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Measuring histone modifications in the human parasite *Schistosoma mansoni*

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Abstract

DNA-binding proteins play critical roles in many major processes such as development and sexual biology of *Schistosoma mansoni,* and are important for the pathogenesis of schistosomiasis. Chromatin immunoprecipitation (ChIP) experiments followed by sequencing (ChIP-seq) are useful to characterize the association of genomic regions with posttranslational chemical modifications of histone proteins. Challenges in the standard ChIP protocol have motivated recent enhancements in this approach, such as reducing the number of cells required and increasing the resolution. In this chapter, we describe the latest advances made by our group in the ChIP methods to improve the standard ChIP protocol to reduce the number of input cells required and to increase the resolution and robustness of ChIP in *S. mansoni*.

Keyword

Native chromatin immunoprecipitation, ChIPmentation, Histone modifications, Schistosomiasis, Trematode, Development

1. INTRODUCTION

Measuring posttranslational histone modifications is particularly useful for studying chromatin profiles and potentially link them to gene expression patterns in many organisms. For the purpose of this method chapter, we will focus on histone methylation, one of the many ways in which histones can be modified in the cell nucleus. When histone methylation occurs, one, two, or three methyl groups are transferred from S-adenosyl-L-methionine to lysine or arginine residues of histone proteins by histone methyltransferases (HMTs). Histone demethylases (HDMs) are enzymes that remove methyl groups from target sites which. Both modifications can lead to changes in chromatin accessibility and can allow to turn genes “on” or “off”. Histone modifications are covalent post-translational modifications which act by decreasing/increasing nucleosome contact (between histone-histone or histone-DNA) and therefore altering structure and stability of the chromatin, and/or by recruiting proteins with chromatin modifying enzyme activity [6]. Specific methylation of histone 3 at lysine 4, 9, 27, 36, or 79 (H3K4me3, H3K9me3, H3K27me3, H3K36me3, respectively) and methylation of histone 4 at lysine 20 (H4K20me1) have been shown to be extremely conserved through all kingdoms and involved in many biological processes.

The human parasite *Schistosoma mansoni* is a digenetic trematode with two consecutive host. The parasite goes through at least five developmental stages during its life cycle; two of them are free living larvae that transit between the two obligatory hosts within a fresh water ecosystem. Each developmental stage is characterized by specific posttranslational histone modifications, in particular methylations. Histone methylation also responds to environmental changes, development, sexual

biology and parasite pathogenesis and seems to be involved in an adaptive response [3, 5, 8–10]. Nowadays, several methods are used for mapping histone methylation on the genome, with chromatin immunoprecipitation being the one with the highest resolution. In cross-linking ChIP (X-ChIP), the chromatin is cross-linked, sonicated or digested into small fragments and then a specific antibody is used to immunoprecipitate the fragments containing the protein of interested. Next, reverse crosslink is needed and the DNA is purified. Specific protein-DNA interaction at known genomic binding sites sequences can be identified by quantitative PCR (ChIP-qPCR) or high-throughput sequencing (ChIP-seq). Alternatively, in native ChIP (nChIP) non-crosslinked chromatin is fragmented by enzymatic digestion and then processed in its native form. Our goal with the present book chapter is to share improved methods and immunoprecipitation protocols on *S. mansoni* samples that will be followed by high-throughput sequencing.

Native ChIP-seq (nChIP-seq) is a powerful technique used to determine the role of histone post-translational modifications, often characterized by the modification's position, abundance, and their type. In contrast to other chromatin immunoprecipitation methods, nChIP uses enzymatic chromatin fragmentation, leaving histone proteins in their native form and keeping the natural interaction with DNA under physiological salt conditions. Recently, ChIPmentation, a method that combines chromatin immunoprecipitation with sequencing library preparation by Tn5 transposase ('tagmentation'), was adapted by our group for nonmodel organisms, among them *S. mansoni* adult worms. Knowing that there are similarities and differences between principles of immunoprecipitation techniques, we will share here methods and protocols used by our group for experiments that will be followed by large-scale sequencing, *i.e.* nChip-seq and ChIPmentation.

