FINAL SCRIPT: APPROVED FOR FILMING

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Title: Generation of Monoclonal Cultures from *Wolbachia*-Infected *Drosophila melanogaster* JW18 Cell Line

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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**

   Videographer: Please capture the videos for the shots labelled SCOPE, using a SCOPE KIT

   SCOPE: 2.5.3, 2.11.1

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: 14
Number of Shots: 28

Introduction

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

1.1. **Navina Mable Francis:** We aim to generate monoclonal JW18 cell lines to study *Wolbachia* phenotypes in homogeneous cell populations. These should have consistent characteristics and produce less experimental variability than mixed cell populations.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What advantage does your protocol offer compared to other techniques?

1.2. **Navina Mable Francis:** Our protocol is comparatively low-cost, avoids specialized equipment, minimizes cell stress, and enables reproducible generation of clonal insect cell cultures.

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

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

1.3. **Ewa Chrostek:** We will be able to follow evolution of Wolbachia bacteria in monoclonal cell lines, and estimate the rate of Drosophila genome evolution in cell culture.

1.3.1. 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.

2. Preparation and Clonal Expansion of JW18 *Drosophila* Cells Using Conditioned Medium in a 96-Well Format

Demonstrator: Navina Mable Francis

NOTE: scope kit was not needed as the microscope in the laboratory was electronic and displayed an image on a screen. Extra shots are mostly close ups with no extra action

2.1. To begin, prepare fresh culture medium by supplementing Shield and Sang Insect medium with 10 percent FBS **[1]**.

2.1.1. WIDE: Talent preparing medium by adding fetal bovine serum to a bottle of Shield and Sang Insect medium using a serological pipette. NOTE: 2.1.1 is splitted for two (WIDE and close up- 2.1.1.A)

2.2. Using a 25 square centimeter flask, grow JW18 cells in fresh FBS-supplemented Shield and Sang Insect medium at 25 degrees Celsius **[1]**. Then, scrape the cells from the flask, resuspend them in fresh medium, add cells to a new flask, and label it **[2].** Incubate the flask until the cells reach confluency, approximately 1 week **[3]**. NOTE: The VO is edited for additional shots

2.2.1. Talent pipetting culture medium into a flask and adding JW18 cells.
Added shot: Talent scraping the cells off the flask, resuspending and adding cells to new flask and labelling the new flask. NOTE: Shots are slated as 2.2.1A, 2.2.1B and 2.2.1C

2.2.2. Shot of the confluent culture flasks. NOTE: extra shot to put after 2.2.2. is: 2.2.2.A

2.3. Now, transfer the cell culture media from the flask to a 50-milliliter tube **[1].** Filter the 1-week-old spent medium first through a 5-micrometer sterile filter **[2]** followed by a 0.2-micrometer sterile filter fitted to a 50-milliliter syringe to remove *Wolbachia*, cell debris, and other contaminants **[3]**. NOTE: The VO is edited for additional shots

Added shot: Talent moving cell culture media from flask to a 50 mL tube. NOTE: Slated as 2.3.1

2.3.1. Talent filtering the culture through 5 µm sterile filter attached to 50 mL syringe. NOTE: Slated as 2.3.1A

2.3.2. Shot of the filtrate being filtered through 0.2 µm sterile filter attached to 50 mL

syringe.

2.4. Now with a serological pipette, pipette 6 milliliters of filtered spent medium and 24 milliliters of fresh medium to prepare conditioned medium **[1-TXT]**.

2.4.1. Talent pipetting spent and fresh medium into a labelled conical tube and mixing gently. **TXT: Total volume: 30 mL per well plate**

2.5. For the preparation of JW18 cell suspension, use a cell scraper to scrape the cells from a confluent, 1-week-old flask **[1].** Resuspend them using a serological pipette **[2]**. Count the cells using a Neubauer chamber **[3].** Dilute the suspension with conditioned medium to a final concentration of 2000 cells per milliliter **[4]**.
Videographer: Please capture the videos for the shots labeled SCOPE, using a scope kit

2.5.1. Talent scraping cells from a flask.

2.5.2. Talent pipetting scraped culture into a tube. NOTE: extra shot to put after 2.5.2 - named as 2.5.3.0

2.5.3. SCOPE: The cell suspension in a Neubauer chamber is being seen. NOTE: extra shots to put after 2.5.3 - it is 2.5.3.A and 2.5.3.B

2.5.4. Talent preparing the diluted culture.

2.6. Transfer the conditioned medium to a reagent reservoir **[1]**. Then use a multichannel pipette to add 100 microliters of conditioned medium to each well of a 96-well plate, except well A1 **[2]**.

2.6.1. Talent pouring medium into a reagent reservoir. NOTE: extra shot to put after 2.6.1 - named as 2.6.1.A

2.6.2. Talent using multichannel pipette to dispense medium into the 96-well plate, skipping well A1.

2.7. Now pipette 200 microliters of the cell suspension to well A1 **[1]**. Using a single-channel pipette, transfer 100 microliters from well A1 to well B1 and mix gently to avoid bubbles **[2]**. Discard the final 100 microliters from the last well **[3]**. NOTE: The VO is edited for the deleted shot

2.7.1. Talent pipetting cell suspension into well A1.

2.7.2. Talent performing serial dilution with careful mixing. NOTE: 2.7.2 and 2.7.3 are combined in a single shot

~~2.7.3. Shot of wells in Column 1 containing diluted cell suspension.~~ NOTE: Not filmed

2.7.4. Talent discarding final volume after last well.

2.8. Next, add 100 microliters of conditioned medium to each well in the first column to achieve a volume of 200 microliters and mix **[1]**. With a multichannel pipette, transfer 100 microliters from the first column to the second and mix gently **[2]**.

