

Submission ID #: 69656

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Title: Genome-Wide Mapping of Histone Modifications and Transcription Factor Binding Sites in Neuroendocrine Small Cell Lung Cancer Cell Lines Using CUT&RUN

Authors and Affiliations:

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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. Filming location:** Will the filming need to take place in multiple locations? **Yes**
If **Yes**, how far apart are the locations? Approximately 100 meters

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 25

Number of Shots: 56

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

Videographer's NOTES:

Talent was very nice, but a little hard to direct. However, I think it went pretty well.

All steps are shot in HD, all intros are 4K (except first take of 1.1)

Note on the general order of shooting:

- Video #4
- Part of video #5
- Video #2
- Video #3
- Back to video #5
- Intros
- Finish up video #5
-

1.1, 1.2 & 1.3 – Hui-Zi Chen went way off script and did some much longer intros. Did at least a couple takes of each.

1.4, 1.5 – Meijun Du wasn't super comfortable on camera. She did a few takes of each.

Captured headshots of all authors, except Angela Mathison (she was on vacation)

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. **Hui-Zi Chen:** Our research maps histone post-translational modifications and lineage-defining transcription factor binding across SCLC subtypes to uncover epigenetic mechanisms and identify potential therapeutic vulnerabilities.

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

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Hui-Zi Chen:** ChIP-seq requires large cell numbers and yields high background noise. This protocol uses CUT&RUN, enabling histone and transcription factor profiling with fewer cells and lower background.

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

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Hui-Zi Chen:** CUT&RUN identifies genomic locations of chromatin-interacting proteins, including histone modifications, TF binding, revealing epigenetic regulatory landscapes.

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

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.4. **Meijun Du:** This protocol allows mapping of histone modifications and DNA-binding profiles of diverse proteins in situ in the cellular genome.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Meijun Du:** In the future, profiling histone PTMs and TF binding across SCLC subtypes may reveal targetable subtype-specific gene programs.

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

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Harvesting and Processing Small Cell Lung Cancer Neuroendocrine Cells

Demonstrator: Meijun Du

- 2.1. To begin, remove the cultured small cell lung cancer neuroendocrine cells from the 37 degrees Celsius incubator [1] and examine them under a microscope to ensure their quality [2].
 - 2.1.1. WIDE: Talent opening the incubator door and removing the cell culture flask.
 - 2.1.2. Talent placing the sample under a microscope.
- 2.2. Then, transfer the cell suspension to a 50-milliliter conical tube [1] and centrifuge the tube at 1,200 *g* for 5 minutes at room temperature [2].
 - 2.2.1. Talent transferring the cell suspension into a labeled 50 milliliter conical tube.
 - 2.2.2. Talent placing the tube into a centrifuge and starting the spin cycle.
- 2.3. Aspirate the medium from the conical tube by using the vacuum in the hood [1] and rinse the resulting cell pellet with 20 milliliters of PBS [2].
 - 2.3.1. Talent carefully aspirates the supernatant without disturbing the pellet using the vacuum in the hood.
 - 2.3.2. Talent adding 20 milliliters of 1× PBS into the same tube.
- 2.4. Centrifuge the tube again at 1,200 *g* for 5 minutes [1] and aspirate the PBS [2]. Add Accutase to digest the cell pellet for 3 to 5 minutes at 37 degrees Celsius, adjusting the time based on pellet size [3].
 - 2.4.1. Talent placing the conical tube into the centrifuge and starting the spin.
 - 2.4.2. Talent aspirating the PBS carefully using the vacuum in the hood.
 - 2.4.3. Talent adding Accutase solution into the tube and placing it in 37 degrees Celsius beads bath. **Videographer's NOTE:** 2.4.3 – Got two different shots of this step, one showing adding of accutase, the other showing the bead bath, use both.
- 2.5. Next, terminate the digestion by adding 5 milliliters of PBS with 10 percent FBS [1] and mixing to obtain a single-cell suspension [2].

