Chromatin immunoprecipitation sequencing (ChIP-seq) protocol for small amounts of frozen biobanked cardiac tissue

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Abstract

Chromatin immunoprecipitation and sequencing (ChIP-seq) is a well-established method to study the epigenetic profile at the genome-wide scale, including histone modifications and DNA-protein interactions. It provides valuable insights to better understand disease mechanisms. Here we present an optimized ChIP-seq protocol suitable for human cardiac tissues, especially the frozen biobanked small biopsy samples.

Keywords: chromatin immunoprecipitation, sequencing, small biopsy, cardiac tissues, antibody, promoters, enhancers

1. Introduction

Chromatin immunoprecipitation (ChIP) is commonly used to study protein-DNA interactions and it was traditionally coupled with quantitative real-time polymerase chain reaction (PCR) to study the enriched DNA regions, which had several limitations, such as long amplicon, primer-related bias, and poor primer integrity ***[1]***. ChIP interrogated with microarrays or ChIP-on-chip was developed to address PCR-related issues, however, it has other drawbacks, including the available microarray probes on the chip and the normalization of the array data ***[2]***. ChIP followed by high-throughput sequencing (ChIP-seq) is adapted from ChIP-on-chip and provides better coverage, fewer artefacts, higher resolution, which subsequently improve the quality of the data ***[3]***. Nowadays, ChIP-seq is commonly used to identify the epigenetic signature at the genome-wide scale, which is a hallmark of the disease ***[4–6]***. Antibodies targeting different histone marks, such as H3 lysine 27 acetylation (H3K27ac), H3 lysine 4 trimethylation (H3K4me3), and H3 lysine 36 trimethylation (H3K36me3), are used in ChIP-seq to capture specific DNA regions (i.e. enhancers and promoters) ***[7]***. Valuable information can be obtained, including motif enrichment analysis to study transcription factors that are likely to bind to those regions, gene expression prediction, and gene-enhancer interaction analysis ***[8]***. Thanks to rapidly developed single-cell assays, in recent years single-cell ChIP-seq is highly used to obtain the cell-specific epigenetic profiles from the bulk samples ***[9–11]***.

Thus far, ChIP-seq has successfully revealed the pathological mechanisms and molecular factors underlying the diseases. For example, the genome-wide DNA replication profile in cultured human colon cancer cells was profiled by ChIP-seq [12]. Key transcription factors in human tumour cells obtained by needle biopsy were also identified using ChIP-seq coupled with the H3K27ac antibody ***[13]***. ChIP-seq coupled with H4K16ac antibody identified normal ageing dependent chromatin modification using postmortem human brain samples from cognitively normal younger individuals, cognitively normal elder individuals, and individuals with Alzheimer's disease ***[14]***. A study used ChIP-seq and showed transcriptional enhancers involved in heart development and function using fresh human fetal and adult hearts ***[15]***. Another study used ChIP-seq to further reveal affected key enhancers and promoters and their enriched biological functions in failing human hearts with dilated cardiomyopathy when compared to the controls ***[16]***. We also employed ChIP-seq coupled with the H3K27ac antibody and revealed the epigenomic reorganization in remodelled human hearts due to severe aortic stenosis when compared ***[17]***.

We further demonstrated that histone acetylation profiles correlated with the transcriptome profile per sample-wise and identified a set of promising transcription factors as potential key upstream regulators during the myocardial remodelling. Besides the bulk level, Churko and colleagues identified a set of DNA regions and nearby genes that interacted with transcription factors of interest using ChIP-seq in human induced pluripotent stem cell-derived cardiomyocytes ***[18]***. Another study further isolated cardiomyocyte nuclei from diseased and non-failing hearts and obtained their epigenetic profiles using ChIP-seq coupled with antibodies targeting H3K27ac, H3K9ac, H3K4me3, H3K36me3, H3K9me3, H3K27me3, and H3K4me1, respectively ***[19]***.

Piling studies are now integrating ChIP-seq data with other data, such as whole-genome data and transcriptome data, to identify novel and key regulatory factors that drive the pathological mechanism of the disease ***[20, 21]***. Nevertheless, several challenges of incorporating ChIP-seq to study heart failure remain and are well summarized in our review ***[22]***. Thus, it is important to have an established ChIP-seq protocol that ensures and/or improves the quantity and quality of the captured DNA fragments from cardiac tissues in the first place. Here, we present a well-optimized ChIP-seq protocol from the manufacturer's instructions for small human cardiac tissues, including the handling of cardiac samples and the visualization of amplified DNA fragments on a gel.

