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Title: A Semiautomated ChIP-Seq Procedure for Large-Scale Epigenetic Studies

Authors and Affiliations: Justin Cayford[1], Sara Herrera-de la Mata[1], Benjamin Joachim Schmiedel[1], Vivek Chandra[1], Pandurangan Vijayanand[1], and Grégory Seumois[1]

[1]Division of Vaccine Discovery, La Jolla Institute for Immunology

Corresponding Author:
Grégory Seumois
Gregory@lji.org

Co-Authors:
jcayford@lji.org
sherrera@lji.org
bschmiedel@lji.org
vchandra@lji.org
vijay@lji.org

Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **52**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Justin Cayford**: This protocol is significant in that it allows for high-throughput ChIP-Seq analysis of primary samples to better understand cis regulatory mechanism regulation and its importance in disease **[1]**.

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

REQUIRED:

1.2. **Justin Cayford**: The main advantages of this method are the reproducibility of the data and the drastic reduction in the amount of hands-on lab time **[1]**.

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

Introduction of Demonstrator on Camera

1.3. **Justin Cayford**: Demonstrating the procedure will be Sara Herrera de la Mata, a Research Technician from my laboratory **[1][2]**.

1.3.1. INTERVIEW: Author saying the above
1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Protocol

2. Chromatin Shearing

2.1. For chromatin shearing, add 70 microliters of fresh, room temperature, complete lysis buffer to a glycine-fixed, snap frozen and thawed cell pellet of interest for a 1-minute incubation at room temperature **[1-TXT]** followed by 1 minute of resuspension without bubbles **[2]**.

2.1.1. WIDE: Talent adding buffer to pellet, with buffer container visible in frame **TEXT: See text for full cell sample preparation details**
2.1.2. Cells being resuspended *Videographer: Important/difficult step*

2.2. Incubate the sample for another minute at room temperature **[1]** before placing the cells on ice **[2]**.

2.2.1. Talent setting timer, with tube visible in frame
2.2.2. Talent placing tube on ice

2.3. Transfer the resuspended pellet into a 0.65-milliliter low binding tube on ice **[1]** before loading the tube onto the tube holder of a sonicator **[2]**.

2.3.1. Talent adding cells to 0.65-mL tube on ice
2.3.2. Talent placing tube onto holder

2.4. Fill any gaps with balance tubes containing 70 microliters of water **[1]** and let the cells stabilize in the water bath for about 1 minute before starting the sonication **[2]**.

2.4.1. Gap(s) being filled
2.4.2. Talent setting timer, with tube(s) visible in frame

2.5. After every 3 cycles, remove the samples from the sonicator for gentle vortexing **[1]** and pulse spin the tubes before placing them back into the holder **[2-TXT]**.

2.5.1. Talent vortexing tube
2.5.2. Talent placing tube into microcentrifuge **TEXT: Caution: Remove bubbles from outside of tube before next cycle**

3. Automated Chromatin Immunoprecipitation Sequencing (ChIP-Seq)

3.1. For histone modification, add 100 microliters of complete tC1 (T-C-one) buffer to each

tube of two ChIP (chip) 8-tube strips **[1]** and transfer 20 microliters of each of 16 chromatin sheared samples into individual tubes of the ChIP 8-tube strips **[2]**.

3.1.1. WIDE: Talent adding buffer to tube(s), with buffer container visible in frame
3.1.2. Talent adding sample(s) to tube(s)

3.2. Wash the chromatin tubes with 80 microliters complete tC1 buffer **[1]** and pool the washes in the appropriate tubes of the ChIP 8-tube strips for a final volume of 200 microliters of solution per tube **[2]**.

3.2.1. Talent washing tube, with buffer container visible in frame
3.2.2. Talent adding wash to tube(s)

3.3. Add the appropriate volume of antibody to 500 microliters of tBW1 (T-B-W-one) buffer **[1]** and quickly vortex and pulse-spin **[2]**.

3.3.1. Talent adding antibody to buffer, with antibody and buffer containers visible in frame
3.3.2. Tube being vortexed

3.4. Then add 70 microliters of tBW1 **[1]** and 30 microliters of antibody solution to each tube of a new ChIP 8-tube strip **[2]**.

3.4.1. Talent adding buffer to tube(s), with buffer container visible in frame
3.4.2. Talent adding antibody to tube(s), with antibody container visible in frame

3.5. Next, thoroughly vortex a container of protein A bead solution **[1]** and add 5 microliters of beads per 0.5 micrograms of antibody to a new tube strip **[2]**

3.5.1. Talent vortexing bead solution
3.5.2. Talent adding beads to tube(s), with bead container visible in frame

3.6. Pulse spin the strip of beads **[1]** and fill the last row of the ChIP liquid-handler with labeled, empty ChIP 8-tube strips **[2]**.

