FINAL SCRIPT: APPROVED FOR FILMING

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Title: Developmental Toxicity Assay Based on Real-Time Monitoring of Fibroblast Growth Factor Signal Disruption in Human Induced Pluripotent Stem Cells

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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? **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: 16
Number of Shots: 27

Introduction

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

1.1. **Koki Murayama:** This study explores an alternative to animal testing that employs cultured cells to assess chemical toxicity. The central question is whether disruptions in signaling pathways of human induced pluripotent stem (iPS) cells can serve as reliable indicators of developmental toxicity.

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

~~What are the most recent developments in your field of research?~~

~~1.2. Enter author name: Most recently, two approaches using human pluripotent stem cells have been reported for an in vitro developmental toxicity assay; one is based on changes in ornithine and cystine and the other is based on differentiation into tissue-specific cells.~~

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

What research gap are you addressing with your protocol?

1.3. **Koki Murayama:** We found that regardless of their mechanism, developmental toxicants disrupt signaling pathways, and their temporal sum serves as an indicator.

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

How will your findings advance research in your field?

1.4. **Koki Murayama:** This iPSC-based assay enables comprehensive evaluation of developmental toxicants, with high accuracy and throughput, and without the use of animals.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.4*

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

Testimonial Questions (OPTIONAL):

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

1.5. Enter author name:

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. Enter author name:

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

2. Cytotoxicity Assay Using Human Induced Pluripotent Stem Cells

Demonstrators: Koki Muaryama, Rieko Matsuura

2.1. To begin, seed cells into an ECM *(E-C-M)*-coated 96-well plate containing 200 microliters of fresh maintenance medium supplemented with 10 micromolar ROCK inhibitor **[1-TXT]**. Seal the plate with a gas-permeable film **[2]**.

2.1.1. WIDE: Talent pipetting cell suspension into each well of an extracellular matrix-coated 96-well plate. **TXT: Seed 2.0 × 10^4 cells/well**

2.1.2. Talent sealing the plate with a transparent, gas-permeable film.

2.2. After 24 hours, replace the medium in each well with 200 microliters of fresh maintenance medium **[1]** and reseal the plate **[2-TXT]**.

2.2.1. Talent aspirating old medium and pipetting 200 microliters of fresh maintenance medium into each well.

2.2.2. Talent resealing the plate with a gas-permeable film. **TXT: After 72 h and 96 h, replace the medium with assay medium**

2.3. Next, dissolve the test chemical in assay medium to its maximum solubility **[1]**.

2.3.1. Talent adding test chemical to a tube of assay medium.

2.4. Prepare a five-point, five-fold serial dilution of the test chemical in assay medium using the previously prepared solution **[1]**.

2.4.1. Talent pipetting test chemical in labeled tubes**. Videographer's NOTE**: We divided this shot into 2 shots:

2.4.1 - Adding 1% DMSO

2.4.1b -Adding other solutions

2.5. Add 11 microliters per well of the prepared chemical solutions **[1]**. For the vehicle control, add 11 microliters per well of assay medium containing the same concentration of DMSO *(D-M-S-O)* **[2]**. After 48 hours of treatment, incubate the plates at room temperature for 30 minutes **[3]**.

2.5.1. Talent pipetting 11 microliters of the test chemical solution into the wells of the

96-well plate. Authors: Please keep the labelled vial of chemical solution in the frame **Videographer's NOTE**: The content of 2.5.1 AND 2.5.2 shots is covered in 1 shot. The 2.5.2 content is found in shot 2.5.1, Take 2 @ 2:04:09 (filename: A003_09291209_C026.mov)

2.5.2. Talent pipetting 11 microliters of vehicle control into designated control wells.

2.5.3. Talent placing the plate into an incubator set at 25 degrees Celsius. **Videographer's NOTE**: Miss-slated as 2.5.5. Take 1 (shot 2.5.3). This shot is missing a slate (filename: A003_09291220_C027.mov), but take 2 is the better take

2.6. Then, add 111 microliters per well of pre-equilibrated ATP-based luminescent cell viability assay reagent at 25 degrees Celsius **[1]**. For the blank well, add 111 microliters of assay medium and 111 microliters of the reagent **[2]**.