2. Materials

Prepare all solutions using ultrapure water and analytical grade reagents. Store all reagents at room temperature, unless otherwise indicated.

2.1 Chemicals and reagents

1. 37% formaldehyde.
2. 1.25 M glycine (store at 4°C).
3. Cold phosphate-buffered saline (PBS).
4. Dynabeads® (store at 4°C).
5. NEXTFLEX® Cleanup Beads 2.0 (store at 4°C).
6. Reverse Crosslinking Buffer (store at 4°C).
7. 20 mg/mL Proteinase K (store at 4°C).
8. MAGnify™ IP Buffer 1 (store at 4°C).
9. MAGnify™ IP Buffer 2 (store at 4°C).
10. MAGnify™ DNA Wash Buffer (store at 4°C).
11. MAGnify™ DNA Elution Buffer (store at 4°C).
12. MAGnify™ DNA Purification Buffer are stored at 4°C (store at 4°C).
13. MAGnify™ Protease Inhibitors (200x) (store at 4°C).
14. MAGnify™ Dilution Buffer (store at 4°C).
15. MAGnify™ Lysis Buffer (store at 4°C).
16. NEXTFLEX® End Repair & Adenylation Buffer Mix 2.0 (store at -20°C).
17. NEXTFLEX® End Repair & Adenylation Enzyme Mix 2.0 (store at -20°C).
18. NEXTFLEX® Ligase Buffer Mix 2.0 (store at -20°C).
19. NEXTFLEX® Ligase Enzyme 2.0 (store at -20°C).
20. NEXTFLEX® PCR Master Mix 2.0 (store at -20°C).
21. NEXTFLEX® Primer Mix 2.0 (store at -20°C).
22. Resuspension Buffer.
23. Nuclease-free Water.
24. 100% ethanol.

25. 80% ethanol.
26. Zymo ChIP DNA Clean & Concentrator Kit.

2.2 Working buffers

1. Lysis Buffer: 74.625 µL of Lysis Buffer stock added with 0.375 µL of 200x Protease Inhibitors per sample.
2. Dilution Buffer: 199 µL of Dilution Buffer stock added with 1 µL of 200x Protease Inhibitors per sample.
3. Diluted Dynabeads® Buffer: 50 µL of Dilution Buffer added with 10 µL of resuspended Dynabeads® per sample.
4. Reverse Crosslinking Solution: 53 µL of Reverse Crosslinking Buffer added with 1 µL of Proteinase K per sample.
5. Purification Buffer: add 5 volumes of DNA Binding Buffer to one volume of sample.

2.3 Equipment

1. Covaris System or other methods for shearing chromatin.
2. Adhesive polymerase chain reaction (PCR) Plate Seal.
3. Magnetic holder.
4. Thermocycler.
5. Nuclease-free barrier pipette tips.
6. 1.5 mL LoBind Eppendorf tubes.

3. Methods

Use 1.5 mL LoBind Eppendorf tubes during the whole procedure from 3.1 to 3.9. Use sterile filter tips during the entire procedure and make sure to always change tips for each sample to avoid possible contamination. Make sure to first vortex shortly and spin down every single tube used in the protocol, including tubes with samples and chemicals.

