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Title: *Ookluc*: A *Plasmodium berghei* line for Identifying Transmission-blocking Compounds

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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

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: **07/07/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 23
Number of Shots: 36

Introduction

REQUIRED:

1.1. **Daniel Y. Bargieri:** Our research aims to identify and characterize transmission-blocking compounds and essential genes in *Plasmodium* sexual stages, using a high-throughput system to support novel strategies for interrupting malaria transmission.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.4.1.*

What research gap are you addressing with your protocol?

1.2. **Daniel Y. Bargieri:** This protocol addresses the lack of standardized, high-throughput methods for identifying Transmission-blocking compounds by using a transgenic *P. berghei* line with luminescence-based, automated detection of post-fertilization parasite development.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.2.*

What advantage does your protocol offer compared to other techniques?

1.3. **Daniel Y. Bargieri:** This protocol enables the screening of Plasmodium sexual stages, detects early transmission-blocking compounds and supports precise IC_{50} determination, offering advantages over conventional methods.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 5B.*

Ethics Title Card

This research has been approved by the Ethics Committee on Animal Use of the Institute of Biological Sciences, University of São Paulo

Protocol

2. Conversion Assay – Screening in 96-Well Plates

Demonstrator: Janaína T. Novais

2.1. To begin, prepare the compound samples by diluting the compound stock to 1 millimolar using ookinete medium **[1]**. Prepare the working samples by diluting this 1 millimolar stock in ookinete medium at a ratio of 1 to 100 to achieve a final concentration of 10 micromolar **[2]**. For one assay, prepare a final volume of 200 microliters of working sample in duplicate **[3]**.

2.1.1. WIDE: Talent pipetting the compound stock solution into a tube and adding ookinete medium to prepare a 1 millimolar solution.

2.1.2. Talent performing a 1 to 100 dilution of the 1 millimolar stock in ookinete medium to create the 10 micromolar working solution.

2.1.3. Talent pipetting out 200 microliters of the 10 micromolar working solution and dispensing it into two tubes labeled for duplicate assays.

2.2. If the compound is dissolved in a solvent other than water, prepare a control sample by diluting the solvent in the ookinete medium in the same proportion used for the test sample **[1]**.

2.2.1. Talent preparing a solvent control in a labelled tube by diluting the solvent in ookinete medium.

2.3. Using two 96-well plates, place 80 microliters of each sample in designated wells for positive control, negative control, solvent control, and test samples in duplicate, following the given layout **[1-TXT].**

2.3.1. Talent pipetting 80 microliters of each sample into wells of two 96-well plates in a specified layout. **TXT: Positive control: 80 µL ookinete medium; Negative control: 80 µL ookinete medium; Solvent control: 80 µL ookinete medium + 1% DMSO; Samples: 80 µL of 10 µM compound in ookinete medium**

2.4. Add 4 microliters of parasitized mouse blood to each well at a 1 to 20 ratio, except the negative control wells **[1]**. Homogenize gently by pipetting up and down **[2]**.

2.4.1. Talent adding 4 microliters of parasitized blood into the wells of the 96-well plate.

2.4.2. Talent gently mixing the contents in each well using a pipette.

2.5. For the negative control, add non-infected mouse blood to each well at a 1 to 20 ratio and homogenize gently by pipetting **[1]**.

2.5.1. Talent adding non-infected mouse blood to the negative control wells and pipetting gently to mix.

2.6. Place the plates in an incubator set to 21 degrees Celsius and incubate for either 6 hours or 24 hours **[1]**.

2.6.1. Talent placing the 96-well plates into a temperature-controlled incubator.

3. Luminescence Assay and Data Analysis

3.1. After incubation, homogenize each sample by pipetting up and down to ensure proper blood-medium mixing **[1]**. Prepare the lysis buffer and luciferase substrate mixture at a 1 to 50 ratio immediately before use **[2]**.

3.1.1. Talent pipetting each well after incubation to homogenize contents.

3.1.2. A shot of the prepared lysis buffer and luciferase substrate mixture.

3.2. Mix this lysis buffer-substrate mixture with each sample at a 1 to 1 ratio, ensuring gentle mixing without introducing bubbles **[1]**. Incubate the plate at 37 degrees Celsius for 3 to 5 minutes **[2]**.

3.2.1. Talent pipetting equal volumes of the sample and substrate mixture in a plate and mixing gently.

3.2.2. Talent placing the mixed plate into a 37 degrees Celsius incubator.

3.3. Now, transfer the samples to a 96-well white flat-bottom plate **[1]** and measure the luminescence using a plate reader **[2-TXT]**.

3.3.1. Talent transferring mixed samples into a white 96-well flat-bottom plate.

3.3.2. Talent placing the plate in plate reader to measure luminescence. **TXT: Parameter configuration:; Emission filter: lens; Measurement interval time: 1 s per well**

3.4. After the luminescence assay, record the raw luminescence data displayed by the plate reader **[1]**.

3.4.1. A shot of the luminescence results displayed in the software window after the measurement completes.

3.5. Using a spreadsheet, calculate the mean of each measurement using the given formula. Replace XX *(ex-ex)* with the address of the first measurement cell and YY with the address of the second measurement cell. Repeat the formula for all samples **[1]**.

