Submission ID #: 61579
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18781643

Title: Quantification of Circulating Pig-Specific DNA in the Blood of a Xenotransplantation Model

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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? **NO**

Current Protocol Length

Number of Steps: 16
Number of Shots: 42

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Lisha Mou:** Xenotransplantation is a feasible method to treat organ failure. However, effective monitoring of the immune rejection of xenotransplantation is a problem for physicians and researchers.

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

1.2. **Ying Lu:** This protocol is a simple, convenient, low cost, and less invasive method to monitor the immune rejection of xenotransplantation.

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

OPTIONAL:

1.3. **Yingying Liang:** This technique can be used in the xenotransplantation field, including pig-to-human islet transplantation, kidney transplantation, liver transplantation, and heart transplantation.

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

Ethics Title Card

1.4. All experiments were performed in accordance with the relevant guidelines and regulations of the Institutional Review Board of Shenzhen Second People's Hospital, First Affiliated Hospital of Shenzhen University.

Protocol

2. Isolating Genomic DNA

2.1. Begin by transferring 500 microliters of blood samples into different microcentrifuge tubes **[1]**. Add 20 microliters of protease K and 500 microliters of lysis buffer into the tubes **[2]**, then thoroughly shake them to mix **[3]**.

2.1.1. WIDE: Establishing shot of talent transferring blood into a centrifuge tube.

2.1.2. Talent adding lysate and proteinase K into a tube.

2.1.3. Talent shaking the tubes.

2.2. Put the tubes in a water bath at 56 degrees Celsius for 10 minutes, shaking them 2 to 3 times during the incubation until the solution becomes clear **[1]**. Centrifuge them briefly to remove the liquid beads from the inner wall of the tube covers **[2]**, then add 500 microliters of anhydrous ethyl alcohol **[3]** and shake thoroughly **[4]**.

2.2.1. Talent placing the tubes in a water bath.

2.2.2. Talent centrifuging the tubes.

2.2.3. Talent adding anhydrous ethyl alcohol to the tubes, with the alcohol container in the shot.

2.2.4. Talent shaking the tube.

2.3. Transfer the mixture into the adsorption column **[1]**, then centrifuge the column for 2 minutes **[2-TXT]** and discard the waste liquid **[3]**.

2.3.1. Talent transferring the mixture to a column.

2.3.2. Talent putting the column in the centrifuge and closing the lid. **TEXT: 12,000 rpm (~13,400 x *g*)**

2.3.3. Talent discarding the waste.

2.4. Add 800 microliters of rinse solution to each adsorption column and centrifuge for 1 minute **[1]**. Leave the columns at room temperature for a few minutes to dry the remaining rinse solution **[2]**.

2.4.1. Talent adding rinse solution to a column, with the rinse solution container in the shot.

2.4.2. Talent taking the columns out of the centrifuge and leaving them on the lab bench.

2.5. Transfer the adsorption columns to a clean centrifugal tube **[1]**, add 50 microliters of elution buffer to the middle of the adsorption films, and place them at room temperature for 2 to 5 minutes **[2]**. Then, centrifuge them for 1 minute **[3]**.

2.5.1. Talent transferring a column to a new centrifuge tube.

2.5.2. Talent adding elution buffer to the column.

2.5.3. Talent putting the columns in the centrifuge and closing the lid.

3. Verification of Primer Specificity

3.1. Prepare the PCR master mix according to manuscript directions, then add 23 microliters of the mix into each 0.6-milliliter microcentrifuge tube **[1]**. Add 2 microliters of genomic DNA to each tube and carefully cap it **[2]**, then briefly mix and centrifuge **[3]**. Place the tubes in a PCR-cycler and start the amplification **[4-TXT]**. *Videographer: This step is important!*

3.1.1. Talent aliquoting the PCR master mix into the tubes.

3.1.2. Talent adding DNA to a tube and capping it.

3.1.3. Talent mixing and centrifuging the tubes.

3.1.4. Talent placing the tube in the PCR machine and closing the lid. **TEXT: Denaturation: 95 °C, 5 s; Annealing: 60 °C, 30 s; Extension: 72 °C, 30 s; 35 cycles.**

3.2. When the reaction is finished, perform agarose electrophoresis. Add 1.2 grams of agarose into a flask containing 100 milliliters of TAE **[1]** and boil it for 5 minutes in the microwave **[2]**. *Videographer: This step is important!*

3.2.1. Talent adding agarose into a flask with TAE.

3.2.2. Talent putting the flask in the microwave and closing the door.

3.3. Once the agarose has cooled to approximately 70 degrees Celsius, add 5 microliters of nucleic acid dye into the flask **[1]**. Slowly pour it into the plate and leave it at room temperature until it solidifies into a gel **[2]**. *Videographer: This step is important!*

3.3.1. Talent adding dye to the flask.

3.3.2. Talent pouring the agarose into a plate.

3.4. Add 5 microliters of each sample and 2-Log DNA Ladder or DNA marker one into the wells of the agarose gel **[1]** and electrophorese it at 120 milliampere until the bands are separated **[2]**. Visualize the gel with an ultraviolet imager **[3]**.

