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Title: High-Resolution Mapping of Protein-DNA Interactions in Mouse Stem Cell-Derived Neurons Using Chromatin Immunoprecipitation-Exonuclease

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **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**

Introduction

1. Introductory Interview Statements

Videographer: Interviewee headshots are required. Take a headshot for each interviewee.

Authors: While filming the interview portion, our videographer will also photograph you for the **JoVE Dedicated Author Webpage**. Please look at this **example**. For questions about the author profile pages and pictures, please contact **author.liaison@jove.com**.

Authors: Please memorize the interview statements prior to your filming day.

1.1. **Kaitlin Montanera:** The main advantage of our ChIP-exo method is the improved mapping resolution of protein-DNA interactions in the cell, compared to the conventional chromatin immunoprecipitation method **[1]**.

1.1.1. INTERVIEW: Named author says the above in an interview-style statement while looking slightly off-camera.

Protocol

2. Antibody Incubation with Beads

2.1. To prepare protein G magnetic beads for ChIP, mix the magnetic beads until homogenous **[1]**. Then, add 25 microliters of the magnetic beads to a 2-milliliter protein low-bind tube **[2]**.

2.1.1. Talent mixes beads until homogenous.
2.1.2. Talent adds beads to 2-mL tube.

2.2. Add 1 milliliter of blocking solution to the beads **[1]**. Mix well by pipetting, then place the tube on a magnetic rack for 1 minute **[2]**. Once the supernatant is clear, remove it **[3]**.

2.2.1. Talent adds blocking solution to beads then mixes well.
2.2.2. Talent places tube on a magnetic rack.
2.2.3. Talent removes supernatant.

2.3. Next, add 1 milliliter of blocking solution to the beads **[1]**. Place the tube on a rocking platform at 4 degrees Celsius, and rock for 10 minutes **[2]**. Then, briefly spin the tube **[3]**. Place tube on the magnetic rack, and remove the supernatant **[4]**.

2.3.1. Talent adds 1 mL of blocking solution.
2.3.2. Talent places the tube on the rocking platform and starts it.
2.3.3. Talent places the tube in centrifuge.
2.3.4. Talent places tube on magnetic rack and removes the supernatant.

2.4. Add 500 microliters of blocking solution to the magnetic beads **[1]**. Briefly spin the antibody against Isl1 *(pronounce **ai**-let one)* **[2]**. Then, add 4 micrograms of the antibody to the tube containing magnetic beads **[3]**.

2.4.1. Talent adds blocking solution to the tube containing magnetic beads.
2.4.2. Talent spins the antibody.
2.4.3. Talent adds antibody to tube containing magnetic beads.

2.5. Place the tube containing magnetic beads and Isl1 *(pronounce **ai**-let one)* antibody on a rocking platform at 4 degrees Celsius, and rock for 6 to 24 hours **[1]**.

2.5.1. Talent places sample on rocking platform and turns it on.

3. Chromatin Immunoprecipitation (ChIP)

3.1. To begin the chromatin immunoprecipitation, wash the antibody-coated beads in 1

milliliter of blocking solution **[1]**. Place the tube containing the antibody-coated beads on a rocking platform at 4 degrees Celsius for 5 minutes **[2]**. After briefly spinning, place the tube on a magnetic rack and remove the supernatant **[3]**.

3.1.1. Talent washes beads in 1 mL of blocking solution.
3.1.2. Talent places sample on rocking platform.
3.1.3. Talent places sample on a magnetic rack and removes the supernatant.

3.2. Resuspend the antibody-coated beads in 50 microliters of blocking solution **[1]**.

3.2.1. Talent adds blocking solution to tube.

3.3. Add 1-milliliter of sonicated lysates to antibody-coated beads **[1].** Then incubate sample on a rocking platform at 4 degrees Celsius overnight **[2]**.

3.3.1. Talent adds 1 milliliter of lysate to tube.
3.3.2. Talent places sample tube on a rocking platform.

3.4. After allowing the sample to incubate overnight, collect liquid from the cap by briefly spinning the tube **[1]**. Then, place the tube on a magnetic rack, and, after 1 minute **[2]**, remove the supernatant carefully with a pipette **[3]**.

3.4.1. Talent places tube in table top centrifuge.
3.4.2. Talent places tube on magnetic rack.
3.4.3. Talent removes supernatant from tube with a pipette.

3.5. Next, perform a series of washes as follows: Add 1 milliliter of cold wash buffer containing CPI to the sample **[1-TXT]**. Mix the sample on a rocking platform at 4 degrees Celsius for 5 minutes **[2]**. Briefly spin the sample tube then place on a magnetic rack **[3]**. After 1 minute, remove the supernatant with a pipette **[4]**.

