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Title: Detection of Aggregation-Prone Behavior in Mutant p53 V157F Breast Cancer Cells Using Multipoint Thioflavin T Fluorescence

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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
Authors: All the protocol videos are already provided by you and are approved to be used

2. Interview statements: Which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interview Statements are read by JoVE's voiceover talent.

Authors: We will internally generate the voice-over for the interview answers.

Current Protocol Length

Number of Steps: 14
Number of Shots: 28

Introduction

NOTE to VO producer: Please generate the VO for the interview answers

INTRODUCTION:

What is the scope of this research?

1.1. This study examines the aggregation propensity of mutant p53 V157F in breast cancer cells and evaluates multipoint plate-reading for detection.

1.1.1. B: Roll: 2.1.3

What are the current experimental challenges?

1.2. Current experimental challenges include accurately detecting p53 aggregation, quantifying amyloid-like structures, and ensuring consistent measurement across cell-based assay surfaces.

1.2.1. B: Roll: 3.4.1

CONCLUSION:

What advantage does this protocol offer compared to other techniques?

1.3. This protocol enables accurate protein aggregation detection in cellular contexts using Thioflavin T staining combined with reliable multipoint fluorescence plate-reading methods.

1.3.1. B: Roll: 4.2.1

2. Seeding the Cells before Staining

Demonstrators: Shao Chin, Zi Zeng and Sih Chen

2.1. To begin, take 10 microliters of Trypan Blue in a microcentrifuge tube **[1]**. Add 10 microliters of resuspended cells in DPBS to the same tube **[2-TXT]**, and mix the contents thoroughly to ensure homogeneity **[3]**.

2.1.1. LAB MEDIA: 1.2. 00:22-00:30 (taking trypan blue) and 00:45-00:48 (adding to a tube).

2.1.2. LAB MEDIA: 1.2. 01:55-02:20. **TXT: DPBS: Dulbecco's Phosphate-Buffered Saline**

2.1.3. LAB MEDIA: 1.3. 00:40-00:45.

2.2. Load 10 microliters of the prepared mixture onto a counter slide **[1]** to count the live cells using an automated cell counter **[2]**.

2.2.1. LAB MEDIA: 1.3. 00:55-01:10.

2.2.2. LAB MEDIA: 1.4. 00-00:05 and 00:15-00:23.

2.3. Based on the viability count, seed 30,000 viable cells per well into a 96-well culture plate **[1]**. Designate 1 well as a non-stained control, and designate the remaining 3 wells for staining **[2-TXT]**.

2.3.1. LAB MEDIA: 1.5. 00:25-00:35.

2.3.2. LAB MEDIA: 1.5. 00:40-00:50. **TXT: Incubation: 37 ºC; 12 h**

3. Thioflavin T Staining and Nuclear Counterstaining

3.1. Weigh 0.02 grams of Thioflavin T or ThT powder using a balance **[1]**. Add the powder to 5 milliliters of deionized water **[2]**, and mix thoroughly until the powder is completely dissolved **[3]**.

3.1.1. LAB MEDIA: 3-1. 00:20-00:30.

3.1.2. LAB MEDIA: 3-2. 00:25-00:32.

3.1.3. LAB MEDIA: 3-2. 00:33-00:41.

3.2. Then, add the staining components to 1 milliliter of DPBS **[1]**. Add ThT and Hoechst stock

solutions to the tube and mix gently to ensure homogeneity **[2-TXT]**.

3.2.1. LAB MEDIA: 4-1. 00:20-00:32.

3.2.2. LAB MEDIA: 4-1. 01:20-01:30 and 4-3.

TXT: ThT: Add 1 µL of 1,000x ThT stock (Final concentration: 12.5 µM)

Hoechst 33342: Add 1 µL of 1 mg/mL stock (Final concentration: ~1 µg/mL =1.6 µM)

3.3. Now, remove the culture medium from each well of the 96-well culture plate **[1]**. Add 100 microliters of the prepared ThT and Hoechst staining buffer to each staining well **[2]**, followed by 100 microliters of DPBS to the non-stained control well **[3]**.

