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Title: Identification of *Mycobacterium* Species by DNA Microarray Chip Method

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

We will need you to record using screen recording software.

We recommend using the screen capture program OBS. JoVE's tutorial for using OBS Studio is provided at this link: https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here:

When you are ready to submit your video files, please contact our China Location Producer, Yuan Yue.

Current Protocol Length

Number of Steps: 17
Number of Shots: 40

Introduction

NOTE: @VO Team, please record the introduction statements

1.1. This research focuses on molecular diagnostics in clinical microbiology. Compared with traditional detection methods, the DNA microarray chip method for identifying *Mycobacterium* species is highly sensitive, specific, and rapid, which can provide important assistance for the early diagnosis of clinical patients **[1]**.

1.1.1. *2.3.1, 2.3.2.*

What are the most recent developments in this field of research?

1.2. Currently, rapid identification methods for non-tuberculous mycobacteria are limited in many grassroots laboratories, which directly impact patient clinical diagnosis and treatment **[1]**.

1.2.1. *2.7*

Ethics Title Card

This research has been approved by the Ethics Committee of Quanzhou First Hospital Affiliated to Fujian Medical University

Protocol

SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Balamurugan) for queries regarding SCREEN timestamps

2. Chip Hybridization

Demonstrator: Chen Zixuan

NOTE: @VO Team, please record the introduction statements (1.1 and 1.2) also

2.1. To begin, set the temperature of the constant temperature water bath to 50 degrees Celsius and preheat it **[1]**.

2.1.1. Talent adjusting the control panel of the water bath and setting the temperature to 50 degrees Celsius.

2.2. To prepare chips, add 200 microliters of distilled water to the bottom of the gene microarray chip hybridization box **[1]**. Place the bracket between the two positioning columns inside the box **[2]**.

2.2.1. Talent pipetting 200 microliters of distilled water into the hybridization box.

2.2.2. Talent inserting the bracket carefully between the positioning columns.

2.3. Carefully place the chip with the front side up on the bracket **[1]** and cover it with the cover slip so that its four bosses face downward, ensuring the end of the cover slip aligns with the label at the end of the chip **[2]**.

2.3.1. Talent placing the chip front-side up on the bracket.

2.3.2. Talent aligning and placing the cover slip with bosses down, ensuring proper orientation with the chip label.

2.4. Next, prepare 200 microliter centrifuge tubes or eight-well strips according to the number of samples and label them properly **[1]**.

2.4.1. A shot of the labeled centrifuge tubes or eight-well strips.

2.5. Heat the hybridization buffer from the kit at 50 degrees Celsius until it is completely melted **[1]**. After thorough mixing, centrifuge it quickly in a microcentrifuge **[2]**. Aliquot 9 microliters of hybridization buffer into each prepared tube or strip **[3]**. Add 6

microliters of the corresponding PCR product to each tube, making a final volume of 15 microliters per reaction mixture **[4]**.

2.5.1. Talent placing a tube containing the hybridization buffer in the preheated water bath.

2.5.2. Talent placing the microcentrifuge tube containing the melted buffer in a microcentrifuge.

2.5.3. Talent pipetting 9 microliters of buffer into the prepared labeled tube or strips.

2.5.4. Talent adding 6 microliters of PCR product into the tube.

2.6. Heat the hybridization reaction mixture to 95 degrees Celsius for 5 minutes to denature it using a PCR instrument or water bath **[1]**. After the denaturation treatment, immediately immerse the mixture in an ice-water mixture and incubate for 3 minutes **[2]**.

2.6.1. Talent placing tubes into a PCR instrument or water bath set to 95 degrees Celsius.

2.6.2. Talent transferring the tubes into a container with an ice-water mixture.

2.7. Remove the hybridization reaction mixture from the ice bath **[1]** and mix it by pipetting up and down twice **[2]**. When no white flocculent precipitate remains **[3]**, add 13.5 microliters of the reaction mixture through the loading hole of the cover slip **[4]**. Quickly cover and seal the hybridization box **[5]** and record the chip number, microarray position, and sample number **[6-TXT]**.