3.1 Preparing tissue and crosslinking the chromatin

1. Set the cryostat to -20°C and cut the frozen cardiac tissue at the thickness of 10 µm (Figure 1). Collect tissues (approximately 10 slides at the size of 2 cm x 2 cm x 10 µm) per sample in a sterile 1.5 mL Eppendorf tube and store on dry ice (see Note 1).
2. In the fume hood, add 21 µL of 37% formaldehyde for every 25 mg of tissue at room temperature.
3. Swirl the tube gently to mix and incubate for exactly 10 minutes (min) at room temperature. Swirl the tube gently every 2 min during incubation.
4. Add 80 µL of 1.25 M glycine for every 25 mg of tissue for a final concentration of 0.125 M.
5. Swirl the tube gently to mix evenly and incubate for 5 min at room temperature, swirl gently every 2 min during incubation.
6. In a cold centrifuge at 4°C, spin the tubes at 600 *g* for 10 min. A pellet will form against the sidewall of the tube.
7. Transfer the tubes to ice and keep them on ice for all subsequent steps.
8. Remove and discard the supernatant from each tube, leaving around 30 µL behind to be sure not to disturb the pellet.
9. Add 500 µL of cold PBS to each tube and resuspend the sample by flicking it with your fingers.
10. Spin at 600 *g* for 10 min at 4°C.
11. Aspirate the PBS and resuspend the sample once more in 500 µL of cold PBS.
12. Spin at 600 *g* for 10 min at 4°C.
13. Aspirate the PBS as much as possible without disturbing the cell pellet.

3.2 Lysing the cells

1. Prepare 75 µL of Lysis Buffer containing Protease Inhibitors per sample (see Note 2). For example, (74.625 µL of Lysis Buffer stock + 0.375 µL of 200x concentrated Protease Inhibitors) x (number of samples +1). Vortex the mixture well to resuspend.
2. Add 75 µL of working Lysis Buffer to each cell pellet from 3.1.
3. Resuspend by mild pulses on the vortex mixer.
4. Incubate the tubes on ice for at least 5 mins.
5. Proceed to 3.3 or snap-freeze the samples in liquid nitrogen and store at -80°C until use.

3.3 Shearing the chromatin

1. Switch on the Covaris 2.5 hours before using it (see Note 3) and set the settings using the S-Series SonoLab Single Software (v2.5) as the following: Duty Cycle: 5%; Cycles: 10; Intensity: 2; Temperature (bath): 4°C; Cyclersper Burst: 200; Power Mode: Frequency Sweeping; Cycle Time: 60 seconds; Degassing Mode: Continuous (see Note 4).
2. Take the lid off a Covaris® MicroTube with AFA fibre and transfer 75 µL of the cell lysate including any remaining tissue from 3.2 into the MicroTube.
3. Insert the tube into a Covaris®-2 series M220 Machine Holder for one 6 mm tube.
4. Sonicate the sample using the settings shown above. The liquid inside the tube should look milky afterwards (Figure 2).
5. Spin the tube briefly (around 5 seconds) to bring the samples to the bottom of the tube and transfer the freshly sonicated chromatin into a new, sterile 1.5 mL microcentrifuge tube.
6. Centrifuge the tubes at 20,000 *g* at 4°C for 5 min.
7. Carefully collect the supernatant that contains the chromatin and transfer it into a new sterile 1.5 mL tube (see Note 5). Discard the original tubes containing the debris and keep the new tubes on ice until further use.
8. Proceed to 3.4 or snap-freeze the samples in liquid nitrogen and store at -80°C until use.

3.4 Diluting the chromatin

1. Prepare 200 µL of working Dilution Buffer containing protease inhibitors per sample (see Note 2). For example, (199 µL of dilution buffer stock + 1 µL of 200x concentrated protease inhibitors) x (number of samples +1).

2. Dilute the sheared chromatin in the cold working buffer by adding 180 µL of the cold working buffer with 20 µL of the sheared chromatin sample to reach the final dilution volume of 200 µL per sample.
3. Of the diluted samples, 100 µL is used for the actual immunoprecipitation reaction and the remaining 100 µL may be used as the input control (required volume = 10 µL).

3.5 Coupling the antibody to Dynabeads®

1. Briefly spin down the tube containing Dynabeads® and resuspend them by gently pipetting up-and-down several times (see Note 6) to make sure there are no bead sediments in the bottom of the tube.
2. For each antibody that is going to be used, prepare a separate master mix of cold working dilution buffer with Dynabeads® at the ratio of 5:1. The final volume of the master mix per sample per antibody is 60 µL. For example, (50 µL of the working dilution buffer + 10 µL resuspended Dynabeads®) x (number of samples +1).
3. Place the tube(s) in the magnetic holder and wait until the beads form a tight pellet.
4. Remove the liquid from the tubes on the magnetic holder (see Notes 7-10).
5. Add 1 µL of an antibody of interest (1 µg/µL) per sample, such as antibodies targeting Histone acetyl K27 or RNA Polymerase II. If there are problems with the chromatin immunoprecipitations, the amount of antibody might be increased up to 10 µg.
6. Close the tubes and flick gently to resuspend the beads.
7. Rotate the tubes end-over-end at 4°C until use in step 5 of section 3.6.

