Submission ID #: 61597
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18787133

Title: High-throughput Screening of Chemical Compounds to Elucidate Their Effects on Bacterial Persistence

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

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: 34

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Prashant Karki:** The protocol described here makes it possible to identify conditions that can significantly impact bacterial persistence in a high-throughput manner.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Prashant Karki:** This method is readily adaptable to screen for various arrays such as drug panels, gene libraries and more.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Prashant Karki:** As the method includes various sub-steps which are to be conducted simultaneously, visual demonstration can aid researchers to manage their timing to obtain precise results.

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

Protocol

2. Microarray Plate Screening

2.1. Begin by preparing microarray-cell cultures **[1]**. Transfer 250 microliters of exponential-phase cells to 25 milliliters of fresh modified LB medium in a 50-milliliter centrifuge tube **[2]**, then gently mix the cell suspension to make it homogeneous **[3]**.

2.1.1. WIDE: Establishing shot of talent walking to the lab bench with the cells in hand.

2.1.2. Talent diluting the cells in LB. **TEXT: Modified LB: LB without NaCl**

2.1.3. Talent gently mixing the tube.

2.2. Transfer the diluted cell suspension into a sterile 50 milliliters reservoir **[1]**. Using a multichannel pipette, transfer 150 microliters of the cell suspension to each well of a microarray plate containing various chemicals. Microarrays can also be generated manually following the method described in the text manuscript **[2]**. *Videographer: This step is important!*

2.2.1. Talent transferring the cells into a reservoir.

2.2.2. Talent adding cells to the wells of the microarray plate.

2.3. Cover the microarray plate with a gas-permeable sealing membrane **[1]** and incubate it in an orbital shaker at 37 degrees Celsius and 250 rpm for 24 hours **[2]**.

2.3.1. Talent covering the plate with a membrane.

2.3.2. Talent putting the plate in the shaker-incubator and closing the door.

2.4. To make persister assay plates, prepare 25 milliliters of modified LB medium containing 5 micrograms per milliliter of OFX in a 50-milliliter centrifuge tube **[1]** and transfer this medium to a sterile reservoir **[2]**. Transfer 190 microliters of the modified LB medium into each well of a generic flat-bottom 96-well plate **[3]**. *Videographer: This step is important!*

2.4.1. Talent adding OFX to LB.

2.4.2. Talent pouring the medium into a reservoir.

2.4.3. Talent transferring the medium to a well plate.

2.5. After the 24-hour incubation, remove the microarray from the shaker **[1]** and transfer 10 microliters of cell cultures from the microarray to the wells of the persister-assay plate containing modified LB medium with OFX **[2]**.

2.5.1. Talent removing the microarray from the shaker.

2.5.2. Talent transferring cell culture into the plate with OFX-LB.

2.6. Take 10 microliters of cell suspensions from the persister-assay plate and serially dilute it three times in 290 microliters of PBS solution using a round-bottom 96-well plate and a multichannel pipette **[1]**. *Videographer: This step is difficult and important!*

2.6.1. Talent making the serial dilutions.

2.7. Then, spot 10 microliters of all serially diluted cell suspensions on antibiotic-free fresh agar plates **[1]**. Cover the persister-assay plate with a gas-permeable sealing membrane **[2]** and incubate it in an orbital shaker at 37 degrees Celsius and 250 rpm for 6 hours **[3]**. *Videographer: This step is difficult and important!*

2.7.1. Talent spotting cells on the agar plate.

2.7.2. Talent covering the persister-assay plate with the membrane.

2.7.3. Talent putting the plate in the incubator and closing the door.

2.8. After the 6-hour incubation, repeat the serial dilution and spotting on agar plates **[1]**.

2.8.1. Talent taking the persister-assay plate out of the incubator.

2.9. Incubate the agar plates for 16 hours at 37 degrees Celsius, then count the colony forming units, or CFUs **[1]**. Use the CFU levels before and 6 hours after the antibiotic treatment to calculate the persister fraction in each well. The CFU counts before the OFX treatment also help assess the effects of osmolytes on *E. coli* viability **[2]**.

2.9.1. Talent taking the agar plate out of the incubator.

2.9.2. Talent counting CFUs.

3. Validating the Identified Conditions

3.1. Transfer 250 microliters of exponential phase cells to 25 milliliters of fresh modified LB medium containing the osmolyte identified from the microarray screening **[1]**. Incubate the flask in an orbital shaker at 250 rpm and 37 degrees Celsius for 24 hours **[2]**.

