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Title: Colony Formation Assay Detecting the Proliferative Capacity of LncRNA-Knockdown Osteosarcoma 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**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 16

Number of Shots: 39

Introduction

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

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Xingyuan Sun:** My research focuses on osteosarcoma and aims to investigate how lncRNA knockdown influences long-term proliferative capacity using colony formation assays.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.2. **Xingyuan Sun:** This protocol assesses single-cell long-term proliferation, providing higher biological relevance and more definitive outcomes than short-term or metabolic-based proliferation assays.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What questions will future research focus on?~~

- 1.3. **Xingyuan Sun:** Future studies will expand this protocol to additional lncRNAs and tumor models to systematically explore mechanisms regulating cancer cell proliferative potential.
 - 1.3.1. 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.

Protocol

2. Plasmid Transfection of 143B Osteosarcoma Cells Using Polyethyleneimine

Demonstrator: Xinyu Zhang

- 2.1. To begin, seed 5×10^5 143B (*One-forty-three B*) osteosarcoma cells into two 10 centimeter culture dishes [1]. Culture the cells in complete DMEM (*D-M-E-M*) supplemented with 10 percent fetal bovine serum [2] at 37 degrees Celsius in a 5 percent carbon dioxide incubator [3].
 - 2.1.1. WIDE: Talent seeding 143B cells into two 10-centimeter culture dishes.
 - 2.1.2. Shot of supplemented DMEM media being added into the dishes.
 - 2.1.3. Talent placing the culture dishes into the incubator.
- 2.2. Prepare the transfection reagents 24 hours later, including plasmids, polyethyleneimine, and Opti-Minimum Essential Medium [1]. Vortex each reagent and allow them to equilibrate to room temperature before use [2].
 - 2.2.1. Talent arranging labeled plasmids, polyethyleneimine, and Opti-Minimum Essential Medium on the bench.
 - 2.2.2. Talent vortexing the reagents and leaving them at room temperature.
- 2.3. Now, label four 1.5 milliliter microcentrifuge tubes for plasmids and polyethyleneimine using a marker pen [1]. Pipette 500 microliters of Opti-Minimum Essential Medium to each tube [2].
 - 2.3.1. Talent labeling four microcentrifuge tubes.
 - 2.3.2. Talent pipetting Opti-Minimum Essential Medium into each tube.
- 2.4. Then add 10 micrograms of plasmid to the corresponding plasmid tube[1] and pipette 30 microliters of polyethyleneimine into each polyethyleneimine tube [2-TXT].
 - 2.4.1. Talent adding plasmid DNA to the labeled tubes.
 - 2.4.2. Shot of polyethyleneimine being pipetted into the PEI tube. **TXT: PEI: 1 $\mu\text{g}/\mu\text{L}$**
- 2.5. Mix each tube gently by pipetting up and down and incubate [1-TXT].
 - 2.5.1. Talent gently mixing the solutions and leaving them to incubate on the bench. **TXT: Incubation: RT, 5 min**
- 2.6. Now combine each plasmid tube with its corresponding polyethyleneimine tube [1], mix gently, and incubate the mixture at room temperature for 20 minutes [2].
 - 2.6.1. Talent combining plasmid and polyethyleneimine solutions.

- 2.6.2. Talent pipetting the suspension up and down.
- 2.7. Gently aspirate the culture medium from the dishes containing the 143B cells [1]. Add 7 milliliters of serum-free medium to each dish [2] and label the dishes according to the plasmid to be added [3].
 - 2.7.1. Talent removing culture medium from the dishes.
 - 2.7.2. Talent adding serum-free medium.
 - 2.7.3. Talent labeling the dishes.
- 2.8. Add the prepared transfection mixture dropwise to the corresponding culture dish [1]. Add the mixture slowly in a circular motion from the outer edge toward the center of the dish [2]. Incubate the cells at 37 degrees Celsius in a 5 percent carbon dioxide incubator [3].
 - 2.8.1. Talent adding the transfection mixture dropwise to the dish.
 - 2.8.2. Shot of the mixture being added in a circular motion.
Videographer's Note: Clip A0163 contains steps 2.8.1-2.8.2
 - 2.8.3. Talent placing the dishes back into the incubator.
- 2.9. Discard the supernatant 8 to 12 hours later [1]. Then add 10 milliliters of complete DMEM to culture dish and incubate [2-TXT].
 - 2.9.1. Shot of the supernatant being pipetted out.
 - 2.9.2. Talent adding 10 mL complete medium. **TXT: Incubation: 37°C, 5% CO₂**
- 2.10. Observe the cells under a fluorescence microscope 72 hours after replacing the medium [1].
 - 2.10.1. Talent viewing cells under a fluorescence microscope.
Videographer's Note: Clip A0171- A0173 are three shots for step 2.10.1

