Title

The study of protein-DNA interactions in CD4 T cells using ChIPmentation

Authors

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Abstract

Chromatin immunoprecipitation (ChIP) coupled with high-throughput sequencing (ChIP-seq) is an invaluable method to profile of enrichment of histone modifications and transcription factor binding sites across the genome. However, standard ChIP-seq protocols require large numbers of cells (> 10^7) as starting material, which are often impossible to obtain for rare immune populations. Here we describe a streamlined ChIP protocol optimised for small cell numbers in conjunction with transposon-tagging mediated sequencing library preparation (ChIPmentation) which allows the analysis of samples of as low as 10^5 cells.

1. Introduction

ChIP is a widely used method to profile genome wide transcription factor binding or histone modification enrichment. The method is based on crosslinking DNA-associated proteins to DNA via formaldehyde treatment of cells. The genomic DNA is then broken up into small fragments either via sonication or micrococcal nuclease digestion and the antigen-bound fragments are immunoprecipitated and purified. Due to the inefficient nature of the immunoprecipitation (IP), it is usually necessary to use tens of millions of cells per ChIP reaction. This is readily achievable

for cell types that can be expanded in vitro. However, this limitation means that this procedure is not applicable to rare immune populations or cell types that can't be expanded in vitro.

To address the problem of limiting cell numbers, several optimised ChIP protocols which reduce the amount of cells required for ChIP have previously been described. One such method, called ChIPmentation ***(1)*** is based on a previously developed method termed tagmentation, the simultaneous fragmentation and sequencing adapter tagging of genomic DNA ***(2)*** or chromatin ***(3)*** using the hyperactive Tn5 transposase which has been used to generate whole genome sequencing libraries and profile regions of open chromatin (ATAC-seq), respectively. ChIPmentation adapts the tagmentation method for ChIP-seq by directly tagging the sonicated and immunoprecipitated chromatin with sequencing adapters to generate ChIP-seq libraries.

Standard ChIP-seq library preparation methods begin by blunting the DNA fragments by exonuclease digestion and fill-in reactions. Then, a poly-A tail is attached to which the PCR amplification linkers are ligated. The limited efficiency of these enzymatic reactions causes incomplete processing of the DNA fragments so that some of the sample is not carried forward to the next step. In addition, after each enzymatic step, the DNA fragments have to be purified, which contributes to the sample loss. In contrast, in the ChIPmentation protocol, the ChIP DNA is directly tagged via an efficient transposon insertion and the tagged DNA is purified only once. Therefore, the ChIPmentation protocol dramatically reduces sample loss during library preparation compared to traditional library preparation methods, resulting in increased enrichment ***(1)***. In a direct comparison of the standard ChIP-seq protocol with ChIPmentation for the transcription factor T-bet in 10^6 Th1 cells, we found that the read-densities obtained by

ChIPmentation were superior and equivalent to the enrichment from a standard ChIP-seq experiment with 10^8 cells (Fig. 1).

The ChIP-protocol described here also benefits from a lysis/sonication buffer optimised for low cells numbers. Most ChIP protocols use sonication buffers containing denaturing detergents, which makes it necessary to dilute the sonicated chromatin before addition of the antibody. In contrast, the sonication buffer used in this protocol requires no dilution due to the absence of denaturing detergents. Therefore, the IP occurs in a small volume allowing for more efficient interaction between the bead/antibody complexes and the chromatin, resulting in increased IP efficiency. The lower sonication efficiency of the ionic detergent-free sonication buffer is offset by using a powerful water bath sonicator (Bioruptor Pico).

We describe here a protocol that allows ChIP-seq from as low as 10^5 CD4 T cells. This protocol uses optimised sample volumes and handling steps for chromatin sonication and immunoprecipitation and makes use of the ChIPmentation method ***(1)*** for generating sequencing libraries.

2. Materials

1. Fixation solution: 11% formaldehyde, 50 mM Hepes, pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA. Prepare freshly on the day of the experiment.
2. Quenching solution: 2.5 M glycine in dH_2O. Filter through a 0.22 µM filter and store at RT.