3.6 Binding chromatin to the beads

1. Divide the antibody/Dynabeads® mixture (51 µL per tube) over an appropriate amount of 1.5 mL tubes.
2. Spin the tubes briefly to bring all liquid down to the bottom of the tube and place the tubes in the magnet holder.
3. Let stand for at least 30 seconds or until the beads form a tight pellet.
4. Remove and discard the liquid from the tubes without disturbing the bead pellet.

5. Remove the tubes from the magnet holder and immediately add 100 µL of diluted chromatin samples from step 7 in section 3.5 to each tube containing the antibody/Dynabeads® complex.
6. Close the tubes and flick gently to resuspend the beads.
7. Rotate the tubes end-over-end at 4°C for 2 hours.

3.7 Washing the bound chromatin

1. Spin the tubes briefly to bring any of the liquid trapped in the cap to the bottom of the tube and place the tubes back to the magnetic holder.
2. Let stand for at least 30 seconds or until the beads form a tight pellet.
3. Remove and discard the liquid from the tubes without disturbing the bead pellet.
4. Remove the tubes from the magnet holder and add 100 µL of IP Buffer 1 to each tube (see Note 11).
5. Close the tubes and flick gently to resuspend the beads.
6. Wash the beads by taking the tubes out of the magnetic holder, turn them around and place them back in the holder a couple of times.
7. Repeat steps 1-6 two more times and always use new 1.5 mL tubes after each round of washing.
8. Spin the tubes briefly to bring any of the liquid trapped in the cap to the bottom of the tube and place the tubes back in the magnetic holder.
9. Let stand for at least 30 seconds or until the beads form a tight pellet.
10. Remove and discard the liquid from the tubes without disturbing the bead pellet.
11. Remove the tubes from the magnet holder and add 100 µL of IP Buffer 2 to each tube.
12. Close the tubes and flick gently to resuspend the beads.
13. Put the tubes on ice for 5 min and gently flick them a couple of times.
14. Repeat steps 8-13 one more time.

3.8 Reversing the crosslinking

1. For each IP sample, prepare 54 µL of reverse crosslinking buffer containing proteinase K (see Note 12). For example, (53 µL of reverse crosslinking buffer stock + 1 µL of proteinase K) x (number of samples +1). Vortex briefly to mix well and keep the working buffer at room temperature until use.

2. For each input control sample from step 3 in section 3.4, which was not coupled to the antibody/Dynabeads® complex, add 43 µL of reverse crosslinking buffer containing proteinase K to 10 µL of the input control sample.
3. Place the tubes from 3.7.14 in the magnetic holder and wait at least 30 seconds or until a pellet forms.
4. Remove and discard the liquid from the tubes without disturbing the bead pellet.
5. Remove the tubes from the magnetic holder and add 54 µL of reverse crosslinking buffer containing proteinase K to each tube. Vortex lightly to fully resuspend the beads.
6. Incubate the IP sample tubes and the input control tubes at 55°C for 15 min in a water bath or other heating source of choice.
7. Spin the tubes briefly.
8. Place the IP sample tubes in the magnetic holder and wait at least 30 seconds for a pellet to form.
9. Carefully transfer the liquid to a new, sterile 1.5 mL tube. Do not discard the liquid, it contains the IP sample.
10. Spin the IP sample tubes and input control tubes briefly, then incubate them at 65°C for 15 min in a water bath or other heating source of choice.
11. Cool down the tubes on ice for 5 min (see Note 13).

3.9 Purifying the DNA

1. Purify the samples with the ChIP DNA Clean & Concentrator Kit by adding 5 volumes of DNA binding buffer to each sample. For example, 250 µL of DNA binding buffer + 50 µL of the sample.
2. Transfer the mixture of sample and binding buffer to a Zymo Spin column on top of a collection tube.
3. Centrifuge at 10,000 g for 30 seconds and discard the flow-through.
4. Add 200 µL of washing buffer to the column.
5. Centrifuge at 10,000 g for 30 seconds and discard the flow-through.
6. Repeat step 4 and 5.
7. Add 20 µL of elution buffer directly to the column matrix and transfer the column to a new, sterile 1.5 mL tube.

