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Scriptwriter Name: Gaurav Vaidya
Supervisor Name: Anastasia Gomez

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Title: Genome-wide Analysis of Histone Modifications Distribution using the Chromatin Immunoprecipitation Sequencing Method in *Magnaporthe oryzae*

Authors and Affiliations:

Zechi Wu[#], Wanyu Sun[#], Sida Zhou, Li Zhang, Xinyu Zhao, Yang Xu, Weixiang Wang*

Beijing Key Laboratory of New Technology in Agricultural Application, National Demonstration Center for Experimental Plant Production Education, Department of Agronomy, Beijing University of Agriculture, Beijing, People's Republic of China

[#]These authors contribute equally to this paper.

Corresponding Authors:

Weixiang Wang (wwxbua@163.com)

Email Addresses for All Authors:

szwzqswy97@163.com
809623995@qq.com
onu1079@163.com
xuyang9510@163.com
1918334969@qq.com
zhangli2625w@163.com
wwxbua@163.com

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?

Response: NO

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

Response: 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**.

☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations?

Response: Yes, some of the latter experiments need to be carried out at the Beijing Institute of Biological Sciences, 2.5 kilometers away from us.

Current Protocol Length

Number of Steps: 22
Number of Shots: 54

Introduction

1. Introductory Interview Statements

NOTE for VO: Please record the introductory interview statements and conclusion statements.

REQUIRED:

1.1. This method can help elucidate the molecular mechanisms underlying the regulation of candidate target genes via epigenetic modifications during fungal pathogenesis in plant pathology.

1.1.1. *3.4.2.*

1.2. Chromatin immunoprecipitation sequencing technology can efficiently detect DNA segments that interact with histones and transcription factors in the whole genome. Compared with other methods, it can improve efficiency and resolution.

1.2.1. *8.2.1.*

Protocol

2. Preparation of Protoplasts from *M. oryzae*

2.1. Begin by adding 1000 microliters of liquid Complete Medium to the oatmeal-tomato agar plates using a 1000 microliter pipette **[1]**. Using an inoculation loop, scrape the mycelia of the wild-type strain and the knockout strain **[2]**. Collect the mycelia debris **[3]** and transfer them to 250 milliliters of liquid Complete Medium **[4]**. *Videographer: This step is important!*

2.1.1. WIDE: Establishing shot of talent, adding liquid complete medium to the agar plates.

2.1.2. Talent scraping the mycelia of both strains.

2.1.3. Talent collecting the mycelia debris.

2.1.4. Talent transferring the debris to the liquid Complete Medium.

2.2. Wash the fungal hyphae with 500 milliliters of 0.7 molar sodium chloride solution **[1]**, then collect the fungal mycelium and weigh it **[2]**. Add approximately 1 milliliter of lysis enzyme permeation solution per 1 gram of the fungal mycelium **[3]**. Place the hyphae for lysing at 28 degrees Celsius for 3 to 4 hours with shaking at 150 rpm **[4]**.

2.2.1. Talent washing the fungal hyphae.

2.2.2. Talent weighing the fungal mycelium.

2.2.3. Talent adding lysis enzyme permeation solution.

2.2.4. Talent placing the hyphae on the shaker for lysing.

2.3. Wash the lysed hyphae with 50 milliliters of 0.7 molar sodium chloride solution **[1]**. Centrifuge the sample and discard the supernatant carefully **[2].** Resuspend the protoplasts in 20 milliliters of 0.7 molar sodium chloride solution **[3]**.

2.3.1. Talent washing the lysed hyphae.

2.3.2. Talent discarding the supernatant.

2.3.3. Talent resuspending the protoplasts.

3. In Vivo Crosslinking and Sonication

3.1. Add 55 microliters of 37 percent formaldehyde drop by drop to 2 milliliters of sodium chloride buffer containing protoplast for crosslinking until the final concentration is 1 percent **[1]**. To quench the unreacted formaldehyde, add 20 microliters of 10x glycine to each tube **[2]**. *Videographer: This step is important!*

3.1.1. Talent adding 37% formaldehyde drop by drop to the protoplast solution.

3.1.2. Talent adding 10x glycine to each tube.

3.2. Discard the supernatant carefully after centrifugation **[1]**. Resuspend the pellet in 1 milliliter of 0.7 molar sodium chloride solution **[2]**. Discard the supernatant carefully after centrifugation and resuspend the pellet in 750 microliters of RIPA buffer **[3]**.

3.2.1. Talent discarding the supernatant.
3.2.2. Talent resuspending the pellet.
3.2.3. Talent resuspending the pellet in RIPA buffer.