2. MATERIALS

2.1. Antibodies

One of the most important parts of ChIP techniques is related to the quality and specificity of the antibodies. Specificity and the strength of antibody-antigen interaction must be experimentally determined since they will affect the quality of the ChIP experiment in terms of specificity and sensitivity (Please check [2] for detailed information). Even if antibodies against histone isoforms (monoclonal or polyclonal) are sold as "ChIP-certified", they must be validated on your focal organism first. By a simple western-blot, it is possible to determine if the antibody can be used or should be avoided in your experiment. Antibody that give multiple bands must be avoided, those that do not reveal a band can potentially be used in ChIP. Keep in mind that all antibodies must be aliquoted on arrival and store (to avoid freezethaw degradation every time the antibody is used) at -20°C. Furthermore, using the correct antibody concentration can significantly improve the signal-to-background ratio of your library. In this

sense, the determination of the amount of antibody required by performing a ChIP experiment is instrumental for each antibody type and concentrations. A titration is necessary to determine the optimal amount of antibody to use with your organism (Please check section 3.2 for detailed information).

In addition, this chapter offers the opportunity to share knowledge on antibodies contamination coming from the production method. In the last two years, our group worked on improving the nChIP technique to reduce the number of cells needed in each sample. In our hands, nChIP had been routinely performed with a sample size of 150,000 cells [2]. However, due to recent demands (e.g. studies using stem cells and reproductive organs), an improvement in sensitivity of our procedures was required. For that, we compared nChIP with other methods proposed in the literature, such as commercially available X-ChIP and ultralow-input native ChIP (ULI-ChIP) [1]. We obtained best signal-to-background ratio for nChIP in all tested conditions. Our experiments were performed on the following samples: 150,000, 80,000, 20,000, 10,000 and 1,000 of *S. mansoni* cells, in duplicate for each antibody (anti-H3K4me3 and anti-H3K27me3) + control, as well as without antibody (background). Although all samples passed our quality control step before sequencing, we observed sometimes high levels of nonspecific DNA for ≤20,000 cells. An unexpected source of contamination were the antibodies themselves. E.g. in anti-H3K4me3 from Merck (cat. Nr. 04-745, several lots) we found high amounts of rabbit DNA. This contamination became predominant when working with less than 100,000 cells where ≥90% of the reads were rabbit DNA. Interestingly, our laboratory does not raise rabbits which means it is very unlikely that this material has been contaminated during the procedure. We suspect the contamination to come directly from the antibodies, which is plausible as many antibodies are produced in rabbits. Therefore, we have adopted in our laboratory an additional procedure for testing all antibody when working with a low number of cells. In that case, we tested the antibodies produced in rabbits by PCR using GAPDH primer (F-$_{5'}$-GCCGCTTCTTCTCGTGCAG-$_{3'}$; R-$_{5'}$ATGGATCATTGATGGCGACAACAT-$_{3'}$). We have tested monoclonal antibodies from three suppliers before selecting the one with the lowest level of contamination. Our group reported this incident for the first time in [10].

2.2. Materials

1. Micro dialysis units (Slide-a-Lyzer 3500 D cut-off, Pierce 69550)
2. 15 ml centrifugation tubes
3. Centrifuges
4. Stirrers
5. Waterbath or similar
6. PCR machine

7. Gel electrophoresis equipment
8. 1 M KCl, autoclaved
9. 5 M NaCl, filtered and autoclaved
10. 1 M MgCl, autoclaved
11. 1 M Tris/Cl pH 7.4 - 7.6, autoclaved
12. 0.5 M EDTA, autoclaved
13. 1 M CaCl2, 10 ml, filtered
14. 100 mM DTT, 1 ml (store at -20°C)
15. Roche Complete Protease Inhibitor (ref: 11 697 498 001)
16. 2.5 M Sodium butyrate (store at 4°C)
17. 25 mM PMSF in isopropanol, 10 ml (store at -20°C)
18. 15 U/µl Micrococccal nuclease (MNase) (USB 70196Y) in sterile 50%(v/v) glycerol, aliquot to ~10 µl and store at -20°C
19. Protein A - sepharose CL-4B (Sigma P3391 250 mg) (store at 4°C)
20. Agarose gel loading buffer
21. 20%(w/v) SDS, filtered
22. 20 g/l glycogen solution (store at -20°C)
23. 2%(w/v) NaN3 in water (store at 4°C), NaN2 is very toxic!