3.6.1. Strip being placed into centrifuge
3.6.2. Talent adding strip(s) to liquid-handler

3.7. Then follow the *ChIP-16-IPure-200D* (chip-sixteen-eye-pure-two hundred-D) program specifications for the placement of the rest of the strips **[1]** and load the buffers into the correct positions **[2-TXT]**.

3.7.1. Talent loading strip(s) *Videographer: Important step*

3.7.2. Talent loading buffers, with buffer container(s) visible in frame **TEXT: Use tW4 instead of tE1 buffer**

4. Library Adaptor Transposase Integration

4.1. After sequencing, capture the beads in an 8-tube-strip magnet **[1]** and transfer 25 microliters of tagmentation buffer to each tube **[2]**.

4.1.1. WIDE: Talent placing tube(s) onto magnet NOTE: Don't use Take 1 or Take 2
4.1.2. Talent adding buffer to tube(s), with buffer container visible in frame *Videographer: Difficult step*

4.2. With the tubes removed from the magnet, mix gently until the bead solutions are homogenous **[1]** before placing the re-capped tubes into a pre-heated thermomixer for 3 minutes **[2]**.

4.2.1. Beads being mixed *Videographer: Important step*
4.2.2. Talent placing tube(s) onto thermomixer *Videographer: Difficult step*

4.3. At the end of the incubation, remove the ChIP 8-tube strips in the last row of the ChIP liquid-handler **[1]** and add 2 microliters RNase A to each sample **[2]**.

4.3.1. Talent removing tubes
4.3.2. Talent adding RNase A to tube(s), with RNase A container visible in frame

4.4. Then pulse spin the re-capped tubes **[1]** and gently mix the until the bead solutions are homogenous **[2-TXT]**.

4.4.1. Talent placing tube(s) into centrifuge
4.4.2. Talent mixing beads **TEXT: Re-cap tubes after mixing**

5. Tagmented DNA Fragment Purification

5.1. For purification of the tagmented DNA fragments, transfer 100 microliters of the decrosslinked DNA samples into individual 1.5-milliliter tubes **[1]** and wash the tubes with 100 microliters of DNA binding buffer per tube **[2]**.

5.1.1. WIDE: Talent transferring sample(s) to tube(s)
5.1.2. Talent washing tube(s), with buffer container visible in frame

5.2. Pool the washes in the appropriate tubes **[1]** and load the samples onto columns **[2]**.

5.2.1. Talent adding wash to tube(s)

5.2.2. Talent adding sample(s) to column(s)

5.3. Place the columns into new 1.5-milliliter collection tubes **[1]** and add 9 microliters of 55-degree Celsius Tris-EDTA Buffer directly to the column matrices **[2]**.

5.3.1. Talent placing column(s) into tube(s)
5.3.2. Talent adding buffer to column(s), with buffer container visible in frame

5.4. After 1 minute, centrifuge the columns **[1-TXT]** and transfer the 9 microliters of eluate from each column into a new 8-tube strip on ice **[2]**.

5.4.1. Talent placing tube(s) into centrifuge **TEXT: 1 min, 10,000 x g, RT** NOTE: Don't use Take 1 - Take 3
5.4.2. Talent adding eluate to tube(s), with columns visible in frame

5.5. Elute the DNA fragments again as demonstrated but with 8 microliters of Tris-EDTA buffer **[1]** and pool the eluate with the previously harvested DNA fragment solution **[2]**.

5.5.1. Talent adding buffer to column(s), with buffer container visible in frame NOTE: DNS use 5.3.2.
5.5.2. Talent adding eluate to tube(s), with columns visible in frame NOTE: DNS use 5.4.2.

6. Purified Sample Amplification and Size-Selection

6.1. To amplify the purified DNA fragment samples, mix the samples with a multichannel pipette **[1]** and perform an amplification program with the appropriate number of cycles **[2]**.

6.1.1. WIDE: Talent mixing samples
6.1.2. Talent placing sample(s) into thermocycler

6.2. After the amplification, use a plate magnet to capture the beads **[1]** and discard the supernatant **[2]**.

6.2.1. Talent placing sample(s) onto magnet
6.2.2. Talent discarding supernatant(s)

6.3. Wash the beads three times with 200 microliters of fresh 80% ethanol per wash without disrupting the bead pellet **[1]**.