3. Colony Formation Assay to Evaluate Long-Term Proliferation of Osteosarcoma Cells

- 3.1. Remove the medium from the culture dishes [1]. Add 1.5 milliliters of trypsin–EDTA acid to digest the cells [2]. Gently pipette to detach the cells [3] then add 5 milliliters of complete medium to terminate digestion [3].
 - 3.1.1. Shot of the media being pipetted out of the culture dishes.
 - 3.1.2. Talent adding 1.5 mL trypsin–EDTA acid to the dish.
 - 3.1.3. Talent pipetting to detach cells
 - 3.1.4. Talent adding 5 mL complete medium to the suspension.
- 3.2. Transfer the cell suspension into a 15-milliliter microcentrifuge tube [1]. Then pipette

20 microliters of the cell suspension into a hemocytometer to count the cells [2-TXT]. Collect 3×10^5 cells for ribonucleic acid extraction to verify knockdown efficiency [3].

3.2.1. Talent transferring the cell suspension into a tube.

3.2.2. Talent pipetting 20 μ L of cell suspension into a hemocytometer. **TXT: Repeat cell count multiple time to ensure consistency between groups**

3.2.3. Talent pipetting cells into a labeled tube for ribonucleic acid analysis.

Videographer's Note: Clip A0186 and A0187 are for step 3.2.3, and the author hopes to show the text on the tube in clip A0187.

3.3. Now seed 2×10^3 cells per well into a 6-well plate [1]. Pipette complete DMEM to a final volume of 2 milliliters [2]. Culture the cells at 37 degrees Celsius in a 5 percent carbon dioxide incubator [3-TXT].

3.3.1. Talent seeding cells into a 6-well plate.

3.3.2. Shot of DMEM being pipetted into the well-plate.

3.3.3. Talent placing the plate into the incubator. **TXT: Replace medium with fresh DMEM every other day**

3.4. Monitor colony formation daily [1]. Terminate the culture when most colonies in the control group contain at least 50 cells or after 10 to 14 days of incubation [2].

3.4.1. Talent observing colonies under the microscope.

3.4.2. Talent checking colony size and density.

3.5. After discarding the supernatant, wash the cells twice with 1 milliliter of PBS per well, discarding the solution after each wash [1]. Fix the cells in 1 milliliter of 10 percent formalin solution for 15 minutes [2]. Discard the fixative then wash the cells twice with 1 milliliter per well of PBS [3].

3.5.1. Talent pipetting 1 mL PBS into each well and discarding it.

3.5.2. Talent adding formalin solution to the wells.

3.5.3. Talent adding 1 mL/well PBS.

3.6. Stain the cells with 1 milliliter of 0.5 percent crystal violet solution for 10 to 30 minutes [1]. Then gently wash the plate with slowly running tap water [2]. Air-dry the cells at room temperature [3]. Capture images of the colonies using a camera [4].