- 2.5.1. Talent adding 5 milliliters of PBS with 10 percent fetal bovine serum to the digested cells.
- 2.5.2. Talent gently pipetting the mixture up and down using a pipette.
- 2.6. After centrifuging the cells again as demonstrated earlier, resuspend the pellet in 5 milliliters of PBS [1]. Count the starting cells and confirm their viability and integrity using the automatic cell counter [2].
 - 2.6.1. Talent adding 5 milliliters of PBS and resuspending the cell pellet.
 - 2.6.2. Talent placing the cell sample in the cell counter.
- 2.7. Calculate the total number of cells required by multiplying the number of reactions by 5×10^5 and add 20 percent excess to account for pipetting errors [1]. Transfer the calculated total number of cells to a 1.5-milliliter tube [2] and centrifuge the tube at 600 *g* for 3 minutes at room temperature [2].
 - 2.7.1. Talent writing in a notebook.
 - 2.7.2. Talent transferring the uniform suspension into a 1.5 milliliter microcentrifuge tube.
 - 2.7.3. Talent placing the cells in a centrifuge and initiating a 600 *g* spin.
- 2.8. Resuspend the cells in wash buffer at a density of 5×10^5 cells per reaction for a total of six reactions [1]. Use 100 microliters per reaction and pipette gently but thoroughly to ensure a uniform suspension [2-TXT].
 - 2.8.1. Talent adding Wash Buffer to the pellet.
 - 2.8.2. Talent gently pipetting the suspension up and down to mix evenly. **TXT: Repeat the wash step once more**

3. Binding Cells to Activated Beads and Antibodies

- 3.1. Aliquot 100 microliters of the washed cells into each tube of an 8-strip set that contains 10 microliters of activated Concanavalin-A beads [1]. Gently pipette to mix the contents [2].
 - 3.1.1. Talent pipetting 100 microliters of washed cells into each tube containing 10 microliters of Concanavalin A beads.

- 3.1.2. Talent gently pipetting to mix the cells and beads thoroughly.
- 3.2. Incubate the bead-cell slurry for 10 minutes at room temperature to allow cell binding to the beads [1] and quickly spin the tubes in a mini centrifuge to collect the slurry without allowing the beads to settle [2].
- 3.2.1. Talent placing the tubes on a benchtop stand for incubation at room temperature.
- 3.1.3,. Talent placing the 8-strip tubes into a mini centrifuge and performing a quick spin. **NOTE: Move 3.1.3 after 3.2.1. VO is moved**
- 3.3. Place the tubes on a 0.2 milliliter tube-magnet rack, allow the slurry to clear [1] and pipette to remove supernatant [2]. Then, remove the tubes from the magnet [3], immediately add 50 microliters of ice-cold Antibody Buffer to each reaction and gently pipette to resuspend the beads [4].
- 3.3.1. Talent placing the tubes on magnetic stand.
- 3.3.2. Talent removing the supernatant.
- 3.3.3. Talent lifting tubes off the magnetic stand and setting them down on the benchtop.
- 3.3.4. Talent adding 50 microliters of ice-cold Antibody Buffer into each tube and gently resuspending the beads by pipetting.
- 3.4. Label the 8-strip tubes appropriately [1]. Add 0.5 micrograms each of H3K4me3 (*H-3-K-4-M-E-3*), H3K4me1 (*H-3-K-4-M-E-1*), and immunoglobulin G antibodies and 0.6 micrograms of E2F7 polyclonal antibody from different sources to each reaction tube [1].
- 3.4.2,. Talent labeling the 8-strip tube clearly with appropriate identifiers. **NOTE: Move 3.4.2 before 3.4.1, VO swapped**
- 3.4.1. Talent pipetting precise volumes of each antibody into all 8-strip reaction tubes.
- 3.5. Gently pipette to mix the contents of each reaction tube thoroughly [1] and incubate them overnight on a nutator at 4 degrees Celsius with tube caps elevated [2-TXT].
- 3.5.1. Talent gently pipetting up and down in each tube to mix the reagents.
- 3.5.2. Talent placing the 8-strip tubes on a nutator, adjusting the caps to remain

elevated.