3.1.1. Talent transferring cells to LB with osmolyte.

3.1.2. Talent putting the cells in the shaker-incubator.

3.2. After 24 hours, remove the flask from the shaker **[1]** and transfer 250 microliters of the cell culture to 25 milliliters of fresh modified LB medium in a 250-milliliter baffled flask **[2]**.

3.2.1. Talent removing the flask from the shaker.

3.2.2. Talent transferring cells to fresh LB.

3.3. Add 25 microliters of OFX stock solution to the cell suspension **[1-TXT]** and shake the flask gently to make the assay culture homogenous **[2]**. Incubate the flask in a shaker at 37 degrees Celsius and 250 rpm **[3]**.

3.3.1. Talent adding OFX stock to the cells. **TEXT: 5 mg/mL** NOTE: 3.3.1 and 3.3.2 may have been merged

3.3.2. Talent shaking the flask.

3.3.3. Talent putting the flask in the shaker-incubator.

3.4. At every hour during the treatment, including a time point before addition of ofloxacin, transfer 1 milliliter of the assay culture from the flask to a 1.5-milliliter microcentrifuge tube **[1]** and centrifuge it at 17,000 *x g* for 3 minutes **[2]**. *Videographer: This step is important!*

3.4.1. Talent transferring 1 mL of cell culture into a microcentrifuge tube.

3.4.2. Talent putting the tube in the centrifuge and closing the lid.

3.5. Remove 950 microliters of supernatant and wash the cells 3 times with 950 microliters of PBS **[1]**. After the final wash, resuspend the cell pellet in 100 microliters of PBS solution **[2]**. Take 10 microliters of the cell suspension and serially dilute it 6 times with 90 microliters of PBS inside a 96 well round bottom plate **[3]**. *Videographer: This step is important!*

3.5.1. Talent removing supernatant and adding PBS.

3.5.2. Talent resuspending cells in PBS.

3.5.3. Talent making serial dilutions.

3.6. Spot 10 microliters of the diluted cell suspensions on an antibiotic-free agar plate **[1]**. To increase the limit of detection, plate the remaining 90 microliters of cell suspension on a fresh agar plate **[2]**. Incubate the plates at 37 degrees Celsius for 16 hours, then count CFUs **[3]**.

3.6.1. Talent spotting the cells on agar.

3.6.2. Talent plating the remaining cell suspension on a fresh agar plate.

3.6.3. Talent putting the agar plates in the incubator and closing the door.

Results

4. Results: Microarray and Validation

4.1. A representative image of agar plates used to determine CFU levels of cell cultures before and after OFX treatment is shown here **[1]**.

4.1.1. LAB MEDIA: Figure 2 A.

4.2. The first column is the control group **[1]**, the second column has cells that were cultured in 100 millimolar sodium nitrate **[2]**, and the third column contains cells that were cultured in 60 millimolar sodium nitrite. For simplicity, only two osmolytes are focused on here **[3]**.

4.2.1. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the first column in each image.*

4.2.2. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the second column in each image.*

4.2.3. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the third column in each image.*

4.3. A graphical representation of the CFU data from the plates **[1]** and persister fractions of the cell cultures tested in 96 well plates are shown here **[2]**. To calculate the fractions, persister counts were normalized to the cell counts obtained before the antibiotic treatments **[3]**.

4.3.1. LAB MEDIA: Figure 2 B and C. Video Editor: Emphasize B.

4.3.2. LAB MEDIA: Figure 2 B and C. Video Editor: Emphasize C.

4.3.3. LAB MEDIA: Figure 2 C.

4.4. To generate biphasic kill curves, the cells were cultured in 25 milliliters of modified LB medium with the indicated osmolytes in baffled flasks for 24 hours, then transferred to persister-assay flasks for persister enumeration **[1]**.

4.4.1. LAB MEDIA: Figure 3.

Conclusion

5. Conclusion Interview Statements

5.1. **Prashant Karki:** When attempting this protocol, keep in mind that spotting the cells on agar plates is labor intensive and requires focus. An error during this step can lead to cross-contamination among the conditions being tested, so it is important to practice spotting multiple samples simultaneously prior to the experiment.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.7.1.*

5.2. **Prashant Karki:** Following this protocol, drug panels and mutant cell libraries can be screened to study their impacts on persistence.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

5.3. **Prashant Karki:** With this screening, we have identified a number of osmolytes and pH conditions that have a significant impact on bacterial persistence.

5.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.