8. Centrifuge at 10,000 g for 30 seconds.
9. Discard the column, label the tubes properly and store them at -20°C until use.

3.10 End-pair & adenylation of the purified DNA fragments

1. Measure DNA concentrations in each sample from section 3.9 and start by taking 1-2 µL per sample (see Note 14).
2. In nuclease-free 96-well PCR strip tubes, add 7.5 µL of NEXTflex™ End-Repair & Adenylation Buffer Mix, 1.5 µL of NEXTflex™ End-Repair & Adenylation Enzyme Mix, 10 ng of purified DNA samples (see Note 15), and nuclease-free water to reach the final volume of 25 µL.
3. Incubate the samples on a thermocycler using the following program: 22°C for 20 min, 72°C for 20 min, and an infinite 4°C hold.

3.11 Adaptor ligation

1. Thaw NEXTflex™ Ligase Enzyme Mix to room temperature and vortex for 5-10 seconds (see Note 16).
2. For 25 µL of each end-repaired DNA sample from section 3.10, add 33.75 µL of NEXTflex™ Ligase Enzyme Mix (see Note 17-18) and 1.5 µL of diluted NEXTflex™ DNA Barcode (see Note 19).
3. Mix thoroughly by pipetting (see Note 20).
4. Apply adhesive PCR plate seal and incubate at room temperature for 15 min or on a thermocycler at 22°C for 15 min (see Note 21).

3.12 Cleanup

1. In case strip tubes are used, transfer the samples from section 3.11 to 1.5 mL tubes.
2. Add 40 µL of AMPure XP Beads to each sample and mix thoroughly until homogenized.
3. Incubate samples at room temperature for 5 min.
4. Place the tubes on the magnetic holder at room temperature for 5 min.
5. Remove and discard the supernatant.
6. Add 200 µL of 80% ethanol to each bead pellet and incubate at room temperature for 30 seconds.
7. Carefully remove ethanol without touching the bead pellet.
8. Remove the tubes from the magnetic holder and let dry at room temperature till seeing the cracks on the pellet (see Note 22).

9. Add 50 µL of Resuspension Buffer to the dried beads and mix thoroughly until homogenized.
10. Incubate samples at room temperature for 5 min.
11. Place the tubes on the magnetic holder at room temperature for 5 min.
12. Collect the clear supernatant and transfer them to new tubes.
13. Repeat steps 2-8.
14. Add 22 µL of Resuspension Buffer to the dried beads and mix thoroughly until homogenized.
15. Incubate samples at room temperature for 5 min.
16. Place the tubes on the magnetic holder at room temperature for 5 min.
17. Transfer 20 µL of clear sample to a new tube.

3.13 PCR amplification

1. Add 51 µL of nuclease-free water, 25 µL NEXTflex™ PCR Master Mix, and 4 µL of NEXTflex™ Primer Mix to 20 µL of the ligated DNA sample from section 3.12 to reach the final volume of 100 µL.
2. Apply adhesive PCR plate seal and incubate the samples on a thermocycler using the following program: 98°C for 2 min, 13 cycles of (98°C for 30 seconds, 65°C for 30 seconds, 72°C for 60 seconds), 72°C for 4 min, and an infinite 4°C hold (see Note 23).
3. Take 4 µL of the sample and mix it with 1 µL of dye and load the mixture to the gel and check the amplified products (Figure 3).
4. Add 80 µL of AMPure XP beads to each sample (beads:sample = 8:10), mix thoroughly until homogenized.
5. Place the tubes on the magnetic holder and incubate at room temperature for 5 min.
6. Remove and discard the supernatant without disturbing the bead pellets.
7. Add 200 µL of 80% ethanol to each pellet and incubate at room temperature for 30 seconds.
8. Carefully remove the ethanol without disturbing the bead pellets.
9. Remove the tubes from the magnetic holder and let dry at room temperature for 5 min or till seeing the cracks on the pellet.
10. Add 21 µL of Resuspension Buffer to dried pellets and mix thoroughly until homogenized.
11. Place the tubes on the magnetic holder and incubate at room temperature for 5 min.