3.4.1. Talent adding the samples or ladder to a few wells.

3.4.2. Talent starting the electrophoresis.

3.4.3. Talent using the imager.

4. Standard Curve of cpsDNA

4.1. After performing transformation as described in the text manuscript, verify the plasmids by double restriction enzyme digestion with EcoR-I *(pronounce 'eco-R-one')* and Bam H-I *(pronounce 'bam-high')* **[1-TXT]**.

4.1.1. Talent putting the restriction reaction into the incubator and closing the door. **TEXT: EcoR I: 15 U/µL ; Bam HI: 15 U/µL**

4.2. Separate the digested products with 1% agarose electrophoresis and expose the gel to UV light **[1]**. Dilute the concentrated plasmid with the fragment of porcine DNA to 2 x 10^{11} copies per milliliter for the starting standard solution using double distilled water **[2]**.

4.2.1. Gel running.

4.2.2. Talent diluting the plasmid.

4.3. To establish a standard curve, prepare a qPCR master mix as described in the text manuscript and add 40 microliters of the mix into each tube of an 8-tube strip **[1]**. Add 10 microliters of standard DNA of different concentrations **[2]**, cap the tubes, then briefly mix and centrifuge them **[3]**.

4.3.1. Talent placing master mix into tubes in a strip.

4.3.2. Talent adding DNA to the tubes.

4.3.3. Talent mixing and centrifuging the tubes.

4.4. Place the tubes in the qPCR machine and perform the amplification **[1]**.

4.4.1. Talent programming the qPCR. *Video Editor: Show Figure 2 here as well.*

5. Quantitation of Circulating Pig-specific DNA

5.1. Use EDTA tubes to collect blood samples of about 400 microliters from the pig-to-monkey artery patch models or 100 microliters from the pig-to-mouse cell transplantation models **[1]**, then transfer the samples to 1.5-milliliter centrifuge tubes **[2]**. *Videographer: This step is important!*

5.1.1. Blood samples in EDTA tubes.

5.1.2. Talent transferring the blood samples to centrifuge tubes.

5.2. Remove the blood cells from the blood samples by centrifugation at low temperature and high speed **[1-TXT]**. Transfer the supernatant to a new 1.5-milliliter centrifuge tube **[2]** and remove the cell debris by centrifugation at 16,000 x *g* for 10 minutes **[3]**. *Videographer: This step is important!*

5.2.1. Talent putting the tubes in the centrifuge and closing the lid. **TEXT: 3,000 x *g*, 4 degrees Celsius, 5 minutes**

5.2.2. Talent transferring the supernatant to a new tube.

5.2.3. Talent putting the tubes in the centrifuge and closing the lid.

5.3. Transfer the supernatant to a new 1.5-milliliter centrifuge tube **[1]** and extract the circulating DNA using a commercial serum or circulating DNA extraction kit **[2]**. Condense the volume of circulating DNA to 40 microliters and store it at -20 degrees Celsius **[3]**. Perform qPCR to quantify the circulating pig-specific DNA **[4]**.
Videographer: This step is difficult and important!

5.3.1. Talent transferring the supernatant to a new tube.

5.3.2. Talent at the lab bench opening the DNA extraction kit.

5.3.3. Talent storing DNA in a freezer.

5.3.4. Talent at the qPCR machine.

Results

6. Results: PCR Validation of Porcine Specific Primers

6.1. Porcine-specific primers were designed and used to quantify circulating pig-specific DNA by qPCR in pig-to-mouse cell transplantation models and pig-to-monkey artery patch transplantation models **[1-TXT]**.

6.1.1. LAB MEDIA: Figure 3. Video Editor: *Label A "***primer #4***" and B "***primer #11***".*

6.2. Agarose electrophoresis was used to isolate amplified DNA fragments. Using PCR, the primers specific for amplified porcine genomic DNA were identified **[1]**.

6.2.1. LAB MEDIA: Figure 4 A.

6.3. Next, species-specificities of these primers in the cohort of monkey or human genomic DNA **[1]** or in the cohort of mouse genomic DNA were confirmed **[2]**.

6.3.1. LAB MEDIA: Figure 4 B. *Video Editor: Emphasize the lanes on the human and monkey gels labeled with a *.*

6.3.2. LAB MEDIA: Figure 4 B. *Video Editor: Emphasize the lanes on the mouse gel labeled with a #.*

6.4. Finally, two species-specific primers were used to amplify pig DNA in the pig-to-monkey artery patch **[1]** and pig-to-mouse cell transplantation models **[2]**.

6.4.1. LAB MEDIA: Figure 4 C. *Video Editor: Emphasize the top gel.*

6.4.2. LAB MEDIA: Figure 4 C. *Video Editor: Emphasize the bottom gel.*

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Conclusion

7. Conclusion Interview Statements

7.1. **Jiao Chen:** The most important thing to remember when attempting this protocol is that contamination of aerosols should be prevented.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.3.1.*

7.2. **Hongyuan Zhang:** This technique paves the way for researchers to explore new questions within xenotransplantation because it is a simple, low cost, and non-invasive method to monitor the immune rejection of xenotransplantation.

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