4. Protein A-G Micrococcal Nuclease Binding

- 4.1. Remove the 8-strip tubes from the nutator [1] and perform a quick spin to collect liquid at the bottom of each tube [2].
 - 4.1.1. Talent retrieving the 8-strip tubes from the cold incubator.
 - 4.1.2. Talent placing the tubes into a mini centrifuge for a quick spin.
- 4.2. Now, place the tubes on the magnet stand until the slurry clears [1]. Then, carefully pipette to remove and discard the supernatant [2].
 - 4.2.1. Talent positioning the tubes onto the magnet stand.
 - 4.2.2. Talent gently aspirating the cleared supernatant from the tube.
- 4.3. Keeping the tubes on the magnet stand, wash each tube twice with 200 microliters of cold Cell Permeabilization Buffer [1] and use a multiple channel pipette to remove the supernatant after each wash [2].
 - 4.3.1. Talent adding 200 microliters of cold Cell Permeabilization Buffer to each tube on the magnet stand.
 - 4.3.2. Talent aspirating the wash buffer from the magnet stand.
- 4.4. Next, remove the tubes from the magnet stand [1]. Immediately add 50 microliters of cold Cell Permeabilization Buffer to each reaction [2] and gently pipette to resuspend the cells and beads [3].
 - 4.4.1. Talent lifting the tubes off the magnet stand and setting them on the benchtop.
 - 4.4.2. Talent adding 50 microliters of buffer to each tube.
 - 4.4.3. Talent gently pipetting up and down to resuspend the beads and cells.
- 4.5. Then, add 2.5 microliters of Protein A-G Micrococcal Nuclease to each reaction [1] and mix well by gently pipetting [2].
 - 4.5.1. Talent pipetting 2.5 microliters of pAG-MNase into each tube.
 - 4.5.2. Talent gently mixing the contents by pipetting.

- 4.6. After a 10-minute incubation, perform a quick spin of the tubes [1]. Place them back on the magnet stand until the slurry clears [2] and then remove the supernatant [3-TXT].
 - 4.6.1. Talent placing the tubes into a mini centrifuge and performing a quick spin.
 - 4.6.2. Talent placing the spun tubes back onto the magnet stand.
 - 4.6.3. Talent aspirating the supernatant from each cleared tube. **TXT: Wash the sample 2x with 200 µL of cold cell permeabilization buffer**

- 4.7. After washing the sample, gently resuspend the beads and cells in 50 microliters of cold Cell Permeabilization Buffer [1] and place the tubes on ice [2].
 - 4.7.1. Talent gently pipetting to resuspend the pellet in 50 microliters of buffer.
 - 4.7.2. Talent placing the tubes onto ice. **Videographer's NOTE: 4.7.1 & 4.7.2 – Talent said it made sense to have this as a single shot. Combined shot is slated as “4.7.1, take 2”**

5. Targeted Chromatin Digestion and Release

- 5.1. While keeping the tubes on ice, add 1 microliter of 100 millimolar Calcium Chloride to each reaction in the 8-tube strip from the previous step [1]. Gently pipette to fully resuspend the beads and promote efficient digestion [2].
 - 5.1.1. Talent pipetting 1 microliter of 100 millimolar Calcium Chloride into each chilled tube.
 - 5.1.2. Talent gently mixing each tube's contents by pipetting to resuspend the beads thoroughly.

- 5.2. Incubate the tubes on a nutator at 4 degrees Celsius for 2 hours, keeping the tube caps elevated throughout the incubation [1].
 - 5.2.1. Talent placing the tubes on a nutator with caps elevated and starting the 2-hour incubation at 4 degrees Celsius.

- 5.3. Now, in a new 1.5-milliliter tube, prepare the Stop Buffer Mix by combining 33 microliters of Stop Buffer with 1 microliter of *Escherichia coli* spike-in DNA at 0.5 nanograms per reaction [1]. Gently vortex to mix the solution [2].
 - 5.3.1. Talent pipettes 33 microliters of Stop Buffer and 1 microliter of spike-in DNA per

reaction and combines five reactions into a fresh 1.5 milliliter tube.

Videographer's NOTE: 5.3.1 – Shot is fine, but incorrectly slated as “5.5.3”

5.3.2. Talent gently vortexing the tube to mix the Stop Buffer Mix.

5.4. At the end of the 2-hour incubation, add 33 microliters of the prepared Stop Buffer mixture to each reaction tube and mix thoroughly [1]. Incubate the reaction tubes in a thermocycler at 37 degrees Celsius for 10 minutes [2].

5.4.1. Talent pipetting 33 microliters of Stop Buffer mixture into the incubated reaction tubes and mixing by pipetting up and down.

5.4.2. Talent placing the tubes into a thermocycler and initiating a 10-minute incubation at 37 degrees Celsius.

5.5. After a quick spin, place the tubes on a magnet rack until the solution becomes clear [1]. Carefully transfer approximately 85 microliters of the supernatant containing the CUT&RUN (*cut and run*)-released DNA [2-TXT] into fresh 1.5-milliliter tubes. Purify the recovered DNA, assess its quantity and perform fragment size distribution for quality control [3]. Then, proceed with CUT&RUN library preparation, quantification, indexed library pooling, and paired-end next-generation sequencing [3 and 4].