WORKING SOLUTION

2X BASE BUFFER	60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 mM Tris/Cl, pH 7.5 Add 2 of Roche protease inhibitor tabletts
BUFFER 1	0.3 M sucrose, 5 mM $CH_3CH_2CH_2COONa$, 0.1 mM PMSF, 0.5 mM DTT, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 mM Tris/Cl, pH 7.5
BUFFER 2	0.3 M sucrose, 5 mM $CH_3CH_2CH_2COONa$, 0.1 mM PMSF, 0.5 mM DTT, 0.8% (v/v) NP40, 60 mM KCl, 15 mM NaCl, 5 mM MgCl2, 0.1 mM EDTA, 15 mM Tris/Cl, pH 7.5
BUFFER 3 FOR 3 CELL SAMPLES	1.2 M sucrose, 5 mM $CH_3CH_2CH_2COONa$, 0.1 mM PMSF, 0.5 mM DTT, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 mM Tris/Cl, pH 7.5

MNASE DIGESTION BUFFER	0.3 M sucrose, 5 mM $CH_3CH_2CH_2COONa$, 0.2 mM PMSF, 4 mM $MgCl_2$, 1 mM $CaCl_2$, 50 mM Tris/Cl, pH 7.5

DIALYSIS BUFFER	5 mM $CH_3CH_2CH_2COONa$, 0.2 mM PMSF, 0.2 mM EDTA, 1 mM Tris/Cl, pH 7.5
ANTIBODY INCUBATION BUFFER	150 mM Nacl, 20 mM Tris/Cl, 20 mM Sodium butyrate, 5 mM EDTA, 100 µM PMSF
ELUTION BUFFER	20 mM Tris/Cl, 50 mM NaCl, 5 mM EDTA, 20 mM sodium butyrate, 100 µM PMSF, 1 % SDS

WASHING BUFFERS

BUFFER A	50 mM Tris/Cl, 10 mM EDTA , 5 mM sodium butyrate, 75 mM NaCl
BUFFER B	50 mM Tris/Cl, 10 mM EDTA , 5 mM sodium butyrate, 125 mM NaCl
BUFFER C	50 mM Tris/Cl, 10 mM EDTA , 5 mM sodium butyrate, 175 mM NaCl

1. 100 mM Dithiothreitol (DTT) in distilled water, aliquot to 500 µl (store at -20°C). 144 DTT is a reducing agent that prevents the formation of disulfide bonds in and between proteins.

2. 25 mM Phenylmethanesulfonylfluoride (PMSF) in isopropanol, 10 ml (store at -20°C). PMSF is a serine protease inhibitor.

3. Roche Complete Protease Inhibitor tablets (ref: 11 697 498 001) (store at 4°C).

4. 2.5 M Sodium butyrate in distilled water (store at 4°C). Sodium butyrate is a histone deacetylase (HDAC) inhibitor. Sodium butyrate solution is not very stable and should not be stored for more than 4 weeks. The product is an irritant and the solution has a nauseating odor.

5. 2%(w/v) NaN_3 in water (store at 4°C) as preservative. Sodium azide is very toxic.

6. 15 U/µl Micrococcal nuclease (MNase) (EC 3.1.31.1) (USB 70196Y) in sterile 50%(v/v) glycerol, aliquot to ~10µl and store at -20°C. Do not refreeze. MNase digests DNA between nucleosomes.

3- METHODS

3-1. Preparation of Sepharose-Protein A

1. Weigh 250 mg Sepharose-protein A in 15 ml falcon tube (See note 1).
2. Wash with 10 ml sterile water .
3. Centrifuge 10 min at 4000 x g at 4°C,
4. Remove supernatant.

