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Title: Fluorescence-Based Detection of FEN1 Nuclease Activity and Screening of Small-Molecule Inhibitors

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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: 12
Number of Shots: 26

Introduction

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

REQUIRED:

1.1. **Rongcheng Li:** We developed a fluorescence-based method to detect FEN1 nuclease activity and screen small-molecule inhibitors, advancing cancer diagnostics and targeted therapy research.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

What are the current experimental challenges?

1.2. **Jiahui Du:** Current experimental challenges include the complexity and safety concerns of conventional methods, along with the high cost and specialized equipment required for existing fluorescence-based techniques.

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. **Rongcheng Li:** This protocol addresses the shortcomings of traditional radiological methods and high-cost fluorescence techniques, providing a safe, efficient and easy-to-use detection solution.

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

What research questions will your laboratory focus on in the future?

1.4. **Songbai Liu:** Our laboratory will focus on optimisatiob of a high-throughput screening platform to explore the *in vivo* applications and mechanisms of FEN1 inhibitors in cancer therapy.

1.4.1. 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.

Testimonial Questions:

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

1.5. **Rongcheng Li:** The video demonstrates the fluorescent labelling experimental process, enhances the reproducibility of the method, attracts the attention of cross-field researchers, and expands the scope of application of the technology.

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?

1.6. **Rongcheng Li:** It is expected that the experimental videos will reduce laboratory training time, decrease operational error rates, promote academic collaboration, and enhance paper citations due to method transparency.

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

Videographer: Please capture the testimonials in both Chinese and in English

Protocol

2. Preparation and Analysis of TAMRA-labeled DNA Substrate for FEN1 Nuclease Activity Assay

Demonstrator: Rongcheng Li

2.1. To begin, DNA oligonucleotide strands based on the specific cleavage activity of FEN1 *(fenn-one)* **[1]**. Mix the single-stranded DNA oligonucleotides in an annealing buffer **[2-TXT]**.
- 2.1.1. WIDE: Talent looking at DNA oligonucleotide strand design on a computer.
- 2.1.2. Talent pipetting DNA oligos into tubes containing annealing buffer. **TXT: 5 µL D1 (10 pmol/µL), 10 µL T1 (10 pmol/µL), and 7.5 µL U2 (10 pmol/µL)**

2.2. Add 10 microliters of annealing buffer and deionised water to make a total volume of 50 microliters.**[1]**. Then heat the reaction mixture at 100 degrees Celsius for 1 minute in a metal bath **[2]**.
- 2.2.1. Talent adding annealing buffer and deionized water.
- 2.2.2. Talent placing the tube into a metal bath preheated to 100 degrees Celsius.

2.3. Immediately transfer the tube to 72 degrees Celsius and incubate for 10 minutes **[1]**. Then turn off the heat source **[2]** and allow the solution to gradually cool to room temperature to form the complete double-stranded DNA substrate **[3]**.
- 2.3.1. Talent transferring the tube to another metal bath set at 72 degrees Celsius.
- 2.3.2. Talent turning off the heat source.
- 2.3.3. Talent leaving the tube on the bench to cool naturally.

2.4. For the FEN1 nuclease activity assay, first combine 2 microliters of the DNA substrate with FEN1 nuclease in the reaction buffer **[1-TXT]**. Incubate the reaction mixture in a metal bath at 37 degrees Celsius for 15 minutes **[2]**.
- 2.4.1. Talent pipetting DNA substrate and FEN1 nuclease into a new microcentrifuge tube. **TXT: Final volume: 20 µL**
- 2.4.2. Talent placing the tube into a metal bath set at 37 degrees Celsius.

2.5. Add 20 microliters of 2x termination buffer to stop the reaction **[1]**. Then load 20 microliters of the terminated sample onto a pre-prepared 12% denaturing polyacrylamide gel **[2]**.
- 2.5.1. Talent pipetting 2x termination buffer into the reaction tube.

2.5.2. Talent loading the sample into wells of the denaturing polyacrylamide gel.

2.6. Electrophorese the gel at 100 volts for 60 minutes **[1]**. Once complete, visualize the electrophoresis results under a fluorescence imaging system **[2]**.
2.6.1. Talent starting the gel electrophoresis and setting the voltage.
2.6.2. Talent placing the gel in a fluorescence imaging system.

3. Screening of Small Molecule Inhibitors of FEN1 Nuclease

3.1. With a pipette, transfer 2 microliters of dimethyl sulfoxide into a 1.5-milliliter microcentrifuge tube **[1]**. Add 2 microliters of FEN1 nuclease **[2]** and 10 microliters of 2X reaction buffer to the tube **[3]** and 6 microliters of deionized water **[4].**
3.1.1. Talent pipetting dimethyl sulfoxide into a labeled 1.5 milliliter microcentrifuge tube. Videographer's NOTE: Clip C2159 captures step 3.1.1 - 3.1.4 all together.

Videographer's NOTE: Clip C2157 is the mixed tube from step 3.1.

3.1.2. Talent adding FEN1 nuclease.
3.1.3. Talent adding 2x reaction buffer into the same tube.
3.1.4. Talent adding microliters of deionized water into the same tube.