3.5.1. Talent adds 1 milliliter of buffer. *Videographer: Please obtain multiple reusable takes; this shot can be reused for 4.2.1, 4.4.1,* ***Error! Reference source not found.****, and 4.7.1.* **TEXT: 1st: lysis buffer 3; 2nd: high salt wash buffer; 3rd: LiCl wash buffer; 4th: 10 mM Tris-HCl buffer** *Video editor: Please keep this text on screen for next 3 shots.*
3.5.2. Talent places sample on rocking platform. *Videographer: Please obtain multiple reusable takes; this shot can be reused for 4.2.2 and 4.4.2.*
3.5.3. Place sample on a magnetic rack. *Videographer: Please obtain multiple reusable takes; this shot can be reused for 4.2.3 and 4.4.3.*
3.5.4. Talent removes sample with a pipette. *Videographer: Please obtain multiple reusable takes; this shot can be reused for 4.2.4 and 4.4.4.*

4. Enzymatic Reactions on Beads

4.1. For the first enzymatic reaction, end-repair and dA-tailing *(pronounce D-A-tailing)*, add

38 microliters of autoclaved double distilled water to the sample **[1]**. Then, add end-prep reaction mix and end-prep enzyme mix **[2]**. Mix the contents of the tube by pipetting **[3].** Then, incubate the sample at 20 degrees Celsius for 30 minutes **[4]**.

4.1.1. Talent adds water to tube
4.1.2. Talent adds end-prep reaction mix and end-prep enzyme mix to tube. **TEXT: 5.6 µL end prep reaction mix, and 2.4 µL end prep enzyme mix**
4.1.3. Mix sample in tube by pipetting.
4.1.4. Talent places sample in incubator.

4.2. Next, wash the sample beads with high salt wash buffer, lithium chloride wash buffer, and 10 millimolar Tris-hydrogen chloride buffer, as described previously **[1][2][3][4]**.

4.2.1. *Videographer/video editor: Reuse footage from 3.5.1. Video editor, please show 4.2.1, 4.2.2, 4.2.3, and 4.2.4 as a multipanel.*
4.2.2. *Videographer/video editor: Reuse footage from 3.5.2.*
4.2.3. *Videographer/video editor: Reuse footage from 3.5.3.*
4.2.4. *Videographer/video editor: Reuse footage from 3.5.4.*

4.3. To perform the index adapter ligation, add 27 microliters of cold 10 millimolar Tris-hydrogen chloride buffer to the sample **[1]**. Then, add index adapter, ligation enhancer, and ligase master mix **[2-TXT]**. Incubate the sample at 20 degrees Celsius for 15 minutes **[3]**.

4.3.1. Talent adds buffer to tube.
4.3.2. Talent adds index adapter, ligation enhancer, and ligase master mix. **TEXT: 2 µL 15 µM index adapter; 0.5 µL ligation enhancer, and 15 µL ligase master mix**
4.3.3. Talent places sample in incubator.

4.4. Again, wash the sample beads with high salt wash buffer, lithium chloride wash buffer, and 10 millimolar Tris-hydrogen chloride buffer **[1][2][3][4]**. Then, add 47 microliters of cold 10 millimolar Tris-hydrogen chloride buffer to the sample **[5]**.

4.4.1. *Videographer/video editor: Reuse footage from 3.5.1. Video editor, please show 4.4.1, 4.4.2, 4.4.3, and 4.4.4 as a multipanel.*
4.4.2. *Videographer/video editor: Reuse footage from 3.5.2.*
4.4.3. *Videographer/video editor: Reuse footage from 3.5.3.*
4.4.4. *Videographer/video editor: Reuse footage from 3.5.4.*
4.4.5. Talent adds buffer to sample tube.

4.5. To repair the nick in the DNA resulting from the missing phosphodiester bond, add 11.1 microliters of the fill-in mix to the sample **[1-TXT]**. Incubate the sample at 30 degrees Celsius for 20 minutes **[2]**.

4.5.1. Talent adds fill-in mix to each sample. **TEXT: Reference Table 4**
4.5.2. Talent places sample in incubator.

4.6. After a series of washes, add 50 microliters of cold, autoclaved double distilled-water to the sample **[1].** To digest the ChIP *(pronounce chip)* DNA in the 5-prime to 3-prime direction, add lambda exonuclease buffer and lambda exonuclease, and mix the sample by pipetting **[2-TXT].** Incubate the sample at 37 degrees Celsius for 30 minutes **[3]**. *Videographer, this is the most important step for viewers to see.*

4.6.1. Talent adds water to the sample tube.
4.6.2. Talent adds buffer and exonuclease to tube and mixes by pipetting. **TEXT: 6 µL 10x lambda exonuclease buffer, and 2 µL 5 U/µL lambda exonuclease**
4.6.3. Talent places sample in incubator.

4.7. After the digestion is complete, repeat the three washes a final time **[1-TXT]**.
4.7.1. *Videographer/video editor: Reuse footage from 3.5.1.* **TEXT: Wash with high salt wash buffer, LiCl wash buffer, and 10 mM Tris-HCl buffer**

5. Elution and Purification

5.1. To elute ChIP sample from the beads, resuspend the sample in 75 microliters of ChIP elution buffer **[1]**, and incubate at 65 degrees Celsius for 15 minutes at 130 times *g* **[2]**.