2.8.1. Talent adding 100 microliters of conditioned medium to wells of the first column and mixing.

2.8.2. Talent transferring 100 microliters from column 1 to the 2nd column.

2.9. Repeat dilutions for columns 1 to 12 across the plate **[1]**. After mixing, discard 100 microliters from the last column so each well contains 100 microliters **[2]**. Add another 100 microliters of culture medium to bring the final volume to 200 microliters per well **[2]**.

2.9.1. Talent diluting samples across the plate.

2.9.2. Talent discarding final volume from column 12.

2.9.3. Talent topping up wells to final volume with culture medium.

2.10. Cover the plate, seal it with parafilm, and label it appropriately **[1]**.

2.10.1. Talent sealing plate with parafilm and applying label.

2.11. Next, observe the plate under a microscope, scanning each well and its edges to confirm the presence of single cells **[1]**. Record the well number of each single cell on a 96-well plate map on paper **[2]**.

2.11.1. SCOPE: Microscope view of single cells in a well, with zoom on edges. NOTE: extra shot to put after 2.11.1 named as 2.11.1.A

2.11.2. Talent marking well locations on a printed plate map.

2.12. After the identification of single cells, place the plate in an incubator set to 25 degrees Celsius **[1-TXT]**.

2.12.1. Talent placing sealed plate in incubator. **TXT: Monitor the wells every 2 days**

2.13. Inspect the wells every 2 to 3 days for bacterial contamination and to ensure the medium has not dried out **[1]**. Top up with fresh medium if needed **[2]**.

2.13.1. Talent examining wells closely for contamination.

2.13.2. Talent pipetting fresh medium into drying wells.

2.14. Once the cells reach confluence in the 96-well plate, transfer them to a 24-well plate

[1]. Subsequently, transfer the cells to a 6-well plate and finally to a flask **[2]**.

2.14.1. Talent pipetting confluent cells into a 24-well plate. NOTE: extra CLOSE UP shots to mix with wider but describing this same procedure 2.14.1 named as 2.14.1.A

2.14.2. Talent transferring cells to a 6-well plate. NOTE: series of extra CLOSE UPs shots to mix with wider but describing this same procedure 2.14.2 named as 2.14.2.A, 2.14.2.B and 2.14.2.C

Results

3. Results

3.1. The first signs of cell division were observed on day 3, with wells containing approximately four cells each **[1]**. By day 14, clonal growth was observed either as a monolayer **[2]**, or as a sphere in JW18-G4 *(J-W-Eighteen-G-Four)* **[3]**.

3.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the day 3 row*

3.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the day 14 images for JW18-B9, JW18-C7, and JW18-E5*

3.1.3. LAB MEDIA: Figure 2. *Video editor: Highlight the day 14 image for JW18-G4*

3.2. On day 28, visible differences in growth rates were most apparent among the clones **[1]**. The JW18-C7 *(J-W-Eighteen-C-Seven)* clone reached confluence by day 45 **[2]**, while the remaining clones achieved continuous growth only by day 77 **[3]**.

3.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the day 28 row*

3.2.2. LAB MEDIA: Figure 2. *Video editor: Highlight the JW18-C7 image in the day 45 row*

3.2.3. LAB MEDIA: Figure 2. *Video editor: Highlight the images of JW18-B9, JW18-E5, and JW18-G4 in the day 77 row*

3.3. By the 30th passage, the four established clones displayed distinct cell morphologies **[1]**.

3.3.1. LAB MEDIA: Figure 3. *Video editor: Sequentially highlight each image panel*

3.4. PCR analysis detected *Wolbachia wsp (W-S-P)* gene in all clones except JW18-C7, indicating its loss of infection **[1]**. FISH *(Fish)* analysis confirmed Wolbachia presence in JW18-B9, JW18-E5, and JW18-G4, but not in JW18-C7 **[2]**.

3.4.1. LAB MEDIA: Figure 4. *Video editor: Highlight the top panel and focus on the absence of the band for JW18-C7 compared to other lanes.*

3.4.2. LAB MEDIA: Figure 5. *Video editor: In the "Merged" column, highlight the magenta signals corresponding to Wolbachia in rows JW18-B9, JW18-E5, and JW18-G4.*

1. serological
Pronunciation link:
https://www.merriam-webster.com/dictionary/serological

IPA: /ˌsɛrəˈlɑdʒɪkəl/
Phonetic Spelling: seh-ruh-LAH-juh-kul

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2. Neubauer
Pronunciation link:
https://www.merriam-webster.com/dictionary/Neubauer
IPA: /ˈnɔɪˌbaʊər/
Phonetic Spelling: nohy-BOW-er

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3. confluent
Pronunciation link:
https://www.merriam-webster.com/dictionary/confluent
IPA: /ˈkɒnfluənt/ (or in American accent /ˈkɑnfluənt/)
Phonetic Spelling: KON-flu-ent

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4. parafilm
Pronunciation link:
https://www.merriam-webster.com/dictionary/Parafilm
IPA: /ˈpærəˌfɪlm/
Phonetic Spelling: PAIR-uh-film

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5. Wolbachia
Pronunciation link:
https://www.merriam-webster.com/dictionary/Wolbachia
IPA: /ˌwɒlbækˈiə/ (American: /ˌwɔlˈbæk.i.ə/)
Phonetic Spelling: wol-BAK-ee-uh

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6. fetal
Pronunciation link:
https://www.merriam-webster.com/dictionary/fetal
IPA: /ˈfitəl/
Phonetic Spelling: FEE-tul

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7. JW18
No confirmed link found (this is a specific clone/strain identifier, not a standard dictionary entry)
IPA: /dʒeɪ-dʌbəl.juː-eɪˈtiːn/
Phonetic Spelling: jey-DOUB-yoo-ay-EEN