FINAL SCRIPT: APPROVED FOR FILMING

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Title: MeRIP-qPCR Assay for Detecting m6A Modification Levels of Specific RNA in 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: 13
Number of Shots: 29

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. I am trying to address the advantages of MeRIP-qPCR compared with other techniques and future research directions.

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:** MeRIP-qPCR sensitively detects low-abundance m6A, enables regional localization, and offers a low-cost alternative to sequencing-based approaches with antibody enrichment efficiency.

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 are expected to further integrate MeRIP-qPCR with nanopore sequencing to improve accuracy, resolution, and functional interpretation of m6A modifications.

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. Methylated RNA Immunoprecipitation from Osteosarcoma Cells for Downstream Purification and Analysis

Demonstrator: Binghan Yan

2.1. To begin, remove the culture medium from a 3.5-centimeter dish containing osteosarcoma cells **[1].** Gently wash the once with pre-chilled PBS **[2]**. Add an appropriate volume of RNA lysis buffer directly to the dish to fully lyse the cells **[3].** Gently pipette the suspension to homogenize **[4]**.

2.1.1. WIDE: Talent removing the culture medium from a 3.5-centimeter dish containing osteosarcoma cells.

2.1.2. Talent adding pre-chilled phosphate-buffered saline to the dish.

2.1.3. Talent adding RNA lysis buffer to the dish.

2.1.4. Talent pipetting gently to homogenize the cells.

2.2. Transfer the cell suspension into a 1.5-milliliter microcentrifuge tube **[1]**. Then centrifuge the microcentrifuge tube at 1,000 *g* for 5 minutes at 4 degrees Celsius **[2]**. Carefully remove the microcentrifuge tube after centrifugation **[3]**.

2.2.1. Talent transferring the homogenized cell suspension into a 1.5-milliliter microcentrifuge tube.

2.2.2. Talent placing the microcentrifuge tube into a centrifuge and setting it to 1,000 g for 5 minutes at 4 degrees Celsius.

2.2.3. Talent removing the microcentrifuge tube from the centrifuge.

2.3. Using a 1-milliliter pipette, aspirate the supernatant completely without disturbing the pellet at the bottom **[1]**. Then add 300 microliters of Lysis Buffer to the cell pellet **[2]**.

2.3.1. Talent carefully aspirating the supernatant with a 1-milliliter pipette, avoiding contact with the cell pellet.

2.3.2. Talent adding 300 microliters of Lysis Buffer to the pellet in the microcentrifuge tube.

2.4. Mix thoroughly by inverting the tube 30 times until the solution appears clear and viscous with no visible clumps **[1]**. Then incubate the tube at room temperature for 2 minutes before RNA precipitation **[2]**.

2.4.1. Talent inverting the microcentrifuge tube 30 times to mix the contents until the

solution is clear and viscous.

2.4.2. Talent placing the tube on the bench for incubation at room temperature.

2.5. For methylated RNA immunoprecipitation, add the listed reagents to the tube **[1].** Close the cap of the PCR tube tightly for immunocapture **[2]**. Then place the PCR tube on a rotator or rolling shaker and incubate **[3-TXT]**.

2.5.1. LAB MEDIA: Table 1

2.5.2. Talent sealing the PCR tube cap containing the reagents tightly. **Videographer's NOTE:** Clip A0032, the effective footage is the last 10 seconds when the tube gets sealed

Note from postshoot integrator: The videographer did not specify the shot numbers for these notes. I have put these together, looking at the shot description, so please check the shot

2.5.3. Talent placing the PCR tube onto a rotator or rolling shaker. **TXT: Incubation: RT, 90 min**

2.6. Now, transfer the PCR tube onto a magnetic stand **[1]**. Allow the tube to sit for 2 minutes until the solution becomes clear and the beads are captured on the tube wall **[2]**. Then carefully aspirate and discard the supernatant without disturbing the beads **[3]**.