2.7.1. Talent retrieving the hybridization mixture from the ice-water mixture.

2.7.2. Talent pipetting the cooled reaction mixture up and down using a micropipette.

2.7.3. A shot of the hybridization mixture with no white flocculent precipitate.

2.7.4. Talent dispensing 13.5 microliters of mixture through the loading hole in the cover slip.

2.7.5. Talent closing and sealing the hybridization box.

2.7.6. Talent recording the chip number, microarray position, and sample number. **TXT: Only one sample can be added to each microarray**

2.8. Now, place the sealed hybridization boxes horizontally into the constant temperature water bath that has been preheated to 50 degrees Celsius. After placing all the boxes inside **[1]**, start the timer for 120 minutes **[2-TXT]**.

2.8.1. Talent placing hybridization boxes into the water bath horizontally.

2.8.2. Talent starting the digital timer on the constant temperature water bath. **TXT: Avoid introducing air bubbles during the loading process; Prevent prolonged air exposure of the liquid on chip microarrays**

2.9. After the hybridization reaction is completed, take out the hybridization boxes horizontally **[1]**, disassemble them, and retrieve the chips **[2]**. Immediately place the chips on the slide rack inside a container filled with Chip Washing Solution I *(one)* that has been equilibrated to room temperature **[3]** and wash them at 80 to 100 rpm on a constant-temperature shaker for 3 minutes at room temperature **[4]**.

2.9.1. Talent removing the hybridization boxes horizontally.

2.9.2. Talent disassembling the hybridization boxes and taking out the chips.

2.9.3. Talent placing chips on a slide rack inside a beaker filled with Chip Washing Solution I.

2.9.4. Talent placing the beaker in a constant-temperature shaker.

2.10. Wash the chips again with Chip Washing Solution II *(two)* **[1-TXT]** on the shaker set to 80 to 100 rpm for 3 minutes at room temperature **[2]**.

2.10.1. Talent transferring chips to Washing Solution II. **TXT: Equilibrate the Chip Washing Solution II to RT before washing** NOTE: Timestamp: 2.10-2.11.MOV 01:20-01:32

2.10.2. Talent placing the beaker in the constant-temperature shaker. NOTE: Timestamp: 2.10-2.11.MOV 03:20-03:38

2.11. Now, place the chips in a centrifuge and spin at 100 *g* for 5 minutes at room temperature **[1]**.

2.11.1. Talent loading chips into a centrifuge and starting spin. NOTE: Timestamp: 2.10-2.11.MOV 15:43-15:58

3. Chip Scanning and Result Interpretation

Demonstrator: Jiang Yancheng

3.1. Turn on the scanner **[1]** and then open the corresponding software. Click the **Laser Control** button to allow it to preheat for 10 minutes **[2]**.

3.1.1. Talent turning on the scanner.

3.1.2. SCREEN: 68234-3.1. 2.MP4 00:03-00:19

3.2. Input sample-related information into the software **[1]**.

3.2.1. SCREEN: 68234-3.2.1.MP4 00:12-00:18; 01:35-01:47

3.3. After the scanner finishes preheating, click the **Eject** button **[1].** Place the dried chip steadily on the small bracket piece, push the chip gently and horizontally into the scanner compartment **[2],** and click the **Load** button **[3]**.

3.3.1. SCREEN: 68234-3.3.1.MP4 00:02-00:09

3.3.2. Talent placing the chip onto the bracket piece and pushing the chip gently and horizontally into the scanner compartment.

3.3.3. SCREEN: 68234-3.3.3.MP4 00:02-00:08

3.4. Now, input the chip number into the software and click to select the **detection area** (**microarray 1–4**) *(detection area, microarray 1 to 4)* **[1]**. Click **Select Sample** to choose the corresponding samples for each microarray **[2]**. Then, click **Start Detection** to begin chip scanning. The scanning results will be displayed on the screen and automatically saved **[3-TXT]**.

3.4.1. SCREEN: 68234-3.4.MP4 00:09-00:25

3.4.2. SCREEN: 68234-3.4.MP4 00:26-00:33

3.4.3. SCREEN: 68234-3.4.MP4 00:35-00:39; 00:42-00:48; 00:53-01:15 *Video editor: Speed as required* **TXT: Repeat the process of inserting the chip and scanning for the next chip; Continue until all chips are scanned**

3.5. Use the **Data Query** page to conduct data queries and printing operations **[1]**.

3.5.1. SCREEN: 68234-3.5.1.MP4 00:02-00:20

3.6. After completing all operations, turn off the laser **[1]**, exit the software **[2]**, and finally, turn off the scanner **[3]**.