12. Transfer 20 µL of clear sample to a new tube.
13. Optional: check the final product on a gel.
14. Measure the concentrations of the final products using Qubit or other suitable methods (see Note 14).

4. Notes

1. Additional slides from the same cardiac sample could be collected for other omics experiments, such as RNA sequencing and proteomics, which will provide extra layers of information next to ChIP-seq.
2. When calculating the volume of needed chemicals, i.e. for the preparation of the master mixes, always count 1 extra sample due to the pipetting error and avoid introducing batch effects of preparing additional chemicals.
3. Covaris machine for shearing the chromatin takes around 2.5 hours to function, so make sure to switch on the machine beforehand to fit the experiment schedule.
4. Make sure the degas button is on.
5. Label the tubes properly to distinguish collected samples throughout the protocol clearly, i.e. sheared chromatin samples in section 3.3, chromatin input controls in section 3.4, and purified DNA in section 3.9.
6. Coupling the antibodies to the Dynabeads takes about 1.5 hours, so make sure the chromatin isolation is ready before preparing the antibody-Dynabeads mixture.
7. Do not vortex and freeze the Dynabeads®, this will damage the beads.
8. Avoid touching the beads with the pipette tip when removing the liquid from the Dynabeads®, because this will disturb the bead pellet.
9. Do not allow the beads to dry out and make sure to resuspend the beads within 1 min after removing the liquid from them.
10. It is acceptable to remove the tubes from the magnetic holder during the washing steps in 3.5.
11. During the washing steps in 3.7, keep the magnets, tubes, and buffers on ice to keep cold.
12. A considerable amount of fibrotic tissue is present in the cardiac samples, especially in diseased hearts. Therefore, it is important to incubate samples with proteinase K to digest muscle fibres ***[23, 24]***.
13. It is important to proceed from step 6 to 11 in section 3.8 without breaks to make sure the reverse crosslinking reaction occurs without sacrificing the sample qualities.

14. We recommend measuring DNA concentration using the Qubit Kit rather than Nanodrop for higher accuracy. For input samples or samples with relatively more starting tissues than the others, it is advised to take 1 µL of sample and diluted in Nuclease-free water at a 1:1 ratio for concentration measurements. For the others, it is advised to start with 2 µL of each sample for concentration measurements.
15. The working range of purified DNA may range from 1 ng to 1 µg, but it needs to be the same among samples.
16. Do not spin down the NEXTflex™ Ligase Enzyme Mix to avoid separating the components of the mix and affect the performance.
17. Depending on the input amount and the starting adapter concentration, dilute NEXTflex™ Adaptor with nuclease-free water.
18. It is recommended to add 1.25 µM Adaptor to 1-10 ng of DNA samples, 3 µM Adaptor is desired for 100 ng of DNA samples, and 25 µM Adaptor is desired for 250-1000 ng of DNA samples.
19. Each sample needs a specific barcode. Do not add the same barcode to different DNA samples in the same experiment.
20. The NEXTflex™ Ligase Enzyme Mix is very viscous. Mixing the DNA samples, NEXTflex™ Ligase Enzyme Mix, and NEXTflex™ DNA Barcode thoroughly is critical to obtain optimal results. It is suggested to pipette the mixture up and down 15 times.
21. Set the lid temperature at 37°C on the thermocycler.
22. It takes 1-3 min to dry and see the cracks appearing on the bead pellet.
23. In the PCR program, start with 13 cycles and check the amplified product on the gel. If the band is too weak, extra PCR cycles could be added. However, it is not advised to add more than 10 cycles, which may introduce PCR-biased effects to the samples.