5. Repeat washing (steps 2-4) four times.
6. Add sterile water to 5 ml.
7. Add NaN3 to 0.02%(w/v) and store at 4°C.

3.2 Antibody titration

Ideally, the amount of immunoprecipitated DNA must not depend on the amount of antibody used meaning that antibody should be in excess over the protein you want to precipitate. The inappropriate antibody concentration would lead to many difficulties in reproducibility of subsequent experiments. For that, a titration procedure is needed before ChIP experiment. In our hands, a titration is is done with a constant quantity of chromatin from our sample incubated with increasing amounts of each antibodies.

The titration procedure is costly because substantial amounts of antibodies are consumed, but it is a critical step and assures the reproducibility of the results. For titration do the following:

1. Prepare a dilution series of your chromatin in MNase buffer starting with 20 - 40 µg chromatin DNA for histone ChIP and use 2-20 µg antibody (if the concentration is not known use 2 - 20 µl antibody).
2. Add appropriate amounts of stock solutions to generate the antibody incubation buffer (150 mM NaCl, 20 mM sodium butyrate, 5 mM EDTA, 0.2 mM PMSF, 20 mM Tris/Cl pH 7.5) by taking into consideration the amount of S1 and S2 and their respective buffers.
3. Dilute S1 and S2 in 1ml final volume of antibody incubation buffer.
4. Add the appropriate amount of antibody. Add an equal volume of water to the control tube without antibody.
5. Incubate overnight at 4°C on a slowly rotating wheel.
6. Proceed starting from ***Day 3*** of the protocol.

Antibody saturation could be detected directly by the amount of precipitated genomic region of interest (bound) in comparison to the input (unbound fraction of control). In general, this ratio is expressed in % input recovery:

$$\% \text{ input recovery} = 100 * \text{PCR-efficiency}\,(_{\text{Ct-input - Ct-bound}})$$

From a certain quantity of antibody on, the amount of immunoprecipitated DNA should remain constant (Figure 1). This approach is sensitive to pipetting errors and requires careful standardization and several technical and biological duplicates should be performed. For a successful ChIP experiment, it is instrumental to test the specificity of the antibodies and to determine the optimal amount by titration.

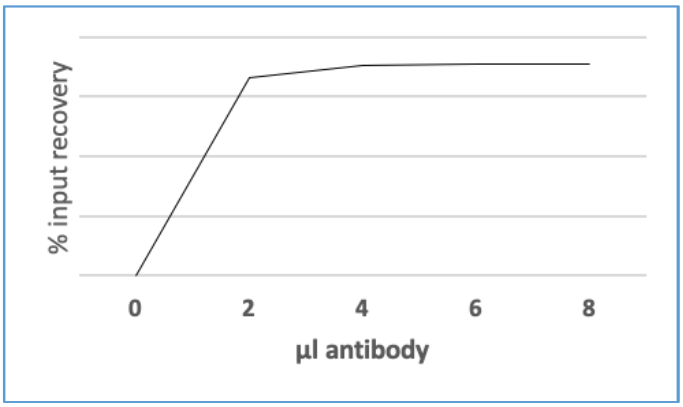

Figure 1. Schematic representation of a titration experiment where antibody saturation was reached at around 4 µl antibody.

3.3 Day 1: Cell Lysis, Chromatin preparation, MNase Digestion (Duration 2 hours)

The following procedure has been improved in sensitivity and can now be performed with 10,000 cells of *S. mansoni*. This corresponds roughly to 100 larvae (miracidia or sporocyst 1). A single adult worm can also be used. Cells can be aliquoted and stored at -80°C for up to 6 months or in liquid nitrogen for up to 12 months. However, it is preferable to use fresh biological material.

1. Cool down centrifuge to 4°C.
2. Prepare a 2%(w/v) agarose gel in 0.5x TBE with 20 µl wells.
3. Preheat a water bath to exactly 37°C.
4. Prepare 2x Base Buffer, Buffers 1, 2, 3, MNase Digestion Buffer and Dialysis Buffer solutions with autoclaved distilled water.
5. Put all buffer solutions on ice (except MNase buffer), which should be pre-heated at 37°C in water bath.