3.6.1. SCREEN: 68234-3.6.1.MP4 00:02-00:07

3.6.2. SCREEN: 68234-3.6.1.MP4 00:02-00:05

3.6.3. Talent pressing the power button on the scanner to turn it off.

Results

4. Results

4.1. The validity of the experimental results is confirmed by quality control **[1]**, with the positive control yielding a detection result of "*Mycobacterium tuberculosis* complex" **[2]**, and the negative control yielding "no *Mycobacterium*" **[3]**.

4.1.1. LAB MEDIA: Figure 3.

4.1.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the second and third rows from the top.*

4.1.3. LAB MEDIA: Figure 3. *Video editor: Highlight 3B.*

4.2. Interpretation of test samples begins with cases where all probes, including the one with the highest signal value, are negative, resulting in a report of "no *Mycobacterium*" **[1]**.

4.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight 4A.*

4.3. In samples where only the *Mycobacterium tuberculosis* complex probe shows a strong signal, the result is identified as positive for that species group **[1]**.

4.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight 4B.*

4.4. If the probe corresponding to *Mycobacterium avium* is the only one with a strong signal, the sample is identified as positive for *Mycobacterium avium* **[1]**.

4.4.1. LAB MEDIA: Figure 4. *Video editor: Highlight 4C.*

4.5. If only the internal control probe is positive and all others are negative, the result is reported as "indeterminate," indicating the presence of a *Mycobacterium* species not covered by the detection kit **[1]**.

4.5.1. LAB MEDIA: Figure 4. *Video editor: Highlight 4D.*

Pronunciation Guide:

1. **centrifuge**
   Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge dictionary.cambridge.org+7youtube.com+7dictionary.cambridge.org+7merriam-webster.com+15merriam-webster.com+15merriam-webster.com+15
   IPA: /ˈsɛn.trəˌfjuʤ/
   Phonetic: SEN-truh-fyooj
2. **microarray**
   Pronunciation link: https://www.merriam-webster.com/dictionary/microarray merriam-webster.com
   IPA: /ˌmaɪ.kroʊ.əˈreɪ/
   Phonetic: MY-kroh-uh-RAY
3. **hybridization**
   Pronunciation link: https://www.merriam-webster.com/dictionary/hybridization youtube.com+2howtopronounce.com+2youtube.com+2dictionary.cambridge.org+1howtopronounce.com+1merriam-webster.com+15merriam-webster.com+15dictionary.cambridge.org+15
   IPA: /ˌhaɪ.brɪ.dəˈzeɪ.ʃən/
   Phonetic: HY-bri-duh-ZAY-shuhn
4. **precipitate**
   Pronunciation link: https://www.merriam-webster.com/dictionary/precipitate merriam-webster.com+15merriam-webster.com+15dictionary.cambridge.org+15youtube.com
   IPA: /prɪˈsɪp.ɪ.teɪt/
   Phonetic: pri-SIP-ih-tayt
5. **flocculent**
   Pronunciation link: https://www.merriam-webster.com/dictionary/flocculent merriam-webster.commerriam-webster.com+1merriam-webster.com+1 *(source mix)*
   IPA: /ˈflɑː.kjə.lənt/
   Phonetic: FLAHK-yuh-luhnt
6. **equilibrate**
   Pronunciation link: https://www.collinsdictionary.com/us/dictionary/english/equilibrate merriam-webster.com+6collinsdictionary.com+6dictionary.cambridge.org+6
   IPA: /ɪˈkwɪl.əˌbreɪt/
   Phonetic: ih-KWIL-uh-brayt
7. **Mycobacterium**
   Pronunciation link: https://www.merriam-webster.com/dictionary/mycobacterium youtube.com+15en.wiktionary.org+15en.wiktionary.org+15
   IPA: /ˌmaɪ.koʊ.bækˈtɪr.i.əm/
   Phonetic: MY-koh-bak-TIR-ee-uhm

8. **Mycobacterium avium**
   Pronunciation link: see “Mycobacterium” above
   IPA: /ˌmaɪ.koʊ.bækˈtɪr.i.əm ˈeɪ.vi.əm/
   Phonetic: MY-koh-bak-TIR-ee-uhm AY-vee-uhm