2.6.1. Talent placing the PCR tube onto the magnetic stand.

2.6.2. Shot of the tube sitting on the magnetic stand as the solution clears and beads gather on the wall.

2.6.3. Talent carefully aspirating and discarding the supernatant while keeping the beads undisturbed.

2.7. While keeping the tube on the magnetic stand, add 150 microliters of Wash Buffer **[1]**. Remove the tube from the magnetic stand and tilt it upside down gently to fully resuspend the beads **[2]**.

2.7.1. Talent adding 150 microliters of Wash Buffer into the PCR tube while it remains on the magnetic stand.

2.7.2. Talent removing the PCR tube and gently tilting it upside down to resuspend the beads.

2.8. Return the tube to the magnetic stand **[1]**. After 2 minutes, when the solution clears, discard the supernatant **[2]**.

2.8.1. Talent placing the tube back on the magnetic stand.

2.8.2. Talent discarding the clear supernatant.

2.9. Now, transfer the PCR tube into a thermal cycler and incubate **[1-TXT]**. Carefully transfer the entire supernatant to a new 0.2-milliliter PCR tube **[2]**.

2.9.1. Talent placing the PCR tube into the thermal cycler and setting the temperature to 55 degrees Celsius. **TXT: Incubation: 55°C, 15 min Videographer's NOTE:** Clip A0045, the effective footage is the last 10 seconds when the temperature reaches 55 degrees.

2.9.2. Talent transferring the 20 microliters of supernatant into a new 0.2-milliliter PCR tube.

2.10. Add 20 microliters of RNA Purification Solution to each sample tube, negative control tube, and input tube **[1]**. Then pipette 160 microliters of 100 percent ethanol **[2]**.

2.10.1. Talent adding 20 microliters of RNA Purification Solution to all designated tubes.

2.10.2. Talent adding 160 microliters of 100 percent ethanol to the tube.

2.11. Vortex the RNA Binding Beads to resuspend them **[1]**. Then transfer 2 microliters of resuspended RNA Binding Beads to each tube **[2]**.

2.11.1. Talent vortexing the tube of RNA Binding Beads.

2.11.2. Talent adding 2 microliters of RNA Binding Beads into each sample tube.

2.12. Place the tube on a magnetic stand **[1]** and add 150 microliters of freshly prepared 90 percent ethanol **[2]**.

2.12.1. Talent placing the tube on the magnetic stand.

2.12.2. Talent adding 150 microliters of 90 percent ethanol.

2.13. After a 30-second incubation, pipette 13 microliters of Elution Buffer to the dried beads **[1]**. Then carefully transfer the entire supernatant to a new RNase-free PCR tube for further analysis **[2]**.

2.13.1. Talent adding 13 microliters of Elution Buffer to the dried beads in the PCR tube.

2.13.2. Talent transferring the supernatant to a new RNase-free PCR tube.

Results

3. Results

3.1. c-Myc *(C- /mɪk/)* enrichment using the anti-N6-methyladenosine antibody was significantly higher than with immunoglobulin G control in both hFOB1.19 *(H-Fob-1-point-One-Nine)* and 143B *(One-forty-Three-B)* cells **[1]**.

3.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the blue and red bars labeled "anti-m⁶A"*

3.2. The c-Myc N6-methyladenosine modification level was significantly higher in 143B cells than in hFOB1.19 cells **[1]**.

3.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the red bar for "anti-m⁶A"*

