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Title: Quantitative Real-Time PCR Evaluation of microRNA Expressions in Mouse Kidney with Unilateral Ureteral Obstruction

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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. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ■ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **Same building, 2 rooms**

Current Protocol Length

Number of Steps: 21
Number of Shots: 54

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Katsunori Yanai**: This method makes it possible to profile microRNA expression in kidneys of mice with a wide range of pathological conditions such as renal fibrosis.

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

1.2. **Katsunori Yanai**: The main advantage of this technique is that it enables miRNA expression profiling with high accuracy and sensitivity by using a simple process that saves time and prevents technical error.

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

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Animal Ethics Committee of Jichi Medical University and were performed in accordance with the Use and Care of Experimental Animals Guidelines from the Jichi Medical University Guide for Laboratory Animals.

Protocol

2. Sham and UUO Surgeries

2.1. To perform the sham surgery, use surgical scissors and tweezers to make an incision in the skin at the abdomen **[1]** and cut the muscle and peritoneal membrane from the bladder to the left lower edge of the ribs **[2]**. *Videographer: This step is important!*

2.1.1. Talent making an incision in the abdomen.

2.1.2. Talent cutting the muscle and peritoneal membrane.

2.2. Moisten two cotton swabs with PBS **[1]** and carefully pull the intestines to the side **[2]**. Place the moistened swabs to identify the left kidney and ureter **[3]**. Close the peritoneal membrane **[4]** and then the incision with 4-0 nylon **[5]**.

2.2.1. Talent moistening the cotton swabs, with the PBS container in the shot.

2.2.2. Talent pulling the intestines to the side.

2.2.3. Talent placing the swabs.

2.2.4. Talent closing the peritoneal membrane.

2.2.5. Talent closing the incision.

2.3. To perform the unilateral ureteral obstruction, or UUO, make an incision in the skin at the abdomen and cut the muscle and peritoneal membrane as demonstrated for the sham surgery **[1]**. *Videographer: This step is important!*

2.3.1. Talent cutting the peritoneal membrane.

2.4. Place a 2.5-milliliter syringe underneath the mouse **[1]**. Moisten 2 cotton swabs with PBS, then pull the intestines carefully to the side with the tweezers and place the swabs to identify the left ureter **[2]**. Use the tweezers to lift the left kidney **[3]**. *Videographer: This step is important!*

2.4.1. Talent placing the syringe under the mouse.

2.4.2. Talent pulling the intestines to the side and placing the swabs.

2.4.3. Talent lifting the left kidney.

2.5. Use 4-0 silk to ligate the left ureter in two places approximately 1 centimeter apart **[1]**. Cut the ureter at the center point of the two ligations **[2]**, and then use 4-0 nylon sutures to close the peritoneal membrane and incision **[3]**. *Videographer: This step is important!*

2.5.1. Talent ligating the left ureter.

2.5.2. Talent cutting the ureter.

2.5.3. Talent closing the peritoneal membrane.

3. Collection of Kidney Samples

3.1. After cutting the peritoneal membrane as previously demonstrated, lift it with tweezers and use surgical scissors to make a sideways incision at the upper edge **[1]**, then continue the incision along the lowest edge of the ribs **[2]**.

3.1.1. Talent making an incision in the peritoneal membrane.

3.1.2. Talent continuing the incision.

3.2. Identify the left kidney and reflux it with PBS until the kidney turns yellow-white **[1]**. Remove the kidney by cutting the left renal artery and vein with the surgical scissors **[2]** and place it in a Petri dish **[3]**, then wash it carefully with PBS **[4]**.

3.2.1. Talent refluxing the left kidney.

3.2.2. Talent removing the kidney.

3.2.3. Talent putting the kidney in the Petri dish.

3.2.4. Talent washing the kidney with PBS.

3.3. Cut the kidney into approximately 10-milligram samples with the surgical scissors and tweezers **[1]**, put each piece of the kidney in its own 1.5-milliliter microcentrifuge tube and close the tube's cap **[2]**.

3.3.1. Talent cutting the kidney.

3.3.2. Talent putting a piece of the kidney in a centrifuge tube and closing the cap.

4. Extraction of Total RNA from the Kidney Samples

4.1. Put a kidney sample in the silicon homogenizer **[1]** and add 700 microliters of the phenol guanidine-based lysis reagent **[2]**.

4.1.1. Talent putting the kidney sample in the homogenizer.

4.1.2. Talent adding lysis reagent to the kidney.

4.2. Slowly press and twist the homogenizer's pestle against the kidney sample to homogenize it **[1]**. Repeat the pressing and twisting until the sample is completely dissolved in the lysis reagent **[2]**. *Videographer: This step is difficult and important!*

4.2.1. Talent homogenizing the sample.

4.2.2. Completely dissolved kidney.

4.3. To further homogenize the sample, transfer the homogenized lysate to the biopolymer spin column **[1]** and spin it at 14,000 x *g* for 3 minutes **[2]**, then transfer the precipitated lysate to an unused 1.5-milliliter microcentrifuge tube **[3]**.

4.3.1. Talent transferring the sample to a spin column.

4.3.2. Talent putting the column in the centrifuge and closing the lid.

4.3.3. Talent transferring the lysate to a tube.

4.4. Combine the lysate in the tube with 140 microliters of chloroform **[1]** and cap the tube tightly. To mix the lysate and chloroform, invert the tube 15 times **[2]**. Incubate the samples for 2 to 3 minutes at room temperature, then spin them at 12,000 x *g* for 15 minutes at 4 degrees Celsius **[3]**.