3.3.1. LAB MEDIA: 5. 00:10-00:20.

3.3.2. LAB MEDIA: 5. 02:39-02:50.

3.3.3. LAB MEDIA: 5. 01:58-02:10.

3.4. Place the culture plate in a dark place at room temperature for 30 minutes **[1]**.

3.4.1. LAB MEDIA: 6.

3.5. Carefully aspirate or discard the staining solution from each well without disturbing the cell monolayer **[1]**.

3.5.1. LAB MEDIA: 7. 00:05-00:20.

3.6. Then, add 100 microliters of DPBS to each well and leave it for 30 seconds **[1]**. Then, discard the DPBS **[2]**.

3.6.1. LAB MEDIA: 7. 00:30-00:40.

3.6.2. LAB MEDIA: 7. 01:10-01:20.

3.7. After repeating the washing step, add 100 microliters of fresh DPBS to each well **[1]**.

3.7.1. LAB MEDIA: 7. 01:55-02:00 and 02:14-02:17.

4. Fluorescence Measurement and Data Analysis

Demonstrators: Sih Chen and Bi Cai

4.1. Remove the cap from the 96-well culture plate before reading the fluorescence signal **[1-TXT]**.

4.1.1. LAB MEDIA: 8. 00:24-00:29. **TXT: Select either a single- or four-point reading method**

4.2. Place the 96-well culture plate into the microplate reader **[1]**. Measure ThT fluorescence with excitation at 450 nanometers and emission at 490 nanometers **[2]**, and measure Hoechst 33342 fluorescence with excitation at 360 nanometers and emission at 460 nanometers **[3]**. Select the desired read area **[4]** and then set the reader parameters, including shaking for 5 seconds before reading, top read direction and an integration time of 140 milliseconds **[5-TXT].**

4.2.1. LAB MEDIA: 8. 00:29-00:34.

4.2.2. LAB MEDIA: 8. 00:08-00:12 (Freeze frame at 00:12). *Video editor: Highlight the numbers 450 and 490 in the boxes*

4.2.3. LAB MEDIA: 8. 00:12 (Freeze frame at 00:12). *Video editor: Highlight the numbers 360 and 460 in the boxes*.

4.2.4. LAB MEDIA: 8. 00:14-00:17.

4.2.5. LAB MEDIA: 8. 00:18-00:22. **TXT: Acquire the fluorescence data**

4.3. After acquiring the fluorescence data, perform calculations to obtain the protein aggregation values **[1]**.

4.3.1. TEXT ON PLAIN BACKGROUND:

$$\text{Aggregation Signal} = \frac{\text{ThT OD}_{\text{staining well}} - \text{ThT OD}_{\text{non-staining well}}}{\text{Hoechst OD}_{\text{staining well}} - \text{Hoechst OD}_{\text{non-staining well}}}$$

4.4. Finally, normalize the ThT fluorescence intensity by setting the ratio in MCF7 cells as 1 to serve as the baseline control **[1]**.

4.4.1. LAB MEDIA: 8. 00:50-00:58. **Authors**: A representative shot will be used here as the actual shot is not available **NOTE**: The authors have approved to use this clip

Results

5. Results

5.1. Measurements were performed using both a single-point **[1]** and a four-point method across stained and non-stained wells in MCF7 and Hs578T cells *(H-S-578-T)* **[2]**.

5.1.1. LAB MEDIA: Figure 1. *Video editor: Show the well diagram marked "1-point" to show the red dot in the center*.

5.1.2. LAB MEDIA: Figure 1. *Video editor: Show the well diagram marked "4-point" to show the four color-coded dots*

5.2. Hs578T breast cancer cells showed a 3.2-fold increase in Thioflavin T fluorescence intensity compared to MCF7 cells using the single-point reading method **[1]**.

5.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the single bar labeled "Hs578T" under the "1 point" group*

5.3. When using the four-point average reading method, Hs578T cells exhibited a 3.86-fold increase in Thioflavin T fluorescence compared to MCF7 cells **[1]**.