3.3. Add 37.5 microliters of 20x protease inhibitor **[1]**. Shear the chromatin by sonication for 8 minutes using an ultrasonic homogenizer **[2-TXT]**. *Videographer: This step is important!*

3.3.1. Talent adding the protease inhibitor.
3.3.2. Talent performing sonication using the ultrasonic homogenizer. **TEXT: 25% W, output 3 s, stop 5 s, 4 °C**

Author's NOTE: 3.2.3 and 3.3.3 were the same; therefore, we only took one shot, 3.3.3.

NOTE: Shot 3.2.2 from the original shot-list was removed, the respective VO narration was also removed; hence 3.3.3 now is 3.3.2.

3.4. After the sample has been ultrasonically broken, take out a part of the sample as "input," containing all DNA and protein released after the sample is sonicated.**[1-TXT]**. To analyze the length of the DNA fragments, run a 1 percent agarose gel electrophoresis after sonication **[2]**. *Videographer: This step is important!*

3.4.1. Talent taking part of the sample out as input. **TEXT: Not used to perform ChIP experiment.**

3.4.2. Talent running the 1% agarose gel electrophoresis.

3.5. After centrifugation, transfer the centrifuged supernatant to a new 1.5-milliliter centrifuge tube **[1]** and store it at -80 degrees Celsius for later use **[2]**. Before performing the IP experiment, dilute each chromatin sample to a ratio of 1:10 with 1x RIPA buffer **[3-TXT]**.

3.5.1. Talent transferring the supernatant to a new centrifuge tube.

3.5.2. Talent placing the tube for storing at -80 degrees Celsius.

3.5.3. Talent diluting the chromatin sample. **TEXT: e.g., add 10 µL of chromatin sample to 1 µL of 1x RIPA buffer**

4. IP of Crosslinked Protein/DNA

4.1. Pipette 50 microliters of superparamagnetic protein beads into a 2-milliliter centrifuge tube **[1]**. Place the tubes on a magnetic stand and let the magnetic beads precipitate. Then, remove the supernatant **[2]**. *Videographer: This step is important!*

4.1.1. Talent pipetting the superparamagnetic protein beads into the centrifuge tube.
4.1.2. Talent placing the tubes on the magnetic stand.

4.2. Add 1 milliliter of pre-cooled 1x RIPA buffer to the tube and wash superparamagnetic protein beads three times **[1]**. Place the tubes on a magnetic stand and remove the supernatant **[2]**. Add 100 microliters of 1x RIPA buffer to each tube **[3]**.

4.2.1. Talent washing the superparamagnetic protein beads.
4.2.2. Talent placing the tubes on the magnetic stand.
4.2.3. Talent adding 1x RIPA buffer to each tube.

4.3. Add 300 microliters of the chromatin sample, 100 microliters of superparamagnetic protein beads, and 4 microliters of H3K4me3 antibody to the tube, mixing them well **[1-TXT]**. Place the samples on a rotary shaker to incubate them overnight at 4 degrees Celsius at 30x *g* **[2]**. *Videographer: This step is important!*

4.3.1. Talent adding chromatin sample, superparamagnetic protein beads, and antibodies to the tube. **TEXT: 2 x 10^7 cells**
4.3.2. Talent placing the tubes on a rotary shaker.

5. Collecting and Rinsing the IP Products

5.1. Wash the superparamagnetic protein bead-antibody and chromatin complex by resuspending the beads in 1 milliliter of 1x RIPA buffer **[1]**. Wash the sample with 1 milliliter of low salt immune complex wash buffer **[2]**, then with 1 milliliter of high salt immune complex wash buffer **[3]**.

5.1.1. Talent resuspending the beads in 1x RIPA buffer.
5.1.2. Talent adding the low salt immune complex wash buffer.
5.1.3. Talent adding the high salt immune complex wash buffer.

5.2. Rinse the beads with 1 milliliter of lithium chloride buffer and remove the supernatant **[1]**, then add 1 milliliter of TE buffer to the tube **[2]**. Wash the tube again with 1 milliliter of TE buffer and remove the supernatant with a pipette **[3]**

5.2.1. Talent sensing the beads with LiCl buffer.
5.2.2. Talent adding TE buffer to the tube.
5.2.3. Talent washing the tube after adding the TE buffer.

6. Elution and Reverse Crosslinking of Protein/DNA Complexes

6.1. Add 100 microliters of elution buffer to each centrifuge tube **[1]**. Perform elution at 65 degrees Celsius for 15 minutes **[2]**. Centrifuge for 1 minute at 10,000 x *g* at 4 degrees Celsius and collect the supernatant into new centrifuge tubes **[3]**.

6.1.1. Talent adding the elution buffer to the centrifuge tube.
6.1.2. A shot of the centrifuge tubes kept for elution.
6.1.3. Talent centrifuging the tubes.