1. Osteosarcoma
   Pronunciation link: https://www.merriam-webster.com/dictionary/osteosarcoma
   IPA: /ˌɑːstioʊˌsɑːrˈkoʊmə/
   Phonetic Spelling: ahs·tee·oh·sar·KOH·muh
2. Phosphate-buffered saline
   Pronunciation link: https://www.merriam-webster.com/dictionary/phosphate-buffered%20saline
   IPA: /ˈfɑːsˌfeɪt ˈbʌfərd səˈliːn/
   Phonetic Spelling: FAHS·fayt BUF·erd suh·LEEN
3. Lysis
   Pronunciation link: https://www.merriam-webster.com/dictionary/lysis
   IPA: /ˈlaɪsɪs/
   Phonetic Spelling: LY·sis
4. Homogenize
   Pronunciation link: https://www.merriam-webster.com/dictionary/homogenize
   IPA: /həˈmɑːdʒəˌnaɪz/
   Phonetic Spelling: huh·MAHJ·uh·nyze
5. Microcentrifuge
   Pronunciation link: https://www.merriam-webster.com/dictionary/microcentrifuge
   IPA: /ˌmaɪkroʊˈsɛntrɪˌfjuːdʒ/
   Phonetic Spelling: MY·kroh·SEN·truh·fyooj
6. Centrifuge
   Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge

IPA: /ˈsɛntrɪˌfjuːdʒ/
Phonetic Spelling: SEN·truh·fyooj

7. Supernatant
   Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
   IPA: /ˌsuːpərˈneɪtənt/
   Phonetic Spelling: SOO·per·NAY·tuhnt
8. Pellet
   Pronunciation link: https://www.merriam-webster.com/dictionary/pellet
   IPA: /ˈpɛlɪt/
   Phonetic Spelling: PEH·lit
9. Immunoprecipitation
   Pronunciation link: https://www.merriam-webster.com/dictionary/immunoprecipitation
   IPA: /ˌɪmjənoʊprɪˌsɪpɪˈteɪʃən/
   Phonetic Spelling: ih·MYOO·noh·pruh·sip·uh·TAY·shuhn
10. Immunocapture
    Pronunciation link: https://www.merriam-webster.com/dictionary/immunocapture
    IPA: /ˌɪmjənoʊˈkæptʃər/
    Phonetic Spelling: ih·MYOO·noh·KAP·chur
11. Methylated
    Pronunciation link: https://www.merriam-webster.com/dictionary/methylated
    IPA: /ˈmɛθəˌleɪtɪd/
    Phonetic Spelling: METH·uh·lay·tid
12. Magnetic
    Pronunciation link: https://www.merriam-webster.com/dictionary/magnetic
    IPA: /mægˈnɛtɪk/
    Phonetic Spelling: mag·NET·ik
13. Thermal cycler
    Pronunciation link: https://www.merriam-webster.com/dictionary/thermal%20cycler
    IPA: /ˈθɝːməl ˈsaɪklər/
    Phonetic Spelling: THUR·muhl SY·kler
14. Ethanol
    Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
    IPA: /ˈɛθəˌnɔːl/
    Phonetic Spelling: ETH·uh·nawl
15. Vortex
    Pronunciation link: https://www.merriam-webster.com/dictionary/vortex
    IPA: /ˈvɔːrtɛks/
    Phonetic Spelling: VOR·teks
16. Elution
    Pronunciation link: https://www.merriam-webster.com/dictionary/elution
    IPA: /ɪˈluːʃən/
    Phonetic Spelling: ih·LOO·shuhn
17. RNase
    Pronunciation link: https://www.merriam-webster.com/dictionary/RNase

IPA: /ˈɑːrˌneɪs/
Phonetic Spelling: AR·nays

18. Enrichment
Pronunciation link: https://www.merriam-webster.com/dictionary/enrichment
IPA: /ɪnˈrɪtʃmənt/
Phonetic Spelling: in·RICH·muhnt
19. N6-methyladenosine
Pronunciation link: https://www.merriam-webster.com/dictionary/methyladenosine
IPA: /ˌmɛθəlˌædəˈnoʊsiːn/
Phonetic Spelling: METH·uhl·AD·uh·NOH·seen
20. Immunoglobulin
Pronunciation link: https://www.merriam-webster.com/dictionary/immunoglobulin
IPA: /ˌɪmjənoʊˈglɑːbjəlɪn/
Phonetic Spelling: ih·MYOO·noh·GLAH·byuh·lin