3.3.1 Cell lysis

1. Prepare aliquots of 10,000 to 100,000 cells, or 1 adult worm (fresh, stored at -80°C or in liquid nitrogen).
2. Remove excess liquid (if any) and resuspend in 1 ml buffer 1, add 1 ml buffer 2 (lysis buffer) and transfer to Dounce.
3. Homogenize for 3 min with Dounce (pestle A) on ice.
4. Put on ice for 7 min.
5. Fill 8 ml of buffer 3 into a 15 ml or 50 ml centrifugation tube that resists 10000 x g,

6. Overlay the 8 ml of buffer 3 with 1 ml cell suspension, so that the tubes are ready for centrifugation 15 min (sporocysts) of 10 min (adults) after buffer 2 has been added to the cells. Do not stir or shake the tube. You should have two distinct phases in the tube.
7. Slightly disturb the interface by passing a pipet tip through it 3-4 times.
8. Use 2 centrifugation tubes for the sporocysts samples that are in 2 ml of buffer 1+ and 2
9. Mark tubes on the side to know on which side to look at the nuclei after centrifugation.
10. Centrifuge at 7800 x g for 20 min at 4°C.
11. Carefully remove the supernatant completely (by pipetting with 10 ml pipette and then, pipetting with micropipettes 1000µl, 100µl, and 10µl).

3.3.2 MNase digestion

1. Resuspend the pellet in 1 ml of MNase Digestion Buffer (1 ml for 10,000 cells)
2. Aliquot 500 µl of this suspension in 1.5 ml microcentrifuge tubes.
3. Add 1 µl MNase (15 U) and incubate 4 min at 37 °C in water bath.
4. To stop the digestion, add 20 µl of0.5 M EDTA to each 500 µl MNase digestion tubes and put the them on ice.
5. Centrifuge 13000 x g for 10 min at 4°C.
6. Transfer the supernatant to a new tube (S1) and keep the pellet (P1).
7. Store S1 at -20°C.
8. Quantify chromatin in S1 with the Qubit® Fluorometer (HS DNA assay) following the manufacturer instructions.

3.3.3 Dialysis of P1

1. Humidify a Slide-a-Lyzer with 50µl dialysis membrane of buffer.
2. Resuspend the pellet P1 in 100 µl of dialysis buffer and dialyze overnight at 4°C in 50 ml dialysis buffer with gentle stirring. The Slide-a-Lyzer should be in a floating device and we work with a magnetic spinner to do the gentle stirring.

3.4 Day 2: Incubation with the antibody (Duration: 30 minutes plus overnight incubation)

1. On the next day, thaw S1.
2. Transfer dialyzed sample into Eppendorf tubes.
3. Centrifuge both tubes side-by-side (S1 and dialysed) at 13,000 g for10 min at 4°C.
4. Transfer the supernatant to a new tube and repeat the centrifugation twice. These triple centrifugations are critical to reduce unspecific background.

5. Supernatant of dialyzed sample is fraction S2.
6. Optional: use 50 µl of S1 and S2 for DNA extraction, centrifuge and load 20 µl of supernatant on a 2% agarose 0.5x TBE gel (100V, for 25 min).
7. Combine supernatants of S1 and S2 into a new tube.
8. Add appropriate amounts of stock solutions to generate the antibody incubation buffer:
9. Dissolve chromatin from S1 (and S2 if you have dialyzed) in a final volume of 1 ml of antibody incubation buffer and adjust the concentrations of the compounds in the antibody incubation buffer using stock solutions and sterile DNA-free water.
10. Add the amount of antibody needed (as determined in Step 3.2, above).
11. Incubate overnight at 4°C on a rotating wheel.