4.4.1. Talent adding chloroform to the tube, with the chloroform container in the shot.

4.4.2. Talent capping the tube and inverting it.

4.4.3. Talent putting the tube in the centrifuge and closing the lid.

4.5. Without disturbing the precipitate, transfer the supernatant to a new 1.5-milliliter microcentrifuge tube **[1]** and add 1.5 times its volume 100% ethanol **[2]**. Vortex the mixture for 5 seconds **[3]**.

4.5.1. Talent transferring the supernatant to a new tube.

4.5.2. Talent adding ethanol to the tube.

4.5.3. Talent vortexing the mixture.

4.6. Load 700 microliters of the sample onto a membrane-anchored spin column in a 2-milliliter collection tube **[1]**. Close the column cap and spin the column at 15,000 x *g* for 15 seconds **[2]**, then throw away the precipitated lysate in the collection tube **[3]**.

4.6.1. Talent loading sample onto spin column.

4.6.2. Talent putting the column in the centrifuge and closing the lid.

4.6.3. Talent taking the tube out of the centrifuge and throwing away the collection tube.

4.7. Wash the sample thoroughly by adding 700 microliters of wash buffer 1 to the membrane-anchored spin column in a 2-milliliter collection tube **[1]**, then repeat the centrifugation and throw away the collection tube **[2]**. Repeat the wash twice with 500 microliters of wash buffer 2 per wash **[3]**.

4.7.1. Talent adding wash buffer 1 to the spin column, with the wash buffer 1 container in the shot.

4.7.2. Talent putting the column in the centrifuge and closing the lid.

4.7.3. Talent adding wash buffer 2 to the spin column, with the wash buffer 2 container in the shot.

4.8. After the last wash, spin the membrane-anchored spin column in a 2-milliliter collection tube at 15,000 x *g* for 1 minute and throw away the precipitated lysate **[1]**.

4.8.1. Talent throwing out the precipitated lysate in the collection tube.

4.9. Transfer the membrane-anchored spin column to a new 1.5-milliliter tube **[1]**. Dissolve the total RNA by adding 30 microliters of RNase-free water to the column, close the column cap **[2]**, and leave it for 5 minutes at room temperature. Then, spin the column at 15,000 x *g* for 1 minute **[3]**.

4.9.1. Talent putting the column in a new collection tube.

4.9.2. Talent adding water to the column and capping it.

4.9.3. Talent putting the column in the centrifuge and closing the lid.

4.10. Transfer the sample containing total RNA to a new microcentrifuge tube on ice **[1]** and measure the concentration of total RNA by spectrophotometry **[2]**.

4.10.1. Talent transferring the RNA to a tube on ice.

4.10.2. Talent using the spectrophotometer.

5. Synthesis of cDNA

5.1. Prepare a master mix solution according to manuscript directions and add 8 microliters to each tube of an 8-well strip tube **[1]**. After adjusting the total RNA density, place a 12-microliter aliquot of total RNA into each tube and close the cap **[2]**. Centrifuge the tubes for 15 seconds **[3]**.

5.1.1. Talent putting master mix into a few tubes on the strip.

5.1.2. Talent adding RNA to the tubes.

5.1.3. Talent centrifuging the tubes.

5.2. Put the tubes in the thermal cycler and incubate them for 60 minutes at 37 degrees Celsius. When finished, immediately incubate them for 5 minutes at 95 degrees Celsius for the synthesis of cDNA **[1]**.

5.2.1. Talent placing the tube in the thermal cycler and programming it.

5.3. Transfer the cDNA into a new 1.5-milliliter microcentrifuge tube and dilute it ten times with distilled water **[1]**. Vortex and centrifuge the tube for 5 seconds **[2]**, then perform quantitative real time PCR as described in the text manuscript **[3]**.

5.3.1. Talent transferring the cDNA to a new tube and diluting it.

5.3.2. Talent vortexing and centrifuging the cDNA.

5.3.3. Talent programming the rtPCR machine.

Results

6. Results: Differentially Expressed miRNAs in the Kidneys of UUO Mice

6.1. This protocol was used to create a unilateral ureteral obstruction mouse model via left ureteral ligation in 8-week-old male mice weighing 20 to 25 grams. Ureters were completely obstructed by double ligation with 4-0 silk sutures. Kidneys were collected at 8 days post-surgery **[1]**.

6.1.1. LAB MEDIA: Figure 1.

6.2. The micro RNA qRT-PCR *(spell out 'Q-R-T-P-C-R')* data indicated that the level of micro RNA-3070-3p was significantly increased **[1]** and the levels of micro RNA-7218-5p and micro RNA-7219-5p were decreased in the kidneys of the UUO mice compared to the controls **[2]**.

6.2.1. LAB MEDIA: Figure 1. *Video Editor: Emphasize the miRNA-3070-3p graph.*

6.2.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize the miRNA-7218-5p and miRNA-7219-5p graphs.*

Conclusion

7. Conclusion Interview Statements

7.1. **Katsunori Yanai**: When attempting this protocol, remember to repeat twisting the kidney sample until it is completely dissolved in the phenol/guanidine-based lysis reagent to achieve a good RNA extraction.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1.*