** and incubate for 10 minutes **[3]**.
   3.5.1. Talent pipetting 100 microliters of PBS-Triton 2 percent into each tube while holding the pestle above.
   3.5.2. Talent placing the tube on a vortex mixer.
   3.5.3. Talent placing the tube aside to incubate.

3.6. Next, dispense three 10 microliter drops of undiluted lysate from each embryo onto LB agar plates **[1]**. Use a multichannel pipette to serially dilute each lysate in a 96-well plate up to a $10^{-3}$ dilution **[2]**. Finally, dispense three 10 microliter drops of the dilutions next to the undiluted spots **[3]** and incubate overnight at 37 degrees Celsius **[4-TXT]**.
   3.6.1. Talent spotting three drops of undiluted embryo lysate on an LB agar plate.
   3.6.2. Talent using a multichannel pipette to perform serial dilutions in a 96-well plate.
   3.6.3. Talent pipetting and spotting the diluted lysates beside the undiluted ones.
   3.6.4. Talent placing the plate into a 37 degrees Celsius incubator. **TXT: Count the fluorescent colonies for each dilution; Calculate CFU/infected embryo**

## 4. Results

4.1. All four cystic fibrosis *Pseudomonas aeruginosa* isolates were significantly less virulent in the injured embryo model **[1]** compared to the PAO1 *(P-A-O-1)* reference strain **[2]**.

4.1.1. LAB MEDIA: Figure 3A and 3B. *Video editor: Highlight the lines for A6520, B6513, C6490 in A and RP73 in B*

4.1.2. LAB MEDIA: Figure 3A and 3B. *Video editor: Highlight the lines for PAO1 in A and B*

4.2. In embryos infected with two isolates, the bacterial load was markedly reduced over 3 days, indicating bacterial elimination **[1]**.

4.2.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the A6520 and C6490 box plots at 67 mark on X-axis*.

4.3. In contrast, the two other isolates, B6513 *(B-sixty five-thirteen)* and RP73 *(R-P-seventy three)*, maintained a relatively stable bacterial load from 18 to 65 hours post-infection following an initial drop, suggesting persistence **[1]**.

4.3.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the B6513 and RP73 box plots.*

4.4. A short 30-minute treatment with tobramycin at 1.5 hours post-infection drastically reduced bacterial load in embryos infected with isolate B6513 **[1]**.

4.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the green-colored box plot for +1.5*.

4.5. Tobramycin had no significant effect on bacterial load when administered at 24 or 48 hours post-infection, demonstrating resistance during persistent infection stages **[1]**.

4.5.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the blue and red box plots at +24 and +48* .

4.6. Ofloxacin treatment at 1.5 hours post-infection significantly reduced bacterial load in B6513, indicating early-stage effectiveness **[1]**.

4.6.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the green-colored box plot for +1.5*.

4.7. Unlike tobramycin, ofloxacin retained partial efficacy at 24 and 48 hours post-infection, reducing bacterial load during persistent stages **[1]**.

4.7.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the blue and red box plots at +24 and +48* .

**Pronunciation guide**:

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## 1. Tricaine

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˈtraɪˌkeɪn/
- **Phonetic Spelling**: TRY-kayn

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## 2. Pseudomonas

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/pseudomonas
- **IPA**: /ˌsuːdəˈmoʊnəs/
- **Phonetic Spelling**: soo-duh-MOH-nuhs(merriam-webster.com)

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## 3. Pasteur pipette

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/pipette
- **IPA**: /pæsˈtɜr paɪˈpɛt/
- **Phonetic Spelling**: pas-TER pie-PET(merriam-webster.com)

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## 4. Triton

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/triton
- **IPA**: /ˈtraɪtən/
- **Phonetic Spelling**: TRY-tun(merriam-webster.com)

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## 5. Ofloxacin

- **Pronunciation link**: https://www.merriam-webster.com/medical/ofloxacin
- **IPA**: /oʊˈflɑːksəsɪn/
- **Phonetic Spelling**: oh-FLOX-uh-sin(merriam-webster.com)

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## 1. Notochord

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/notochord
- **IPA**: /ˈnoʊ.təˌkɔrd/
- **Phonetic Spelling**: NOH-tuh-kord

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## 2. Colony-forming units

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/colony
- **IPA**: /ˈkɑː.lə.ni ˈfɔːr.mɪŋ ˈjuː.nɪts/
- **Phonetic Spelling**: KAH-luh-nee FOR-ming YOO-nits(merriam-webster.com)

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## 3. Microcentrifuge

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/centrifuge
- **IPA**: /ˌmaɪ.kroʊˈsɛn.trəˌfjuːdʒ/
- **Phonetic Spelling**: MY-kroh-SEN-truh-fyooj(merriam-webster.com)

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## 4. Planktonic

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/planktonic
- **IPA**: /plæŋkˈtɑː.nɪk/
- **Phonetic Spelling**: plank-TAH-nik

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## 5. Lysate

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/lysate
- **IPA**: /ˈlaɪ.seɪt/
- **Phonetic Spelling**: LYE-sayt(merriam-webster.com, merriam-webster.com)

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## 6. Diluent

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/diluent
- **IPA**: /ˈdɪl.ju.ənt/

- **Phonetic Spelling**: DIL-yoo-uhnt(merriam-webster.com)

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## 7. Tobramycin

- **Pronunciation link**: https://www.merriam-webster.com/medical/tobramycin
- **IPA**: /ˌtoʊ.brəˈmaɪ.sɪn/
- **Phonetic Spelling**: TOH-bruh-MY-sin

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