3.2. Mix the solution thoroughly **[1]**. Then incubate it on ice for 10 minutes **[2]**. After incubation, pipette 2 microliters of DNA substrate to reach a final reaction volume of 20 microliters **[3].** Videographer's NOTE: Steps 3.2 and 3.5 are repetitive so only filmed with the author once.
3.2.1. Talent gently vortexing or flicking the tube to mix.
3.2.2. Shot of the tube being placed on ice.
3.2.3. Talent adding DNA substrate to the tube.

3.3. Next, prepare a working dilution of the FEN1 nuclease inhibitor, FEN1-IN-4 *(Fen-one-in-Four)*, by performing a two-fold serial dilution from a 1 molar stock solution in dimethyl sulfoxide **[1]**.
3.3.1. Talent using pipette to perform sequential two-fold serial dilutions of the FEN1-IN-4 stock in multiple tubes. Videographer's NOTE: C2158 is the mixed tube from step 3.3

3.4. Pipette 2 microliters each of FEN1 nuclease and the diluted FEN1-IN-4 inhibitor into a new 1.5-milliliter microcentrifuge tube **[1]**. Then pipette 10 microliters of 2x reaction buffer and 4 microliters of deionized water **[2]**.

3.4.1. Talent pipetting FEN1 nuclease and diluted inhibitor into a fresh microcentrifuge tube. Videographer's NOTE: Clip C2156 captures step 3.4.1 and 3.4.2, but was labeled wrong.

3.4.2. Talent adding reaction buffer and deionized water into the same tube.

3.5. Mix gently and incubate the mixture on ice for 10 minutes **[1]**. Now add 2 microliters of DNA substrate to the tube to make the final volume to 20 microliters **[2]**.
Videographer's NOTE: Steps 3.2 and 3.5 are repetitive so only filmed with the author once.

3.5.1. Talent gently mixing and placing the tube on ice.

3.5.2. Talent adding DNA substrate to the reaction tube.

3.6. Incubate the reaction mixture at 37 degrees Celsius in a metal bath for 15 minutes **[1]**. Then terminate the reaction by adding 20 microliters of termination buffer **[2]**.

3.6.1. Talent placing the tube into a 37 degree Celsius metal bath.

3.6.2. Talent adding 2× termination buffer into the tube after incubation.
Videographer's NOTE: Clip C2167 captures step 3.6.2, but was labeled wrong.

Results

4. Results

4.1. A clear protein band at approximately 45 kilodaltons confirmed successful purification of the FEN1 nuclease from induced expression **[1]**.

4.1.1. LAB MEDIA: Figure 2A. *Video editor: Please highlight the blue band in lane 6 around the 45 kDa marker.*

4.2. The fluorescein-labeled double-stranded DNA substrate appeared at 80 kiloDaltons, confirming successful synthesis **[1]** compared to the single-stranded substrate at 59 kiloDaltons **[2]**.

4.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight lane 2 showing the lower band at 59 nt labeled D1.*

4.2.2. LAB MEDIA: Figure 2B. *Video editor: Highlight lane 3 showing the higher band at 80 nt labeled SF.*

4.3. FEN1 nuclease cleaved the 80-nucleotide double-stranded DNA substrate in a dose-dependent manner **[1]**, with complete cleavage observed at 0.5 micrograms per microliter **[2]**.

4.3.1. LAB MEDIA: Figure 2C. *Video editor: Please highlight lane 3 showing intact 80-nt substrate in the SF control.*

4.3.2. LAB MEDIA: Figure 2C. *Video editor: Please highlight lane 5*

4.4. The FEN1-IN-4 compound significantly inhibited the cleavage of double-stranded DNA substrate by FEN1, as shown by the retention of the 80-nucleotide band **[1]**.

4.4.1. LAB MEDIA: Figure 2D. *Video editor: Please highlight lane 4*

4.5. FEN1-IN-4 reduced FEN1 nuclease activity in a concentration-dependent manner, with a half-maximal inhibitory concentration of 14.03 micromolar **[1]**.

4.5.1. LAB MEDIA: Figure 2F. *Video editor: Focus on the downward slope of the curve*

Pronunciation Guides:

1. FEN1

- **Pronunciation link:** https://www.howtopronounce.com/fen1
- **IPA:** /fɛn wʌn/
- **Phonetic Spelling:** fen-wuhn

2. Oligonucleotide

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/oligonucleotide
- **IPA:** /ˌɑː.lɪ.goʊˈnuː.kliːˌtaɪd/
- **Phonetic Spelling:** ah-li-goh-noo-klee-tyd

3. Dimethyl sulfoxide

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/dimethyl%20sulfoxide
- **IPA:** /ˌdaɪˌmɛθəl ˈsʌlfˌoʊˌsaɪd/
- **Phonetic Spelling:** dye-meth-uhl sull-fox-ide

4. Annealing

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/annealing
- **IPA:** /əˈniː.lɪŋ/
- **Phonetic Spelling:** uh-nee-ling

5. Polyacrylamide

- **Pronunciation link:** https://www.howtopronounce.com/polyacrylamide
- **IPA:** /ˌpɑː.li.əˈkrɪ.ləˌmaɪd/
- **Phonetic Spelling:** pah-lee-uh-kril-uh-mide

6. Electrophorese

- **Pronunciation link:** https://www.howtopronounce.com/electrophorese
- **IPA:** /ɪˌlɛk.trə.fəˈriːz/
- **Phonetic Spelling:** ih-lek-troh-fuh-reez