3.5 Day 3: Precipitation and DNA extraction (Duration 3 hours)

3.5.1 Precipitation

1. Prepare 50 µl of protein A - sepharose for each tube
2. Wash the beads with ChIP grade water in a1.5 ml Eppendorf centrifuge 4,000 x g at 4°C for 5 minutes, remove the supernatant and replace with an equal volume of sterile water.
3. Repeat the washing two more times. It is very important resuspend the protein A – sepharose carefully to maintain a homogeneous solution in the stock tube
4. Add 50 µl of protein A - sepharose to each tube.
5. Incubate for at least 4 h at 4°C on a rotating wheel.
6. Prepare washing buffers (10 ml / tube) and cool down to 4°C or on ice:
7. Centrifuge chromatin/antibody mixture for 10 min at 4°C, 11600 x g.
8. Keep the supernatant in a 2 ml tube. This is the unbound fraction UB.
9. Resuspend the pellet in approx. 1 ml of washing buffer A and transfer into a 15 ml Falcon tube containing 9 ml of washing buffer A.
10. Mix for 10 min on a rotating wheel at 4°C.
11. Centrifuge for10 min at 3400 x g and4°C and pour off supernatant (by inversion).
12. Add 10 ml of washing buffer B, mix for 10 min on a rotating wheel at 4°C and centrifuge for 10 min, 4000 x g, at 4°C.
13. Pour off the supernatant (by inversion).
14. Add 10 ml of washing buffer C, mix for 10 min on a rotating wheel at 4°C and centrifuge for 10 min at 4000 x g, at 4°C.
15. Pour off supernatant (by inversion).

16. Centrifuge for 10 min, 4000 x g, at 4°C.
17. Remove the remaining supernatant completely (not by inversion but by pipetting with micropipettes 1000 µl, then 100 µl, then 10µl).
18. Resuspend the pellet in 500 µl elution buffer:
19. Transfer the suspension to a 1.5 ml microcentrifuge tube.
20. Incubate 15 min at room temperature on a rotating wheel.
21. Centrifuge for 10 min, 11600 x g, at 18°C.
22. Transfer the supernatant to a 1.5 ml microcentrifuge tube. This is the bound fraction B.

3.5.2 DNA extraction

1. Perform DNA purification on fractions using the QiaQuick PCR Purification Kit (Qiagen, Cat. No. 28104) or similar, following manufacturer instructions, but adding the following slight modifications.
2. Step 3 and 4: Centrifugation time of 60 s. Step 5: Centrifuge the column in a 2 ml collection tube for 1 min, without the cap.
3. Step 7: Before the centrifugation, let the column stand for 2 min at room temperature.
   Elution volume: 50 µl.
4. In order to obtain more DNA, it is possible to proceed to a second elution in 30 µl.
5. Quantify purified DNA with accurate and sensitive DNA quantification methods following manufacturer instructions.
6. Use 1 µl of this DNA for PCR in 25 µl reactions (quantitative real-time PCR).

3.6 ChIPmentation

ChIPmentation is based on chromatin immunoprecipitation with sequencing library preparation by Tn5 transposase ('tagmentation'). ChIPmentation introduces sequencingcompatible adaptors in a single-step reaction directly on bead-bound chromatin, which reduces time and cell input in comparison to nChIP. There are ChIPmentation kits available and the method can even be automated on suitable pipetting robots or made manually. In our hands, the Diagenode Auto ChIPmentation Kit for Histones (Diagenode, Cat. No.
C01011000) on an IP-Star® Compact Automated System (Diagenode, Cat. No. B03000002) gave excellent results. The procedure can be performed routinely in two days with up to 16 samples in parallel. We followed the provided user manual and will, in the following, only describe handling instructions specific to schistosomes. A single worm is sufficient for each antibody.

1. Shear the chromatin by sonication using the Bioruptor® or other sonication device. Choose the protocol which results in fragments of about 1,000 bp in length, e.g.when using the Bioruptor® Pico shear for single adult worm, use 5 cycles [30 seconds “ON”,
   30 seconds “OFF”].
2. Library amplification varies from 16 to 19 cycles with C_t values dependent on the qPCR machine and calculation method. We observed better results when using 1/3 of slope = number of amplifications. Instead of using Ct that depends on the qPCR analysis algorithm we recommend to display the fluorescence intensity as a function of amplification cycles and use the number of cycles for library amplification that correspond to 1/3 of of the slope at the exponential phase of PCR amplification

4. NOTES

Note 1: 250 mg Protein A - Sepharose swells to approximately 1 ml gel and binds about 20 mg human IgG. You will need 50 µl of the homogeneously mixed sepharose-protein A per ChIP. Protein A has strong affinity to human, mouse and rabbit IgG. For antibodies raised in goat and sheep, Sepharose-protein G or protein A/G mixtures should be used. Paramagnetic Sepharose particles are available, but in our hands, background was on average 20 times higher than in a centrifugation-based separation.