3.5.1. SCREEN: 68466_screenshot_1.mp4. 00:04-00:27. **TXT:** $= \boldsymbol{AVERAGE}(\boldsymbol{XX}:\boldsymbol{YY})$

3.6. Convert the data into percentage inhibition using the given formula, where A is the sample relative light unit value and B is the control relative light unit value **[1]**. Select compounds that show more than 95 percent inhibition for the inhibitory concentration 50% determination **[2]**.

3.6.1. TEXT on PLAIN BACKGROUND:

$$\% \text{ Inhibition} = 1 - (A/B) \times 100$$

A: Sample relative light unit value

B: Control relative light unit value

3.6.2. SCREEN: 68466_screenshot_2.mp4 00:04-end.

4. Conversion Assay — Determining IC_{50}

4.1. After infecting mice with Ookluc and collecting parasitized blood **[1]**, prepare compound samples using ookinete medium as a diluent at the initial concentration that showed 100 percent inhibition of conversion **[2]**.

4.1.1. A shot of the collected parasitized blood.

4.1.2. Talent preparing the compound solution using ookinete medium.

4.2. Pipette the samples in triplicate into a 96-well plate, following the specified layout **[1,2]**.

4.2.1. Talent pipetting designated volumes into the wells of the 96-well plate following the layout instructions.

AND,

4.2.2. TEXT on PLAIN BACKGROUND:

Positive control (C+): 80 µL ookinete medium

Negative control (C-): 80 µL ookinete medium

Sample first dilution (Cpd D1): 160 µL compound in ookinete medium at 100% inhibitory concentration

D2 - D12 wells: 80 µL ookinete medium

Video Editor: Show the text of 4.2.2 as an inset during 4.2.1.

4.3. Using a pipette, perform two-fold serial dilutions by transferring 80 microliters from

well D1 into D2, mixing thoroughly, then transferring 80 microliters from D2 to D3, and continuing this process through to D12 **[1]**. Discard the final 80 microliters removed from D12 to maintain 80 microliters per well **[2]**.

4.3.1. Talent pipetting 80 microliters from well D1 into well D2, mixing by pipetting up and down, and again transferring 80 microliters from D2 to D3.

4.3.2. Talent discarding the final 80 microliters removed from D12.

4.4. Now, add parasitized mouse blood to each well at a 1 to 20 ratio and homogenize gently by pipetting **[1]**.

4.4.1. Talent pipetting parasitized blood into each well and gently mixing.

4.5. In the negative control wells, add non-infected mouse blood at a 1 to 20 ratio and homogenize gently **[1]**.

4.5.1. Talent pipetting non-infected mouse blood into negative control wells and gently mixing.

4.6. Place the plate in an incubator set to 21 degrees Celsius and incubate for either 6 or 24 hours before performing the luminescence assay **[1]**.

4.6.1. Talent placing the plate into a 21 degrees Celsius incubator.

5. Data Analysis — IC_{50} Determination

5.1. After calculating percentage inhibition, open the analysis software and create an XY table for plotting **[1]**. Set the Y-axis to **Enter 3 replicate values in side-by-side subcolumns [2]**.

5.1.1. SCREEN: 68466_screenshot_3.mp4 00:00-00:06.

5.1.2. SCREEN: 68466_screenshot_3.mp4 00:07-end.

5.2. Paste the data into the table with X representing compound concentration and Y representing percentage inhibition **[1]**.

5.2.1. SCREEN: 68466_screenshot_4.mp4 00:00-00:06.

5.3. Now, click on **Analyze**, select **Nonlinear regression-curve fit**, then choose **Dose-response curves Inhibition** followed by **[inhibitor] vs. normalized response -- variable slope** *(Inhibitor versus normalized response variable slope)*, and click **OK [1]**.

5.3.1. SCREEN: 68466_screenshot_5.mp4 00:00-end.

5.4. View the dose-response curve on the left panel and apply customizations such as color or point format changes **[1]**.

5.4.1. SCREEN: 68466_screenshot_6.mp4 00:00-00:15.

5.5. Locate the **Nonlin fit** *(Nonlinear fit)* table to find the calculated IC_{50} *(I-C-fifty)* and 95 percent confidence interval **[1]**.

5.5.1. SCREEN: 68466_screenshot_7.mp4 00:00-00:12.

Results

6. Results

6.1. Atovaquone demonstrated a clear concentration-dependent inhibition of target activity **[1]**, with greater than 95% inhibition observed at concentrations from 0.125 to 1 micromolar **[2]**, and complete loss of activity below 0.007 micromolar **[3]**, suggesting a sharp threshold effect in its inhibitory response **[4]**.

6.1.1. LAB MEDIA: Figure 5A.

6.1.2. LAB MEDIA: Figure 5A. *Video Editor: Highlight the entire rows from D1 to D4.*

6.1.3. LAB MEDIA: Figure 5A. *Video Editor: Highlight the entire rows from D9 to D12.*

6.1.4. LAB MEDIA: Figure 5A.

6.2. The dose-response curve fitted to the Atovaquone data yielded an IC_{50} value of 0.013 micromolar **[1]**, indicating high potency in inhibiting the targeted activity **[2]**.

6.2.1. LAB MEDIA: Figure 5B. Video editor: Highlight the curve and the text "IC_{50} 0.013 µM".

6.2.2. LAB MEDIA: Figure 5B.