FINAL SCRIPT: APPROVED FOR FILMING

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Title: Assessment of DNase Activity by Ratiometric Fluorescence Resonance Energy Transfer

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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**

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

Current Protocol Length
Number of Steps: 11
Number of Shots: 16

Introduction

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

REQUIRED:

1.1. **Vladimir V. Didenko**: We are addressing the need for sensitive detection of weak nuclease activity that requires prolonged observation and various pH conditions.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.1, 2.4.1, 2.4.2.*

What research gap are you addressing with your protocol?

1.2. **Vladimir V. Didenko:** We developed this ratiometric FRET assay to detect and measure DNase activity of different biomolecules with high sensitivity and for extended time periods.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 2D, 2E.*

How will your findings advance research in your field?

1.3. **Candace L. Minchew:** This assay enables the systematic evaluation of how experimental parameters like pH, temperature, and buffer composition affect DNase activity in real-time.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.2.*

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

Testimonial Questions:

Videographer: Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.

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

1.4. **Vladimir V. Didenko, Associate Professor, Michael E. DeBakey Veterans Affairs Medical Center:** Publishing with JoVE offers unique benefits. Its video format makes my research easier to understand, helping more people apply it in their own work.

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

Protocol

2. Preparation of Incubation Mix and Induction of DNase Reaction Using a Ratiometric FRET Probe

Demonstrator: Candace L. Minchew

2.1. To begin, dilute the protein sample to an appropriate concentration in each buffer solution ranging from pH 4.8 to pH 6.5 **[1]**.

2.1.1. WIDE: Talent pipetting the protein sample into separate tubes containing buffers of pH 4.8 to pH 6.5. *Videographer: Please make sure the labels on the tubes are visible in the frame.*

2.2. Prepare the ratiometric FRET *(Fret)* probe solution by dissolving the FRET probe in nuclease-free water at a concentration close to 1 micromolar, or 1 picomole per microliter **[1-TXT]**.

2.2.1. Talent pipetting nuclease-free water into a tube containing the FRET probe and gently mixing. **TXT: Rapidly heat and cool the probe solution to dissociate unwanted base pairings**

2.3. Now, prepare three wells in a 96-well plate for each pH condition to be tested by adding 70 microliters of nuclease-free water and 10 microliters of 10x *(ten-ex)* buffer to each well **[1]**. In three separate wells, prepare the positive controls by combining the specified reagents. For the negative controls, substitute the protein sample with nuclease-free water **[2-TXT]**.

2.3.1. Talent pipetting 70 microliters of nuclease-free water and 10 microliters of buffer into the wells of pH series.

2.3.2. A shot of all specified reagents displayed on the bench as the talent pipetting them into the positive control wells. **TXT: 79 µL nuclease-free water, 10 µL 10x DNase II buffer, 0.33 µL DNase II (1 U/µL stock), 10 µL ratiometric probe solution**

2.4. Induce the DNase *(D-N-ase)* reaction by adding the probe solutions to the mixtures containing DNase protein at different pH values **[1]**. Mix all samples thoroughly, ensuring bubbles do not form **[2]**.

2.4.1. Talent adding the FRET probe solution into each test and control well.

2.4.2. SCREEN: 2,4,2-Shaking-SCREEN.mp4. 00:00-00:44. *Video Editor: Please remove*

00:29-00:40.

2.5. Then, incubate the DNase samples containing the ratiometric FRET probe at 37 degrees Celsius using either a water bath or a temperature-controlled incubator for various durations depending on the strength of the DNase activity **[1]**.

2.5.1. Talent placing the 96-well plate containing samples into a water bath or incubator set to 37 degrees Celsius.

2.6. Terminate all incubation reactions by adding 100 microliters of 250 millimolar Tris-Hydrochloric acid buffer at pH 8 to each well. This step also equalizes the pH across all samples **[1]**.

2.6.1. Talent pipetting 100 microliters of Tris-HCl buffer into each well of the 96-well plate.

3. Fluorometric Signal Detection, Calculation of FRET Ratios, and Cleavage Verification

3.1. Immediately after pH equalization, use a spectrofluorometer to record the emission spectra of both donor and acceptor fluorophores **[1]**. Perform measurements directly in the 96-well plate by exciting the donor fluorophore at 488 nanometers and simultaneously detecting emissions at 525 nanometers for the donor and 580 nanometers for the FRET-derived acceptor signal **[2]**.

