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A new protocol for multispecies bacterial infections in zebrafish and their monitoring through automated image analysis

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PLOS ONE Lab Protocols | Spotlight series

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We use this collection and it's working

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Keywords: image analysis pipeline work both for single pathogen, bacterial infections in zebrafish, zebrafish by imaging, image analysis the zebrafish danio rerio, multispecies bacterial infections in the inner ear structure, live imaging of infection dynamic, automated segmentation of the otic vesicle, bacterial infection, investigating pathogen, pathogen frequencies through fluorescence intensity measure, zebrafish model, infection dynamic, pathogen, segmentation of the otic vesicle, image analysis pipeline, zebrafish, quantifying pathogen frequency, multispecies infection, suitable image analysis pipeline, pseudomonas aeruginosa, automated image analysis, lack of suitable image analysis pipeline, infection protocol, image analysis pipeline work, multispecies infection protocol, single pathogen, established multispecies infection protocol, acinetobacter baumannii, zebrafish danio rerio, pairwise pathogen combination, deep learning for the automated segmentation, automated image processing, pathogenhost interaction, e

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Disclaimer

Spotlight Video

The video below is a supplement with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.

https://www.youtube.com/embed/bmwnGq-VbWc?si=QtnG0ljbdYSV5yVx

Abstract

The zebrafish *Danio rerio* has become a popular model host to explore disease pathology caused by infectious agents. A main advantage is its transparency at an early age, which enables live imaging of infection dynamics. While multispecies infections are common in patients, the zebrafish model is rarely used to study them, although the model would be ideal for investigating pathogen-pathogen and pathogenhost interactions. This may be due to the absence of an established multispecies infection protocol for a defined organ and the lack of suitable image analysis pipelines for automated image processing. To address these issues, we developed a protocol for establishing and tracking single and multispecies bacterial infections in the inner ear structure (otic vesicle) of the zebrafish by imaging. Subsequently, we generated an image analysis pipeline that involved deep learning for the automated segmentation of the otic vesicle, and scripts for quantifying pathogen frequencies through fluorescence intensity measures. We used *Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae*, three of the difficult-to-treat ESKAPE pathogens, to show that our infection protocol and image analysis pipeline work both for single pathogens and pairwise pathogen combinations. Thus, our protocols provide a comprehensive toolbox for studying single and multispecies infections in real-time in zebrafish.

Troubleshooting

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https://www.youtube.com/embed/bmwnGq-VbWc?si=QtnG0IjbdYSV5yVx

Files

SEARCH

Protocol

NAME

Protocol (A): Zebrafish infections into the otic vesicle (2 dpf)

VERSION 1

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Protocol

NAME

Protocol (B): Zebrafish embedding and imaging (3 dpf)

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Protocol (C): Automated segmentation of the otic vesicle and image analysis

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