5.5.1. Talent positioning the tubes onto the magnet rack and show the slurry becoming clear. **Videographer's NOTE:** 5.5.1 – Got additional closeup of liquid in case you wanted to include in this step.

5.5.2. Close-up of aspirating the clear supernatant from the tube without disturbing the beads. **TXT: CUT&RUN: Cleavage Under Targets and Release Using Nuclease** **Videographer's NOTE:** 5.5.2 & 5.5.3 – Combined into a single shot

5.5.3. Talent transfers the supernatant into labeled fresh 1.5 milliliter tube.

5.5.4. TEXT ON 5.5.3 or 4.7.2'S BACKGROUND:

- Construct CUT&RUN libraries, followed by library quantification and fragment analysis
- Pool indexed libraries and perform paired-end next-generation sequencing

Authors: We are already above the 55-shot limit and hence these steps have been summarized in text only

Results

6. Results

- 6.1. Achaete-scute homologue 1-high cell lines DMS79 and H146, and neurogenic differentiation factor 1-high cell lines H82 grew as non-adherent aggregates or compact spheroids in suspension [1] and H446 exhibited mixed morphology, with approximately 80% adherent cells and 20% in suspension [2].
 - 6.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the image panels A, B, and C to show the round cell clusters floating in liquid.*
 - 6.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the image panel D.*
- 6.2. CUT&RUN DNA fragment analysis revealed that the enhancer histone mark H3K4me1 yielded the highest DNA amounts across all cell lines [1], followed by H3K4me3 [2], with E2F7 and IgG (I-G-G) yielding the lowest amounts [3].
 - 6.2.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the tallest bar labeled H3K4me1 across all four cell lines DMS79, H146, and H82 and H446.*
 - 6.2.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the medium-height bars labeled H3K4me3 in all 4 groups.*
 - 6.2.3. LAB MEDIA: Figure 3A. *Video editor: Highlight the shortest bars for E2F7 and IgG in all 4 groups.*
- 6.3. For extracted CUT&RUN DNA, the fragment size distribution for H3K4me1 and H3K4me3 showed clear peaks for mono-nucleosome and di-nucleosome along with genomic DNA [1]. In contrast, no distinct peaks were detected for E2F7 or the IgG control [2].
 - 6.3.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the peaks in the H3K4me1 and H3K4me3 graphs labeled 'mono-nucleosome' and 'di-nucleosome' with red dotted lines.*
 - 6.3.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the graphs for E2F7 and IgG*
- 6.4. Despite low DNA input, next-generation sequencing libraries were successfully constructed for all CUT&RUN targets, including E2F7 and IgG [1].
 - 6.4.1. LAB MEDIA: Figure 4A.

6.5. Heatmap analysis showed that H3K4me3 and E2F7 signals were enriched near transcription start sites [1]. H3K4me1 signals were more broadly distributed in DMS79-wild type cells [2].

6.5.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the H3K4me3 and E2F7 panels under DMS79-WT.*

6.5.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the H3K4me1 panel under DMS79-WT.*

6.6. Genome browser visualization at a randomly selected chromatin region showed distinct peaks for H3K4me3, H3K4me1, and E2F7 [1], while IgG showed no enrichment [2].

6.6.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the sharp coloured peaks in the genome browser tracks for H3K4me3, H3K4me1, and E2F7.*

6.6.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the flat baseline track for IgG showing no peaks.*

1. Neuroendocrine
Pronunciation link: <https://www.merriam-webster.com/dictionary/neuroendocrine>
IPA: /ˌnɒr.ooˈɛn.də.krɪn/
Phonetic Spelling: nur·oh·en·duh·krin
2. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsɛn.trəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
3. Aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /ˈæs.pəˌreɪt/
Phonetic Spelling: as·puh·rayt
4. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuː.pəˈnei.tənt/
Phonetic Spelling: soo·per·nay·tuhnt
5. Accutase
Pronunciation link: <https://www.howtopronounce.com/accutase>
IPA: /ˈæk.juˌteɪz/
Phonetic Spelling: ak·yuh·tayz
6. Fetal
Pronunciation link: <https://www.merriam-webster.com/dictionary/fetal>
IPA: /ˈfiː.təl/
Phonetic Spelling: fee·tuhl