3.1.1. Talent placing the 96-well plate into the spectrofluorometer.

3.1.2. SCREEN: 3.1.2-Scan-Settings-SCREEN.mp4 00:00-00:26.

3.2. Use the recorded fluorescence intensities to assess DNase activity for each sample **[1]**.

3.2.1. SCREEN: 3.2.1-Excel-SCREEN-UPDATED-REPLACEMENT.mp4 00:08-00:15, 00:30-00:39.

3.3. Then, calculate the FRET ratio using the given formula **[1]**.

3.3.1. TEXT on PLAIN BACKGROUND:

$$R_{FRET} = \frac{\text{Donor emission intensity at 525 nm}}{\text{Acceptor emission intensity at 580 nm}}$$

3.4. Verify the cleavage of the FRET probe by loading 15 microliters of each sample onto a 20% denaturing polyacrylamide gel **[1]**.

3.4.1. Talent pipetting 15 microliters of sample onto a 20% denaturing polyacrylamide gel.

3.5. Run the gel for 1 hour at 100 volts **[1].** Finally, place the gel on the tray of the documentation system equipped with a high-resolution color camera **[2]** and acquire gel images **[3]**.

3.5.1. Talent setting the gel apparatus to 100 volts and starting the run.

3.5.2. Talent placing the gel into the tray of the system.

3.5.3. Talent clicking the display screen to capture images. *Videographer: Please record the screen for this shot.*

Results

4. Results

4.1. Denaturing polyacrylamide gel analysis revealed that **[1]** incubation of the FRET probe with leukocyte elastase inhibitor or LEI *(L-E-I)* at pH 4.8 for 24 hours led to its cleavage, producing distinct fluorescein amidite or FAM *(Fam)* and tetramethylrhodamine or TAM *(tam)*-labeled fragments of different lengths **[2]**.

4.1.1. LAB MEDIA: Figure 2A.

4.1.2. LAB MEDIA: Figure 2A. *Video Editor: Highlight the 3rd image from the left (LEI).*

4.2. The size and labeling of the cleavage products indicated specific cuts near the apexes of both hairpins in the probe, consistent with the unique fragmentation pattern produced by LEI **[1]**, and distinct from that of DNase II **[2]**.

4.2.1. LAB MEDIA: Figure 2A, 2B. *Video Editor: Highlight the 3rd image from the left (LEI) in 2A*.

4.2.2. LAB MEDIA: Figure 2B, 2C. *Video Editor: Highlight 2C.*

4.3. Emission spectra of LEI-treated FRET probes showed **[1]** maximal signal change at pH 5.2 **[2]**, followed by pH 4.8 **[3]** and then pH 5.5 **[4]**, indicating peak DNase activity in this acidic range **[5]**, with no significant activity in the control sample **[6]**.

4.3.1. LAB MEDIA: Figure 2D.

4.3.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the curve for pH 5.2*.

4.3.3. LAB MEDIA: Figure 2D. *Video editor: Highlight the curve for pH 4.8.*

4.3.4. LAB MEDIA: Figure 2D. *Video editor: Highlight the curve for pH 5.5.*

4.3.5. LAB MEDIA: Figure 2D.

4.3.6. LAB MEDIA: Figure 2D. *Video editor: Highlight the curve for Control.*

4.4. Quantitative analysis of FRET ratios confirmed that **[1]** LEI exhibited significantly higher activity at pH 5.2 than at pH 4.8 **[2]**, and both were markedly higher than at pH 5.5 **[3]**, with activity dropping sharply above pH 5.5 **[4]**.

4.4.1. LAB MEDIA: Figure 2E.

4.4.2. LAB MEDIA: Figure 2E. *Video editor: Highlight the bars at pH 5.2 and pH 4.8 in the top graph (LEI).*

4.4.3. LAB MEDIA: Figure 2E. *Video editor: Highlight the bars at pH 5.2, pH 4.8, and 5.5 in the top graph (LEI).*

4.4.4. LAB MEDIA: Figure 2E. *Video editor: Highlight the bars at pH 5.8, 6.0, 6.2 and 6.5 in the top graph (LEI).*