5.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the bar labeled "Hs578T" under the "4 points (average)" group*

5.4. Thioflavin T fluorescence in Hs578T cells was consistently elevated at all four measurement points compared to MCF7 cells **[1]**.

5.4.1. LAB MEDIA: Figure 3. *Video editor: Highlight the 4 bars for "Hs578T"*

1. Microliter
   Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
   IPA: /ˈmaɪ.kroʊˌliː.tər/
   Phonetic Spelling: my·kroh·lee·ter
2. Trypan Blue
   Pronunciation link: https://www.merriam-webster.com/dictionary/trypan%20blue
   IPA: /ˈtrɪpˌæn bluː/
   Phonetic Spelling: trip·an bloo
3. Microcentrifuge
   Pronunciation link: https://www.merriam-webster.com/dictionary/microcentrifuge

IPA: /ˌmaɪ.kroʊˈsɛn.trəˌfjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj

4. Homogeneity
Pronunciation link: https://www.merriam-webster.com/dictionary/homogeneity
IPA: /ˌhoʊ.mə.dʒəˈniː.ə.ti/
Phonetic Spelling: hoh·muh·juh·nee·uh·tee
5. DPBS
Pronunciation link: No confirmed link found
IPA: /ˌdiː piː biː ˈɛs/
Phonetic Spelling: dee·pee·bee·ess
6. Dulbecco's Phosphate-Buffered Saline
Pronunciation link: https://www.howtopronounce.com/dulbecco
IPA: /dʌlˈbɛkoʊz ˈfɑs.feɪt ˈbʌf.ərd səˈliːn/
Phonetic Spelling: dull·BECK·ohz foss·fayt buf·erd suh·leen
7. Automated
Pronunciation link: https://www.merriam-webster.com/dictionary/automated
IPA: /ˈɔː.təˌmeɪ.tɪd/
Phonetic Spelling: aw·tuh·may·tid
8. Thioflavin T
Pronunciation link: https://www.howtopronounce.com/thioflavin
IPA: /ˌθaɪ.oʊˈfleɪ.vɪn tiː/
Phonetic Spelling: thigh·oh·flay·vin tee
9. Deionized
Pronunciation link: https://www.merriam-webster.com/dictionary/deionized
IPA: /diːˈaɪ.əˌnaɪzd/
Phonetic Spelling: dee·eye·uh·nyzd
10. Hoechst
Pronunciation link: https://www.howtopronounce.com/hoechst
IPA: /ˈhɛkst/
Phonetic Spelling: hekست
11. Fluorescence
Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescence
IPA: /flʊˈrɛs.əns/
Phonetic Spelling: floor·ESS·uhns
12. Microplate
Pronunciation link: https://www.merriam-webster.com/dictionary/microplate
IPA: /ˈmaɪ.kroʊˌpleɪt/
Phonetic Spelling: my·kroh·playt
13. Nanometer
Pronunciation link: https://www.merriam-webster.com/dictionary/nanometer
IPA: /ˈnæn.oʊˌmiː.tər/
Phonetic Spelling: nan·oh·mee·ter
14. Integration
Pronunciation link: https://www.merriam-webster.com/dictionary/integration

IPA: /ˌɪn.təˈgreɪ.ʃən/
Phonetic Spelling: in·tuh·GRAY·shuhn

15. Millisecond
Pronunciation link: https://www.merriam-webster.com/dictionary/millisecond
IPA: /ˈmɪl.iˌsɛk.ənd/
Phonetic Spelling: mil·ih·sek·uhnd
16. Aggregation
Pronunciation link: https://www.merriam-webster.com/dictionary/aggregation
IPA: /ˌæg.rəˈgeɪ.ʃən/
Phonetic Spelling: ag·ruh·GAY·shuhn
17. Normalize
Pronunciation link: https://www.merriam-webster.com/dictionary/normalize
IPA: /ˈnɔːr.məˌlaɪz/
Phonetic Spelling: NOR·muh·lyze