6.3.1. Talent adding ethanol to tube(s), with ethanol container visible in frame

6.4. After the last wash, use a 20-microliter pipette tip to remove any excess ethanol **[1]** and allow the beads to dry for 10 minutes or until cracks appear in the bead pellets **[2]**.

6.4.1. Ethanol being removed
6.4.2. ECU: Shot of crack(s) in bead pellet *Videographer: Important step*

6.5. Add 40 microliters of pre-warmed water to each dried sample **[1]** and seal the plate before thoroughly vortexing and briefly pulse-spinning **[2]**.

6.5.1. Talent adding water to well(s)
6.5.2. Talent sealing plate

6.6. Then quantify the DNA using a fluorescence quantifying assay according to standard protocols **[1]**.

6.6.1. Talent loading sample onto analyzer

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

2.1.2., 3.7.1., 4.2.1., 6.4.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?

2.1.2. Once again, proper lyses of the cell pellet. Without proper cell lysing and then subsequent sonication, the experiment is likely not going to general high-quality data.

4.1.2/4.2.2. Proper tagmentation of the samples. If the beads are resuspended poorly or too harshly, there is a drop in data quality. Making sure that all of the samples are tagmented for the same time is also important.

Results

7. Results: Representative Semiautomated ChIP-Seq

7.1. Sheared chromatin fragment size measurements demonstrate great reproducibility **[1]**, with greater than 70% of the samples being observed between 100-500 base pairs for 14 cycles **[2]**.

7.1.1. LAB MEDIA: Figure 2A
7.1.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize bands between red lines*

7.2. As illustrated, the cycle at which the intensity of the tagmented sample is half the average maximum is optimal for cycle determination **[1]**.

7.2.1. LAB MEDIA: Figure 2B *Video Editor: please emphasize ½ intensity text and arrow*

7.3. Best sequencing data are obtained when more than 85% of the DNA fragments range between 200 to 1000 base pairs **[1]**.

7.3.1. LAB MEDIA: Figure 2C *Video Editor: please emphasize >85% peak in Ideal graph*

7.4. In these representative enrichment tracks for four gene loci **[1]**, individual tracks for each sample show a high mapping quality and signal-to-noise ratio **[2]**.

7.4.1. LAB MEDIA: Figure 3A
7.4.2. LAB MEDIA: Figure 3A *Video Editor: please sequentially emphasize CD4, MO, and NK data line*

7.5. The first two loci harbor well-expressed genes in these cell types **[1]**, while the genes in the last two loci are not expressed and serve as background controls **[2]**.

7.5.1. LAB MEDIA: Figure 3A *Video Editor: please emphasize first two columns of data*
7.5.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize last two columns of data*

7.6. For the majority of the pairwise comparisons **[1]**, Pearson correlation indexes show more than a 90% correlation, suggesting a high level of consistency between the biological replicates **[2]**.

7.6.1. LAB MEDIA: Figure 3B
7.6.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize red, blue, and yellow squares*

7.7. While cell type-specific loci exhibit a high enrichment in the appropriate cells **[1]**, a house-keeping gene shows very consistent histone modification **[2]**.

7.7.1. LAB MEDIA: Figure 3C *Video Editor: please emphasize IL17R, CCR2, and KLRC1 peaks*

7.7.2. LAB MEDIA: Figure 3C *Video Editor: please emphasize B2M peaks*

7.8. Correlation analysis between ChIP-Seq (chip-seek) dataset performed from samples with less than 100,000 cells **[1]** still demonstrate a high reproducibility and correlation down to 10,000 cells **[2]**.

7.8.1. LAB MEDIA: Figure 4A *Video Editor: please emphasize red data line*

7.8.2. LAB MEDIA: Figure 4A *Video Editor: please sequentially emphasize green and blue data lines*

7.9. However, there is an increased background as the cell numbers are reduced **[1]** as well as a decrease in the correlation coefficients **[2]**.

7.9.1. LAB MEDIA: Figure 4B *Video Editor: please sequentially emphasize graphs from red to green to blue*

7.9.2. LAB MEDIA: Figure 4B *Video Editor: please sequentially add/emphasize R2 equations from red to blue graphs*

Conclusion

8. Conclusion Interview Statements

8.1. **Sara Herrera-de La Mata**: Proper lysing of the cells is essential for generating high quality data, as we have found that incomplete cell lysis effects the entire rest of the protocol **[1]**.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.1.2)

8.2. **Sara Herrera-de La Mata**: Using this method, many different histone modifications and transcription factors can be tested on various cell types of interest to further our understanding of cis-regulatory elements **[1]**.

8.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*