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Figures and figure legends

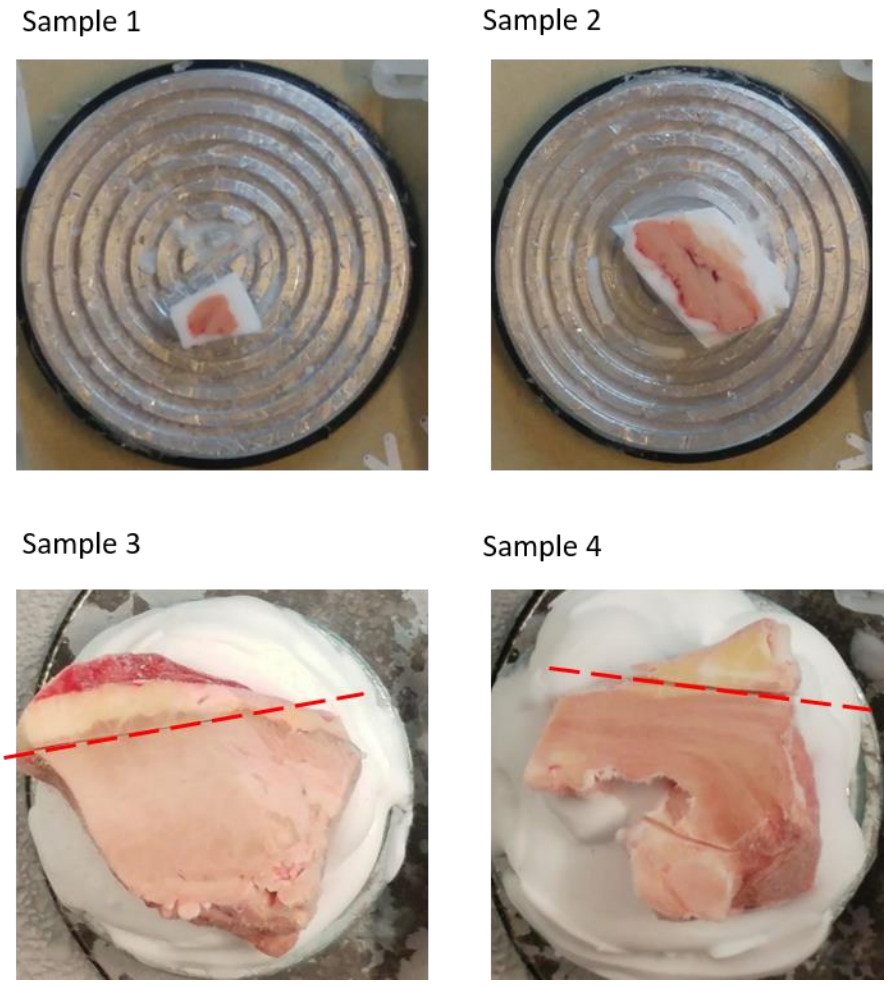

Figure 1. Examples of snap-frozen human cardiac samples. Samples were sliced at a thickness of 10 μm. However, the number of sectioned slides ranged from 10 to more than 50, depending on the sample size. At least 80 slides of sample 1, around 50 slides of sample 2, and around 15 slides of samples 3 and 4 are estimated to provide an adequate amount of cells for the following experiment. Since cell compositions change during the disease progression, it is advised to use a sterile scalpel and remove the fibrotic and/or fatty region of the cardiac tissue, which subsequently maximizes the chromatin signals derived from cardiomyocytes. Red dashed lines in samples 3 and 4 indicate the possible cutting line by the scalpel.