5.1.1. Talent adds elution buffer to tube.
5.1.2. Talent incubates the tube at 65 degrees Celsius at 130 times g.

5.2. Add 2.5 microliters of 20 milligrams per milliliter proteinase K to the sample **[1]**. Vortex the sample briefly **[2]**, then incubate overnight at 65 degrees Celsius **[3]**.

5.2.1. Talent adds proteinase K to sample.
5.2.2. Talent vortexes sample.
5.2.3. Talent places sample in incubator.

5.3. Briefly spin the sample and place on a magnetic rack **[1]**. After 1 minute, transfer the supernatant to a new 1.5-milliliter tube **[2]**.

5.3.1. Talent removes sample from centrifuge and place on magnetic rack.
5.3.2. Talent transfers supernatant to new tube.

5.4. Purify and elute the DNA **[1]**.

5.4.1. Talent begins process of DNA purification.

6. Library Preparation

6.1. After purifying the eluted DNA, transfer 16 microliters of the extracted DNA sample to a PCR tube **[1]**. Add 1.2 microliters of denaturing and primer annealing mix to the sample **[2-TXT]**. Using the program described in Table 6 of the manuscript, denature

and anneal primers to the template DNA **[3]**.

6.1.1. Talent transfers sample to PCR tube.
6.1.2. Talent adds mix to DNA **TEXT: Reference Table 6**.
6.1.3. Talent places tube in PCR machine.

6.2. Add 3 microliters of primer extension mix to the sample, and run the PCR program described in Table 7 of the manuscript **[1-TXT][2]**.

6.2.1. Talent adds primer extension mix to the sample. **TEXT: Reference Table 7** *Video editor, please show 6.2.1 and 6.2.2 as a multipanel.*
6.2.2. Talent places tube in PCR machine.

6.3. Add 4.1 microliters of dA-tailing *(pronounce D-A-tailing)* mix to the sample, and run sample using the PCR program in Table 8 of the manuscript **[1-TXT][2]**.

6.3.1. Talent adds dA-tailing mix to sample. **TEXT: Reference Table 8** *Video editor, please show 6.3.1 and 6.3.2 as a multipanel.*
6.3.2. Talent places tube in PCR machine.

6.4. Then, add 21.5 microliters of universal adapter ligation mix to the sample **[1-TXT]**, and incubate for 15 minutes at 20 degrees Celsius **[2]**.

6.4.1. Talent adds ligation mix to each sample. **TEXT: Reference Table 9**
6.4.2. Talent places samples in incubator.

6.5. Add 29 microliters of LM-PCR mix to the sample **[1-TXT]**, then run sample using the program from Table 10 of the manuscript **[2]**.

6.5.1. Talent adds LM-PCR mix to the sample. **TEXT: Reference Table 10**
6.5.2. Talent places sample in PCR machine.

Results

7. Results: ChIP-exo Produces High Mapping Resolution

7.1. Following 18 cycles of LM-PCR, ChIP-exo samples from mouse motor neurons were electrophoresed on a 1.5 percent agarose gel **[1]**. The results for ChIP-exo Isl1 showed amplified DNA libraries around 200 to 400 base pairs, indicating that the ChIP-exo libraries were successfully amplified by LM-PCR. **[2]**.

7.1.1. LAB MEDIA: Figure 2. *Video editor, show Figure 2B only.*
7.1.2. LAB MEDIA: Figure 2. *Video editor, show Figure 2B only, and emphasize the third column.*

7.2. The band around 100 base pairs represents artifacts from adapters and PCR primers **[1]**. The no antibody control demonstrates that nonspecific, background DNA was digested by the lambda exonuclease treatment **[2]**.

7.2.1. LAB MEDIA: Figure 2. *Video editor, show Figure 2B only, and emphasize the lowest bands.*
7.2.2. LAB MEDIA: Figure 2. *Video editor, show Figure 2B only, and emphasize the second column.*

7.3. Using ChIP-exo and ChIP-seq, Isl1-bound locations were identified **[1]**. The ChIP-exo signal was highly focused at Isl1-binding sites, detecting multiple clustered Isl1 transcription factor binding patterns **[2]**. The ChIP-seq signal displayed broader signals, indicating that ChIP-exo had higher mapping resolution than ChIP-seq for Isl1 **[3]**.

7.3.1. LAB MEDIA: Figure 3.
7.3.2. LAB MEDIA: Figure 3. *Video editor, emphasize red graphs.*
7.3.3. LAB MEDIA: Figure 3. *Video editor, emphasize blue graphs.*

Conclusion

8. Conclusion Interview Statement

Authors: Please memorize the interview statements prior to your filming day.

8.1. **Ho Sung Rhee:** Multiple transcription factors often bind to DNA next to each other as a protein-DNA complex. ChIP-exo allows us to study how multiple transcription factors recognize and regulate target DNA. **[1].**

8.1.1. INTERVIEW: Named author says the above in an interview-style statement while looking slightly off-camera.