2.6.1. Talent pipetting 111 microliters of cell viability reagent into each well of the plate. Authors: Please keep the labelled vial of chemical solution in the frame

2.6.2. Talent pipetting 111 microliters of assay medium and 111 microliters of reagent into the blank well.

2.7. Using a microplate shaker, shake the plate at 300 revolutions per minute for 2 minutes **[1]**.

2.7.1. Talent placing the plate on a microplate shaker and starting the 2-minute shake cycle.

2.8. Transfer 200 microliters of the mixture from each well to a black plate for luminescence measurement **[1]** and incubate the black plate at 25 degrees Celsius for 10 minutes **[2]**.

2.8.1. Talent transferring 200 microliters of mixture from each well to a black 96-well plate.

2.8.2. Talent placing the black plate at room temperature (on bench).

2.9. Then, measure the luminescence intensity **[1]**.

2.9.1. Show the luminometer software interface as the luminescence readings are acquired.

2.10. For the determination test, after incubating the cells for 96 hours, replace the medium in each well with 100 microliters of assay medium **[1]**. Dissolve the test chemical in assay

medium based on the results obtained from the preliminary test **[2]**.

2.10.1. Talent aspirating medium from the wells and pipetting 100 microliters of fresh assay medium.

2.10.2. Talent dissolving the test chemical in assay medium.

2.11. Prepare a nine-point, two-fold serial dilution of the test chemical in assay medium using the previously prepared solution **[1]**.

2.11.1. Talent performing a nine-point, two-fold serial dilution using pipettes and a set of labeled tubes.

2.12. Add 11 microliters per well of the prepared chemical solutions **[1]**. For the vehicle control, add 11 microliters per well of assay medium containing the same concentration of DMSO **[2-TXT]**.

2.12.1. Talent pipetting 11 microliters of diluted chemical solutions into the wells. **Videographer's NOTE**: 2.12.1 - 2.12.2 - Shots combined. The content of these 2 shots is covered in 1 shot

2.12.2. Talent pipetting 11 microliters of vehicle control solution into control wells. **TXT: Incubate for 48 h and proceed similarly to the preliminary test**

3. Real-Time Reporter Assay

3.1. After incubating the cells for 96 hours, replace the medium in each well with 100 microliters of assay medium containing 1 percent luminescent substrate **[1]**. Dissolve the test chemical in assay medium at the concentration determined from the previous section **[2]**.

3.1.1. Talent aspirating old medium and pipetting 100 microliters of assay medium with luminescent substrate into each well.

3.1.2. Talent preparing the test chemical solution in assay medium based on recorded concentration data.

3.2. Prepare an eight-point, two-fold serial dilution of the test chemical in assay medium using the previously prepared solution **[1]**.

3.2.1. Talent performing eight-point, two-fold serial dilution using pipettes and labeled tubes.

3.3. Two hours after the medium exchange, add 11 microliters per well of the serially diluted chemical solutions **[1]**. For the vehicle control, add 11 microliters per well of assay medium containing the same concentration of DMSO **[2]**.

3.3.1. Talent pipetting 11 microliters of diluted test chemical into experimental wells. **Videographer's NOTE**: 3.3.1 - 3.3.2 - Shots combined. The content of these 2 shots is covered in 1 shot

3.3.2. Talent pipetting 11 microliters of vehicle control solution into control wells.

3.4. One hour after starting chemical exposure, add 12 microliters per well of assay medium containing basic fibroblast growth factor **[1-TXT]**. Measure luminescence continuously for 48 hours using a real-time luminescence measurement system under 5 percent carbon dioxide at 37 degrees Celsius **[2]**.