7. Microcentrifuge
 Pronunciation link: <https://www.howtopronounce.com/microcentrifuge>
 IPA: /,maɪ.kroʊ'sɛn.trəˌfjuːdʒ/
 Phonetic Spelling: my·kroh·sen·truh·fyooj
8. Concanavalin-A
 Pronunciation link: <https://www.howtopronounce.com/concanavalin>
 IPA: /,kɒŋ.kə'næv.ə.lɪn/
 Phonetic Spelling: kong·kuh·nav·uh·lin
9. Immunoglobulin
 Pronunciation link: <https://www.merriam-webster.com/dictionary/immunoglobulin>
 IPA: /ɪˌmjuː.noʊ'glɒb.jə.lɪn/
 Phonetic Spelling: ih·myoo·noh·glob·yuh·lin
10. Polyclonal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polyclonal>
 IPA: /,pɑː.li'kloo.nəl/
 Phonetic Spelling: pah·lee·kloh·nuhl
11. Nutator
 Pronunciation link: <https://www.howtopronounce.com/nutator>
 IPA: /'nuː.teɪ.tə/
 Phonetic Spelling: noo·tay·ter
12. Micrococcal
 Pronunciation link: <https://www.merriam-webster.com/medical/micrococcal>
 IPA: /,maɪ.kroʊ'kɑː.kəl/
 Phonetic Spelling: my·kroh·kah·kuhl
13. Nuclease
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclease>
 IPA: /'nuː.kliː.ɛs/
 Phonetic Spelling: noo·klee·ays
14. Permeabilization
 Pronunciation link: <https://www.howtopronounce.com/permeabilization>
 IPA: /,pɜː.mi.ə.bə.lə'zeɪ.fən/
 Phonetic Spelling: per·mee·uh·buh·luh·zay·shuhn
15. Chromatin
 Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatin>
 IPA: /'kroʊ.mə.tɪn/
 Phonetic Spelling: kroh·muh·tin
16. Escherichia
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Escherichia>
 IPA: /,ɛʃ.ə'rik.i.ə/
 Phonetic Spelling: esh·uh·rik·ee·uh
17. Thermocycler
 Pronunciation link: <https://www.howtopronounce.com/thermocycler>
 IPA: /,θɜː.moʊ'saɪ.klɜː/
 Phonetic Spelling: ther·moh·sy·kler

18. Quantification
 Pronunciation link: <https://www.merriam-webster.com/dictionary/quantification>
 IPA: /ˌkwɑːn.tə.fəˈkeɪ.ʃən/
 Phonetic Spelling: kwon·tuh·fuh·kay·shuhn
19. Spheroids
 Pronunciation link: <https://www.merriam-webster.com/dictionary/spheroid>
 IPA: /ˈsfɪr.ɔɪd/
 Phonetic Spelling: sfeer·oydz
20. Morphology
 Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>
 IPA: /mɔːrˈfɑːlə.dʒi/
 Phonetic Spelling: mor·fa·luh·jee
21. Enhancer
 Pronunciation link: <https://www.merriam-webster.com/dictionary/enhancer>
 IPA: /ɪnˈhæn.sə/
 Phonetic Spelling: in·han·ser
22. Histone
 Pronunciation link: <https://www.merriam-webster.com/dictionary/histone>
 IPA: /ˈhɪs.toun/
 Phonetic Spelling: his·tohn
23. Nucleosome
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleosome>
 IPA: /ˈnuː.kli.əˌsoʊm/
 Phonetic Spelling: noo·klee·uh·sohm
24. Genomic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/genomic>
 IPA: /dʒəˈnoʊ.mɪk/
 Phonetic Spelling: juh·noh·mik
25. Sequencing
 Pronunciation link: <https://www.merriam-webster.com/dictionary/sequencing>
 IPA: /ˈsiː.kwən.sɪŋ/
 Phonetic Spelling: see·kwuhn·sing
26. Transcription
 Pronunciation link: <https://www.merriam-webster.com/dictionary/transcription>
 IPA: /trænˈskɹɪp.ʃən/
 Phonetic Spelling: tran·skrip·shuhn
27. Genome
 Pronunciation link: <https://www.merriam-webster.com/dictionary/genome>
 IPA: /ˈdʒiː.noʊm/
 Phonetic Spelling: jee·nohm