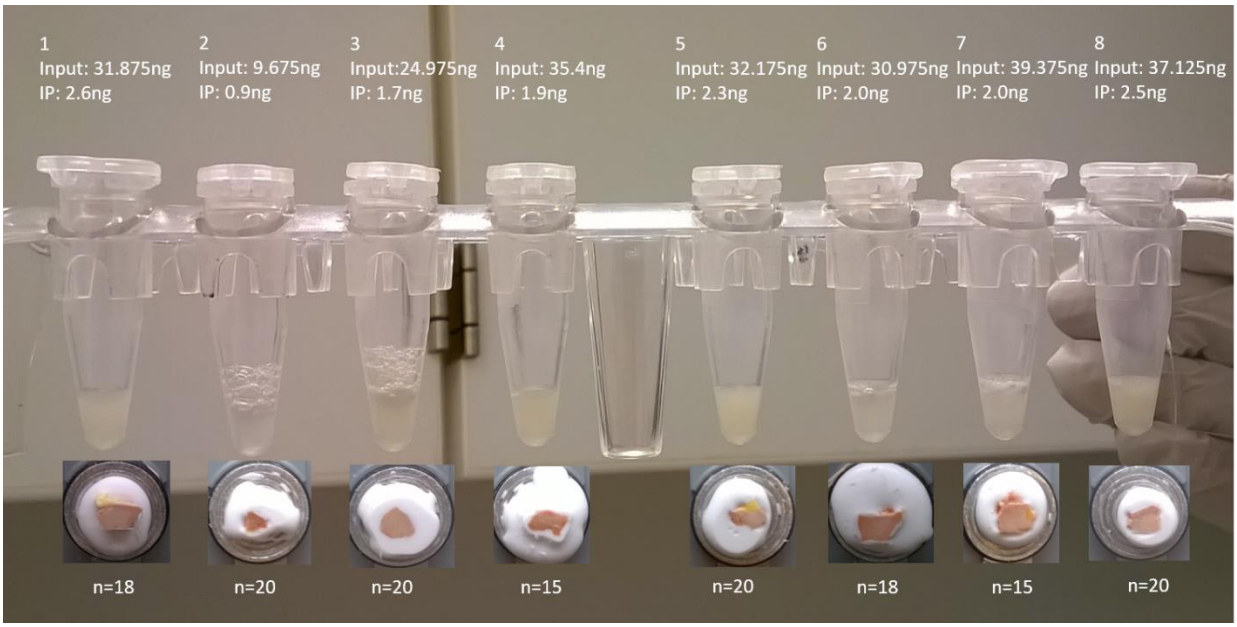

Figure 2. Examples of sonicated chromatin samples from 8 hearts. The DNA concentrations of the input control without coupling to the antibody/Dynabeads®complex and the chromatin immunoprecipitated sample from the same heart were measured by Qubit and shown in the upper panel. The size of each heart and the included number of slides per heart are shown in the lower panel.

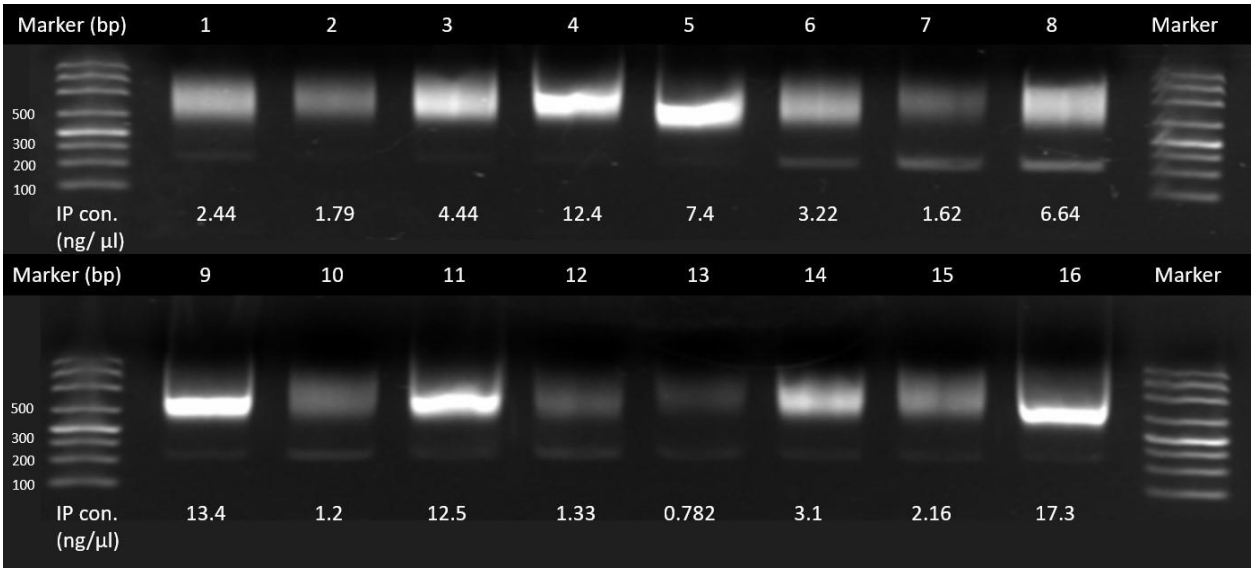

Figure 3. Examples of checking amplified DNA products of 16 samples after 13 cycles of polymerase chain reaction cycles. The sample concentration per chromatin immunoprecipitated (IP) sample is shown below each band.