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Title: Live Cell Imaging with Time Lapse Photography to Study Epidermal Keratinocyte Proliferation Kinetics

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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? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 25
Number of Shots: 56 (40 SC)

Introduction

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

1.1. **Ruby Ghadially MD:** This research focuses on hyper- and hypoproliferative diseases such as psoriasis and aging, with an emphasis on how these conditions affect stem cell and progenitor cell proliferation. Identifying the specific sites of proliferative defects will enable the development of more precisely targeted therapies. **NOTE**: The statement was edited

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

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

1.2. **Ruby Ghadially MD:** For human epidermis we have not had techniques that allow tracking of committed progenitors for multiple generations. Finally, live cell imaging lets us follow a committed progenitor through multiple generations *in vitro* and has provided many insights into human committed progenitor behavior.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.2*

What significant findings have you established in your field?

1.3. **Ruby Ghadially MD:** We have used live cell imaging to show that while we know that stem cells divide less frequently than committed progenitors, when they do divide they divide more rapidly. When tissue damage occurs, stem cells can respond using rapid turnover.

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

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

Ethics Title Card

All human tissue was obtained after approval by the University of California, San Francisco (UCSF) Institutional Review Board (IRB), and consent was obtained for all the tissues used

Protocol

2. Preparing Passage 0 Human Keratinocytes for Imaging

Demonstrator: Brook Abegaze

2.1. To begin, obtain keratinocytes from fresh human skin **[1]**.

2.1.1. WIDE: Talent picking up the tube/dish with isolated keratinocytes.

2.2. Determine the appropriate seeding density for the assay and run pilot studies using samples at multiple dilutions from different donors **[1]**.

2.2.1. Talent adding cells to various tube from donor cell samples.

2.3. To achieve an even distribution of cells, aliquot the total media required for all wells at a specific dilution into a microtube **[1]**. Pipette the total number of cells needed to reach the desired density into the aliquot **[2]** and gently invert the microtube to homogenously distribute the cells **[3]**. Now add the suspension onto the plates **[4]**.

2.3.1. Talent pipetting media into the tube. **NOTE**: 2.3.1, 2.3.2 and 2.3.3 were shot together

2.3.2. Talent adding cell suspension into the same microtube.

2.3.3. Talent gently inverting the tube for even distribution.

2.3.4. Talent adding cell-suspension on a plate.

2.4. After plating, move the plate in a cross pattern three times—up-down, then left-right **[1]** and carefully transfer the plate to the incubator **[2-TXT]**. After incubation, change the media **[3]**.

2.4.1. Talent moving the plate in a cross-pattern on the bench. **TXT: 24 h; 37 °C; 5% CO_2**

2.4.2. Talent placing the plate inside the incubator gently.

2.4.3. Talent removing old media and adding with fresh media to the plate. *Videographer: Please obtain multiple reusable shots for this step. It will be used again in 3.11.1*

3. Time Lapse Photography of Passage 0 Human Keratinocytes

3.1. To open the incubator of the imaging system, press the large triangular button on the bottom left when the light turns green **[1].** Place the vessel in an open bay **[2]** and close the tray using the same button **[3]**.

3.1.1. Talent pressing the triangular button to open the incubator tray.

3.1.2. Talent placing the vessel in an open bay.

3.1.3. Talent pressing the button again to close the tray.

3.2. Now, open the application on the computer **[1]** and log in using the appropriate user identification and password **[2]**. Then, select **Schedule [3]**.

3.2.1. SCREEN: 3.1.1.

3.2.2. SCREEN: 3.1.2.

3.2.3. SCREEN: 3.2.3.

3.3. Press the **+** *(plus)* button underneath the schedule to add the plate to the schedule **[1]**.

3.3.1. SCREEN: 3.3.1 00:00-00:07.

3.4. Select **Scan on Schedule**, then click **Next [1]**.

3.4.1. SCREEN: 3.4.1 00:00-00:06.

3.5. Then, select **New [1]** and choose **Standard** for scan type **[2]**.

3.5.1. SCREEN: 3.5.1.

3.5.2. SCREEN: 3.5.2.

3.6. For scan settings, select **Adherent Cell-by-Cell** using the phase channel at 10x objective, then click **Next [1]**.

3.6.1. SCREEN: 3.6.1 00:00-00:08.

3.7. Next, choose the vessel, then click **Next [1]**. ~~Most common vessels are compatible with the machine, although to fit in the time-lapse microscope's bays **[2]**~~.