Note 2: **Brief overview of comparative and analytical approach.** We present here sequencing results of nChIP-seq and ChIPmentation for adult male worms of *S. mansoni* following the protocol described above. Antibodies were carefully tested for specificity as previously described and were used in saturating quantities (4 µL for each replicate). Two biological replicates were used to perform immunoprecipitation using antibodies against H3K4me3 (Diagenode, C15410003, Lot: A5051-001P). For each experiment, we used a control without antibody to assess nonspecific background (bound fraction) and input (unbound fraction). Inputs were used for normalization in all subsequent bioinformatics analyses. Both products were sequenced as paired-end 75 bp reads on an Illumina HiSeq 2500. All data processing was performed in parallel on a local GALAXY instance (http://bioinfo.univ-perp.fr). Read quality were verified using the FastQC toolbox
(https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Sequences were aligned to the *S. mansoni* reference genome v7 with Bowtie v2.1 using parameters–end-to-end,–sensitive,– gbar 4[7]. BAM files generated by Bowtie2 were sorted and then filtered for unique matches with samtools v1.3.1 [4]. PCR duplicates were also removed using samtools (samtools rmdup). Wiggle files generated in the process were visualized with IGV [12]. We started our analyses by characterizing the genome-wide distribution of narrow posttranslational histone modification. Our choice to compare narrow histone

mark is due to the fact that they are highly specific and related to transcriptional start site (TSS), reducing the possibility of false positive detection. In addition, it has been demonstrated that the histone mark H3K4me3 is typically restricted to narrow regions over specific functional genomic motifs being less dynamic than others marks already tested for S. mansoni [10]. Peak detection and comparative analysis were generated by ChromstaR, an R package that uses Hidden Markov Models (HMM) to perform computational inference of discrete combinatorial chromatin state dynamics over the whole genome [11]. BAM files were processed under the differential mode, with a false discovery rate (FDR) cutoff of 1e-4 and bin size of 150. We used the input as negative controls for comparative analyses. TSS of genes were defined as 3 bp (one base pair upstream and downstream of the +1 transcription site) based on the genome annotation file v.7. First, we analysed the correlation between all samples from each experiment to understand how the distribution of H3K4me3, spanning the entire genome, changed from one approach to another (Figure 2). Both approaches showed a similar profile with over 75% of peak positions being conserved throughout all samples. We conducted our analyses by comparing the genome-wide abundance of detected peaks and their frequency for narrow H3K4me3 (Table 1). Differential frequency of H3K4me3 was lower than 0.3% with just one peak exclusively detected to a specific procedure (Table 1). Genome-wide analyses revealed high similarity between both techniques. In both techniques, H3K4me3 enrichment starts around 500 bp upstream of TSS and do not spread more than 3,000 bp downstream (Figure 3A). Wig files were produced to visualize the distribution of H3K4me3 (Figure 3B). We highlight here the instrumental importance of good quality of antibodies to obtain high quality and deeply sequenced input DNA libraries which has fundamental implication for data analysis. We also show that nChIP-seq and ChIPmentation deliver similar results. The minimum number of cells for reliable peakcall with our nChIP technique is 10,000 cells, and we recommend using ≥20,000 in the routine.