3.4.1. Talent pipetting 12 microliters of assay medium with bFGF into each well. **TXT: Final concentration: 2 ng/mL**

3.4.2. LAB MEDIA: Screen-shot2.png.

Results

4. Results

4.1. Phase contrast imaging confirmed a confluent cell layer covering nearly the entire surface of the wells both before **[1]** and after chemical exposure **[2].**

4.1.1. LAB MEDIA: Figure 2A. *Video editor: Show the "Before exposure" panel*

4.1.2. LAB MEDIA: Figure 2A**.** *Video editor: Show the "After exposure" panel*

4.2. Live and dead staining showed that while the number of propidium iodide-stained dead cells increased after chemical exposure **[1],** the majority of cells remained viable and were stained with calcein-AM **[2].**

4.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the "After exposure" panel.*

4.2.2. LAB MEDIA: Figure 2B**.** *Video editor: Emphasize the dominant green fluorescence in the "After exposure" panel*

4.3. In the absence of test chemicals, luminescence intensity showed two distinct peaks across all six replicate wells, demonstrating high reproducibility and temporal resolution **[1].**

4.3.1. LAB MEDIA: Figure 3A.

4.4. Automated real-time monitoring revealed that valproic acid disrupted fibroblast growth factor signaling in a concentration-dependent manner **[1],** with stronger suppression at higher concentrations **[2].**

4.4.1. LAB MEDIA: Figure 3B (center panel, "Automatic"). *Video editor: Highlight the gradual suppression of luminescence curves from low (blue) to high (red) concentrations.*

4.4.2. LAB MEDIA: Figure 3B (center panel, "Automatic"). *Video editor: Emphasize the red curve which shows the most pronounced signal change*.

4.5. Manual luminescence measurements captured a general suppression pattern by valproic acid but lacked the temporal resolution seen in automated detection **[1].**

4.5.1. LAB MEDIA: Figure 3B (right panel, "Manual"). *Video editor: Highlight the rising red and orange curves (higher VPA doses).*

5. • **extracellular**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/extracellular
   IPA: /ˌɛk·strəˈsɛl·jə·lər/
   Phonetic spelling: **ek-struh-SELL-yuh-lur**
6. • **maintenance**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/maintenance
   IPA: /ˈmeɪn·tə·nəns/
   Phonetic spelling: **MAYN-tuh-nuhns**
7. • **supplemented**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/supplemented
   IPA: /ˈsʌp·lə·mən·tɪd/
   Phonetic spelling: **SUP-luh-men-tid**
8. • **serial**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/serial
   IPA: /ˈsɪ·ri·əl/
   Phonetic spelling: **SEER-ee-uhl**
9. • **luminescent**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/luminescent
   IPA: /ˌlu·məˈnɛs·ənt/
   Phonetic spelling: **loo-muh-NESS-uhnt**
10. • **viability**
    Pronunciation link:
    https://www.merriam-webster.com/dictionary/viability
    IPA: /ˌvaɪ·əˈbɪl·ə·ti/
    Phonetic spelling: **vy-uh-BIL-uh-tee**
11. • **temporal**
    Pronunciation link:
    https://www.merriam-webster.com/dictionary/temporal
    IPA: /ˈtɛm·pə·rəl/
    Phonetic spelling: **TEM-puh-rul**
12. • **confluent**
    Pronunciation link:
    https://www.merriam-webster.com/dictionary/confluent
    IPA: /ˈkɒn·flu·ənt/
    Phonetic spelling: **KON-floo-uhnt**
13. • **fluorescence**
    Pronunciation link:
    https://www.merriam-webster.com/dictionary/fluorescence
    IPA: /flʊəˈrɛs·əns/
    Phonetic spelling: **flu-oh-RESS-uhns**

14. • **automated**
Pronunciation link:
https://www.merriam-webster.com/dictionary/automated
IPA: /ˈɔ·tə·meɪ·tɪd/
Phonetic spelling: **AW-tuh-may-tid**