3.7.1. SCREEN: 3.7.1. **TXT: Non-microplates may need special attachments**

3.7.2. ~~Shot of talent attaching a non-microplate vessel into the microscope bay using an adapter~~. **NOTE**: Delete this shot

3.8. Select an empty bay to place the vessel, then click **Next [1]**. Select the wells to be scanned as well as the number of images per well, then click **Next [2]**.

3.8.1. SCREEN: 3.8.1.

3.8.2. SCREEN: 3.8.2.

3.9. Name the experiment using the desired naming convention **[1]**. Create a plate map of the experiment for future reference, then click **Okay [2]**. Now, click **Next** to continue **[3]**. Defer analysis until after data collection, as the cell-by-cell analysis must be initiated post scanning **[4]**. Click **Next** again **[5]**.

3.9.1. SCREEN: 3.9.1 00:00-00:10.

3.9.2. SCREEN: 3.9.2 00:09-00:12 and 01:10-01:14.

3.9.3. SCREEN: 3.9.3.

3.9.4. SCREEN: 3.9.4 00:00-00:05.

3.9.5. SCREEN: 3.9.5.

3.10. Set the frequency of imaging to 20-minute intervals to reliably track mitoses, which occur approximately every 30 minutes **[1]**. Press **Next** and confirm the settings to begin the experiment **[2]**.

3.10.1. SCREEN: 3.10.1.

3.10.2. SCREEN: 3.10.2 00:06-00:15.

3.11. Change media for keratinocytes every 48 hours **[1]**. To ensure correct plate orientation, wait for the first scan to complete each time a vessel is placed in the machine **[2]**. Place the plate so that the letters denoting the rows are on the left side of the bay **[3]**.

3.11.1. *Reuse 2.4.3*.

3.11.2. SCREEN: 3.11.2 01:19-01:26.

3.11.3. Shot of placing plate into the bay with row letters visible on the left side.

3.12. Follow adult colonies for approximately 2 weeks and neonatal colonies for around 10 days, at which time crowding reduces analytical quality **[2]**.

3.12.1. SCREEN: 3.12.1 00:05-00:14.

3.13. Once all wells have become quiescent or reached confluence, click the **View** tab and double-click the experiment under the list of recent scans **[1]**. Click the **Landscape** icon with the arrow and wait for the export tool to launch **[2]**.

3.13.1. SCREEN: 3.13.1.

3.13.2. SCREEN: 3.13.2.

3.14. Click **As Displayed**, then click **Next [1]**. Then, manually click each field of view to export, then click **Next [2]**.

3.14.1. SCREEN: 3.14.1 00:00-00:06 .

3.14.2. SCREEN: 3.14.2 00:04-00:14.

3.15. Select whether a movie or a series of images is desired **[1]**. For keratinocyte lineage tracing, choose the movie option and select all scans using the **Select all** icon and click **Next [2]**.

3.15.1. SCREEN: 3.15.1.

3.15.2. SCREEN: 3.15.2.

3.16. Export at 1 frame per second at maximum quality after adjusting the bar next to quality **[1]**. After choosing the target folder and file type **[2],** name the file and click **Export [3]**.

3.16.1. SCREEN: 3.16.1 00:01-00:09.

3.16.2. SCREEN: 3.16.2 00:02-00:13.

3.16.3. SCREEN: 3.16.3 00:10-00:18.

4. Constructing Lineage Trees and Data Sheets Using Time-Lapse Imaging

4.1. Open the video file using a media player **[1]** and scroll through the videos and identify growing colonies **[2]**. With VLC, take a screenshot of the colony to be tracked and label it **[3]**.

4.1.1. SCREEN: 4.1.1 00:01-00:08.

4.1.2. SCREEN: 4.1.2 00:10-00:16.

4.1.3. SCREEN: 4.1.3 00:15-00:18 and 00:40-00:43.

4.2. Identify a colony of interest either at the end of or after some days of video recording **[1]**. Rewind the video and identify the colony forming cell **[2]**. Pause the video when the cell is about to divide as it appears to condense **[3]**. Record the timestamp shown in the

bottom left corner **[4]**.

4.2.1. SCREEN: 4.2.1 00:12-00:17.

4.2.2. SCREEN: 4.2.2 00:04-00:13.

4.2.3. SCREEN: 4.2.3 00:20-00:25.

4.2.4. SCREEN: 4.2.4 00:07-00:13 .

4.3. Take a screenshot of the division and label it the same as the colony **[1]**. Document each division using a hand-drawn lineage diagram **[2-TXT]**.

4.3.1. SCREEN: 00:07-00:09 and 00:20-00:24.

4.3.2. Talent drawing a lineage diagram on paper. **TXT: Track and document as many generations as possible**

4.4. Transcribe the manual lineage tree into data sheets, referred to as green sheets due to the spreadsheet's color **[1]**.