6.2. Add 8 microliters of 5 molar sodium chloride to all the tubes and incubate at 65 degrees Celsius for 4 to 5 hours or overnight to reverse the DNA-protein crosslinks **[1]**. Add 1 microliter of RNase A and incubate for 30 minutes at 37 degrees Celsius **[2]**.

6.2.1. Talent adding 5 molar NaCl to all the tubes.
6.2.2. Talent adding RNase A to the tubes.

6.3. Add 4 microliters of Proteinase K to each tube and incubate at 45 degrees Celsius for 1 to 2 hours **[1-TXT]**.

6.3.1. Talent adding Proteinase K to each tube. **TEXT: Dissolved in H_2O at 20 mg/mL and stored at -20 °C**

7. Purification and Recovery of DNA

7.1. Add 550 microliters of the phenol, chloroform, and isoamyl alcohol mixture to the centrifuge tube **[1-TXT]**. Centrifuge for 15 minutes at 10,000 x *g* and aspirate the supernatant **[2]**. Add 1/10th volume of 3 molar sodium acetate solution, 2.5 volumes of absolute ethanol, and 3 microliters of glycogen to the tube **[3]**.

7.1.1. Talent adding the mixture to the centrifuge tube. **TEXT: Ratio of 25:24:1**
7.1.2. Talent aspirating the tubes.
7.1.3. Talent adding the sodium acetate solution, absolute ethanol, and glycogen to the tube.

7.2. Place the sample in a refrigerator at -20 degrees Celsius overnight for precipitation **[1]**. On the next day, centrifuge the tubes and discard the supernatant. Wash the pellet three times with 1 milliliter of freshly prepared 75 percent ethanol at 10,000 x g **[2]**. Add 50 microliters of sterile deionized water to sufficiently dissolve the precipitate **[3]**.

7.2.1. Talent placing the sample in the refrigerator.
7.2.2. Talent washing the pellets using 75% ethanol.
7.2.3. Talent adding sterile deionized water.

8. DNA repair and Solexa library Construction

8.1. Repair the DNA ends to generate blunt-ended DNA using a DNA end-repair kit and

instructions mentioned in the text manuscript **[1]**.

8.1.1. Talent using a DNA-repair kit to generate blunt-ended DNA.

8.2. To add adenine bases to the 3' ends, add 30 microliters of DNA, 2 microliters of water, 5 microliters of 10x Taq *(pronounce "tack")* buffer, 10 microliters of 1 millimolar dATP, and 3 microliters of Taq DNA polymerase to a 0.2-milliliter PCR centrifuge tube **[1]**. Mix them and react in a PCR machine at 72 degrees Celsius for 10 minutes **[2]**.

8.2.1. Talent adding the reagents to a PCR centrifuge tube.
8.2.2. Talent placing the tube into the PCR machine.

8.3. Perform linker ligation by mixing 10 microliters of DNA, 9.9 microliters of water, 2.5 microliters of T4 DNA ligase buffer, 0.1 microliter of adapter oligo mix, and 2.5 microliters of T4 DNA ligase in a 0.2-milliliter PCR centrifuge tube **[1]**. Incubate the tube at 16 degrees Celsius for 4 hours **[2]**.

8.3.1. Talent adding the individual reagents for linker ligation to the PCR centrifuge tube.
8.3.2. Talent placing the tube for incubation.

8.4. To amplify the DNA using PCR primers, add 10.5 microliters of DNA, 12.5 microliters of 2x high fidelity master mix, 1 microliter of PCR primer PE1.0, and 1 microliter of PCR primer PE2.0 to a 0.2 milliliter PCR centrifuge tube and mix well **[1]**.

8.4.1. Talent adding the individual reagents to the PCR centrifuge tube.

Results

9. Results: Chromatin Immunoprecipitation Sequencing Method for Genome-wide Histone Modification Analysis

8.5. The H3K4me3 signals of the Δ*mobre2*, Δ*mospp1*, and Δ*moswd2 (pronounce 'mobre2', 'mospp1', and 'moswd2'as they are spelled)* deletion mutants were significantly decreased at its functional target regions.

8.5.1. LAB MEDIA: Figure 2.

8.6. Compared to the wild-type strain P131, the signals of enriched H3K4me3 chromatin immunoprecipitation sequencing reads in the Δ*mobre2,* Δ*mospp1*, and Δ*moswd2* deletion mutants were largely decreased.

8.6.1. LAB MEDIA: Figure 2.

Conclusion

9. Conclusion Interview Statements

9.1. Following this procedure, ChIP-on-chip and ChIP-qPCR can be performed. They are generally used to screen the downstream target gene of a protein and study protein-DNA interactions at known genome binding sites.

9.1.1. *8.4.1.*