3.6.1. Talent adding 1ml 0.5% crystal violet staining solution to each well.

3.6.2. Talent rinsing the plate under running water.

3.6.3. Talent leaving the plate to dry on the bench.

3.6.4. Talent photographing the stained colonies.

Results

4. Results

- 4.1. Transfection efficiency of 143B cells was confirmed by fluorescence microscopy 72 hours after transfection [1], indicating efficient plasmid delivery in both conditions [2].
 - 4.1.1. LAB MEDIA: Figure 2.
 - 4.1.2. LAB MEDIA: Figure 2. *Video editor: Sequentially highlight both images*
- 4.2. RT-qPCR (R-T Q-P-C-R) reaction analysis showed that SNHG6 (S-N-H-G Six) expression was significantly reduced in 143B cells transfected with shSNHG6 (S-H S-N-H-G Six) [1] compared with the shNC (S-H N-C) group [2].
 - 4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the red bar labeled "shSNHG6,"*
 - 4.2.2. LAB MEDIA: Figure 3 *Video editor: Highlight the blue bar labeled "shNC"*
- 4.3. Colony formation assays revealed a significant reduction in the number and size of colonies in the SNHG6 knockdown group compared to the control group [1].
 - 4.3.1. LAB MEDIA: Figure 4 and Figure 5 . *Video editor: Highlight the plate labeled "shSNHG6," in Figure 4 and the red column in Figure 5*

Pronunciation Guide:

Osteosarcoma

Pronunciation link: <https://www.merriam-webster.com/dictionary/osteosarcoma>

IPA: /ˌɑːsti.ʊs.əˈrˌkoʊ.mə/

Phonetic Spelling: ahs·tee·oh·saar·koh·muh

IncRNA

Pronunciation link: No confirmed link found

IPA: /ˌɛl.ɛnˌsiː.ɑːrˈɛnˈeɪ/

Phonetic Spelling: el·en·see·ar·en·ay

Plasmid

Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>

IPA: /ˈplæz.mɪd/

Phonetic Spelling: plaz·mid

Transfection

Pronunciation link: <https://www.merriam-webster.com/dictionary/transfection>

IPA: /trænˈsfɛk.ʃən/

Phonetic Spelling: tran·sfek·shun

Polyethyleneimine

Pronunciation link: <https://www.merriam-webster.com/dictionary/polyethyleneimine>

IPA: /ˌpɑːli.ɛθ.əˈliːn.iˌmaɪn/

Phonetic Spelling: pah·lee·eth·uh·leen·uh·mine

Dulbecco's Modified Eagle Medium (DMEM)

Pronunciation link: <https://www.merriam-webster.com/dictionary/Dulbecco>

IPA: /duːlˈbɛk.oʊz/

Phonetic Spelling: dool·BEK·ohz

Microcentrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>

IPA: /ˌmaɪ.kroʊˈsɛn.trəˌfjuːdʒ/

Phonetic Spelling: my·kroh·sen·truh·fyooj

Hemocytometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/hemocytometer>

IPA: /ˌhiː.məʊ.saiˈtɑː.mə.tə/

Phonetic Spelling: hee·moh·sigh·tah·muh·ter

Ribonucleic Acid

Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic%20acid>

IPA: /ˌraɪ.boʊ.nuː.kliː.ɪk ˈæs.ɪd/

Phonetic Spelling: rye·boh·noo·klee·ik as·id

Trypsin–EDTA

Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>

IPA: /ˈtrip.sɪn/

Phonetic Spelling: trip·sin

Formalin

Pronunciation link: <https://www.merriam-webster.com/dictionary/formalin>

IPA: /'fɔːr.mə.lɪn/

Phonetic Spelling: for·muh·lin

❓ Crystal Violet

Pronunciation link: <https://www.merriam-webster.com/dictionary/crystal%20violet>

IPA: /'krɪs.təl 'vaɪ.əl.ət/

Phonetic Spelling: kris·tuhl vye·uh·luht

❓ Fluorescence

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>

IPA: /flʊ'res.əns/

Phonetic Spelling: floo·res·uhns

❓ RT-qPCR

Pronunciation link: No confirmed link found

IPA: /,ɑːrˌtiːˌkjuːˌpiːˌsiːˈɑːr/

Phonetic Spelling: ar·tee kyoo·pee·see·ar

❓ SNHG6

Pronunciation link: <https://www.howtopronounce.com/snhg6>

IPA: /,ɛs,ɛn,ɛɪtʃˈdʒiː sɪks/

Phonetic Spelling: es·en·aych·jee six

❓ Incubator

Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>

IPA: /'ɪn.kjəˌbeɪ.tə/

Phonetic Spelling: in·kyuh·bay·ter