4.4.1. SCREEN: 4.4.1.

4.5. Finally, use the green sheets to calculate the proportions of proliferative and differentiation divisions **[1]**. Identify stem cell and committed progenitor colonies and determine cell cycle duration **[2]**.

4.5.1. SCREEN: 4.5.1. 00:10-00:16

4.5.2. SCREEN: 4.5.2 00:20-00:25 .

Results

5. Results

5.1. Primary keratinocytes showed a stereotypical progression in appearance, starting as small rounded cells upon initial seeding **[1]**, then becoming flattened and mobile **[2]**. They begin condensing **[3]** prior to division **[4]** and form proliferative colonies **[5]** or terminally differentiated colonies with non-uniform morphology **[6]**.

5.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the small round cells marked by the arrow in the top image panel labeled "Initial seeding"*

5.1.2. LAB MEDIA: Figure 1. *Video editor: Highlight the flattened cells pointed to by the arrow in the "Flattened morphology" panel*

5.1.3. LAB MEDIA: Figure 1. *Video editor: Mark the cell indicated by the arrow in the "Condensing cell" panel; focus on its denser, darker center*

5.1.4. LAB MEDIA: Figure 1. *Video editor: Highlight the cluster of dividing cells circled in red in the "Cell Division" panel.*

5.1.5. LAB MEDIA: Figure 1*. Video editor: Highlight "Proliferating colony" panel*,

5.1.6. LAB MEDIA: Figure 1. *Video editor: Highlight "Differentiated colony" panel*

5.2. Once the green sheet is constructed, one can determine which colonies originate from stem cells and which originate from committed progenitors **[1]**.

5.2.1. LAB MEDIA: Figure 3 and Supplementary File 1 *Video editor: Show "COLONY 1" data in the worksheet*

Pronunciation guide :

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1. Keratinocytes

Pronunciation link:
https://www.merriam-webster.com/medical/keratinocyte
IPA: /ˌkɛr.əˈtɪn.əˌsaɪt/
Phonetic Spelling: keh-ruh-tin-uh-syte

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2. Pipette

Pronunciation link:
https://www.merriam-webster.com/dictionary/pipette
IPA: /paɪˈpɛt/
Phonetic Spelling: pie-pet

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3. Aliquot

Pronunciation link:
https://www.merriam-webster.com/dictionary/aliquot
IPA: /ˈælɪˌkwɒt/
Phonetic Spelling: al-ih-kwaht

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4. Homogenously *(Note: Not always listed separately from "homogeneous")*

Pronunciation link:
https://www.howtopronounce.com/homogenously
IPA: /həˈmɑː.dʒə.nəs.li/
Phonetic Spelling: huh-mah-juh-nuhs-lee

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5. Incubator

Pronunciation link:
https://www.merriam-webster.com/dictionary/incubator
IPA: /ˈɪn.kjəˌbeɪ.tər/
Phonetic Spelling: in-kyuh-bay-ter

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6. Adherent

Pronunciation link:
https://www.merriam-webster.com/dictionary/adherent
IPA: /ədˈhɪr.ənt/
Phonetic Spelling: uhd-hear-uhnt

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7. Mitoses *(plural of mitosis)*

Pronunciation link:
https://www.merriam-webster.com/dictionary/mitosis
IPA: /maɪˈtoʊ.siːz/
Phonetic Spelling: my-toh-seez

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8. Confluence

Pronunciation link:
https://www.merriam-webster.com/dictionary/confluence
IPA: /ˈkɑːn.flu.əns/
Phonetic Spelling: kahn-floo-uhns

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9. Lineage

Pronunciation link:
https://www.merriam-webster.com/dictionary/lineage
IPA: /ˈlɪn.i.ɪdʒ/
Phonetic Spelling: lin-ee-ij

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10. Proliferative

Pronunciation link:
https://www.howtopronounce.com/proliferative
IPA: /prəˈlɪf.əˌreɪ.tɪv/
Phonetic Spelling: pruh-lif-uh-ray-tiv

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11. Differentiated

Pronunciation link:
https://www.howtopronounce.com/differentiated
IPA: /ˌdɪ.fəˈrɛn.ʃi.eɪ.tɪd/
Phonetic Spelling: dih-fuh-ren-shee-ay-tid

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12. Progenitor

Pronunciation link:
https://www.merriam-webster.com/dictionary/progenitor
IPA: /proʊˈdʒɛn.ɪ.tər/
Phonetic Spelling: proh-jen-ih-ter