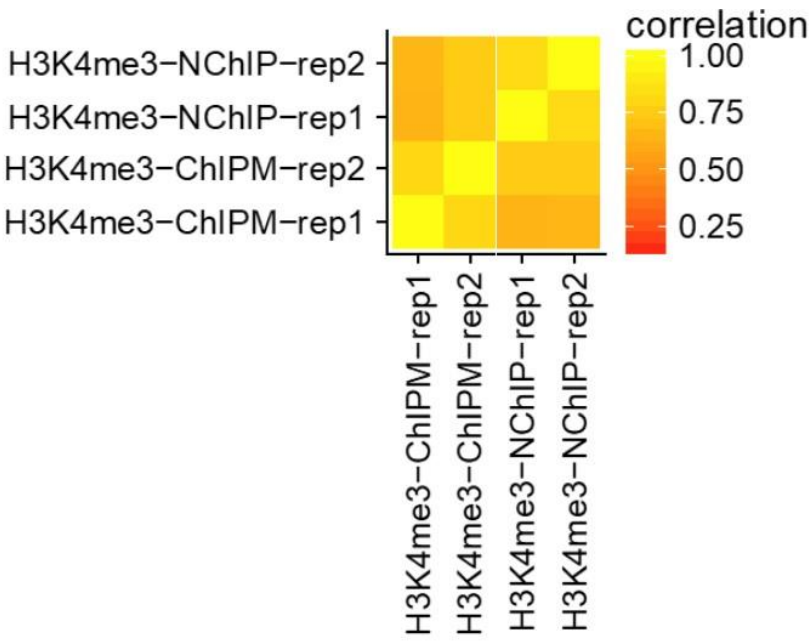

Figure 1. Heatmap of both nChIP and ChIPmentation for H3K4me3 representing similarity of 396 genome wide profiles and peak positions.

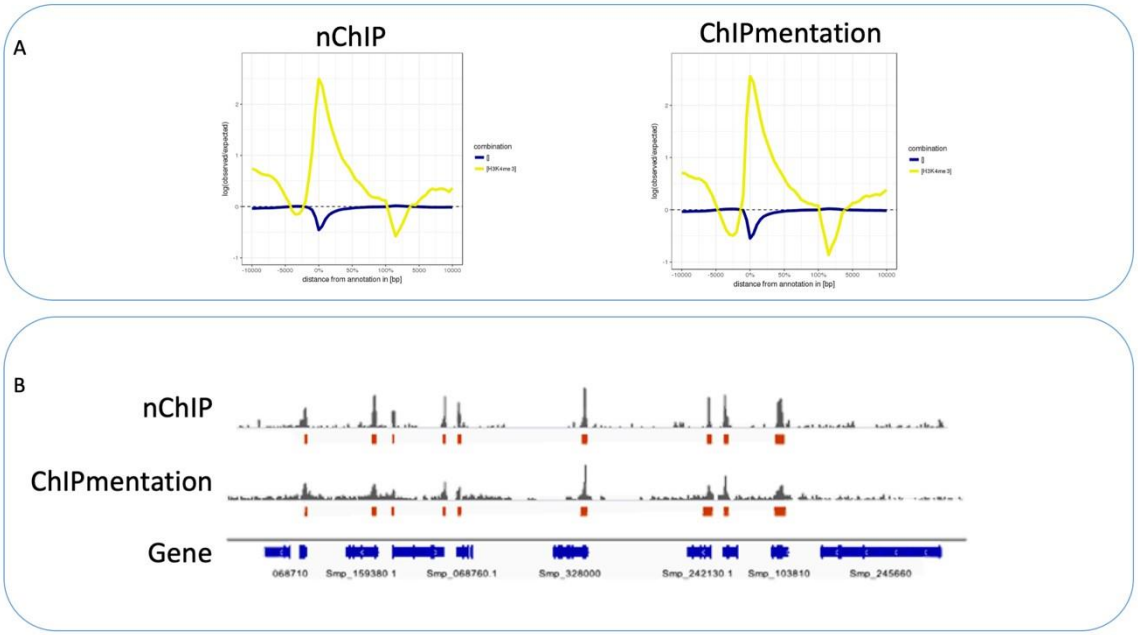

Figure 2. (A) Metagen profile of enrichment of H3K4me3 (yellow line) over genes of *S.mansoni*. X-axis over genes in % of length with 0% transcription start site (TSS) and 100% transcription end site, upstream and downstream in base-pairs (B) genome browser snapshot showing an example of H3K4me3 enrichment (grey peaks) and peak detection (red peak) at TSS.

	nChIP	ChIPmentation
Frequency	3.42%	3.19%
Number of Peaks	6099	6098

Table 1. Genome-wide description of frequency and number of peaks for H3K4me3 of both 399 nChIP and ChIPmentation.

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