3. Antibody binding buffer: 1X PBS + 0.5% BSA. Add 50 ml 1X PBS to 50 ml tube and add0.25 g of BSA on top. Let dissolve at RT. Filter through 0.22 µM syringe filter into a new 50 ml tube. Chill on ice before use.
4. 2X Lysis buffer: 20 mM Tris pH 8.0, 200 mM NaCl, 2 mM EDTA, 1 mM EGTA, 0.2% sodium deoxycholate, 1% N-lauryl sarcosine. Before use, freshly add 2X protease inhibitor cocktail and 2 mM PMSF. Chill on ice before use. If using lysis buffer at 1X concertation, supplement with 1X protease inhibitor cocktail and 1 mM PMSF.
5. Wash buffer I: 50 mM Hepes pH 7.5, 1 mM EDTA, 0.5 M LiCl, 0.7% sodium deoxycholate, 1% IGEPAL CA-630. Chill on ice before use.
6. Wash buffer II: 10 mM Tris pH 8.0. Chill on ice before use.
7. Low-salt TE buffer: 10 mM Tris pH 8.0, 1 mM EDTA pH 8.0, 50 mM NaCl. Chill on ice before use.
8. 5X Tagmentation buffer: 50 mM Tris pH 8.0, 25 mM $MgCl_2$, 50 % (v/v) dimethylformamide. Store at RT.
9. Tagmentation reaction mix: Dilute 5X Tagmentation buffer in dH_2O to 1X concentration with required amount of Illumina TDE1 Tagment DNA Enzyme (Illumina) per 25 µl final volume. Prepare at RT.
10. Tagmentation dilution buffer: 10 mM Tris pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate, 0.5% N-lauryl sarcosine, 0.1% SDS. Chill on ice before use.
11. Elution buffer: 50 mM Tris pH 8.0, 10 mM EDTA, 1% SDS. Store at RT.
12. RNaseA: Dilute in dH_2O to 10 µg/µl final concentration. Aliquot and store at -20°C.

13. Proteinase K: Dilute in dH_2O to 20 µg/µl stock final concentration. Aliquot and store at -20°C.
14. Ethanol wash solution: 80% ethanol. Prepare freshly on the day of the experiment.
15. 50X protease inhibitor cocktail: Dissolve one complete EDTA-free protease inhibitor cocktail tablet (Sigma) in 1 ml dH_2O. Aliquot and freeze at -20°C.
16. PMSF: 100 mM in isopropanol
17. KAPA HiFi HotStart Real-Time Library Amp Kit (Roche).
18. KAPA HiFi HotStart ReadyMix (Roche).
19. Solid phase reverse immobilisation (SPRI) beads: KAPA Pure beads (Roche).
20. Magnetic stands for 8-tube PCR strips and 1.5 ml tubes.
21. APEX NoStick tubes (alpha laboratories)
22. Axygen 1.7 ml Maxymum Recovery (MR) tubes (Corning).
23. Low-retention tips. These tips reduce sample loss at all steps at which chromatin or beads are pipetted.
24. Bioruptor Pico sonicator (Diagenode).
25. Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific).
26. Agilent DNA 1000 Kit (Agilent).
27. NEBNext Library Quant Kit for Illumina (New England BioLabs).

Methods

3.1. Crosslinking of cells

1. Prepare cells under required culture conditions in 500 µl medium (see Note 1).
2. Add 50 µl fixation solution (1/10 volume) to cells for a final concentration of 1% formaldehyde.

3. Incubate on a seesaw rocker for 20 min at RT.
4. Stop fixation by adding 25 µl (1/20 volume) quenching solution.
5. Transfer cells to 1.5 ml tube.
6. Pellet cells at 800xg for 5 min at 4°C
7. Remove supernatant (SN) but leave the last 50 µl in the tube.
8. Wash 2X with 1.5 ml ice-cold PBS, pelleting cells at 800xg for 5 min at 4°C each time.
9. Either freeze the cells at -80°C in 50 µl remaining volume or directly proceed to step 2.3.

3.2. Preparation of antibody/bead complexes

1. Add protein G magnetic beads (see Note 2) to a 1.5 ml APEX NoStick tube containing chilled antibody binding buffer. The combined volume of beads and antibody binding buffer is 1 ml.
2. Spin 300xg for 5 sec to pellet the beads, place tubes in a magnetic holder and allow the beads to be drawn to the magnet.
3. Remove the SN with a pipette and add 1 ml antibody binding buffer.
4. Return the tube to the magnetic stand, turning the tube by 180° to pull any beads still stuck to the side of the tube through the buffer.
5. Repeat the wash twice more for a total of three washes. After the final wash, resuspend beads in 350 µl antibody binding buffer.
6. Add antibody beads (see Note 3).
7. Incubate beads for at least 3 hrs in an overhead shaker in a cold room.

3.3. Cell lysis and sonication

1. Either thaw crosslinked cells on ice or use freshly crosslinked cells.
2. Spin cells at 1,000xg for 1 min at 4°C.
3. Use a pipette to reduce the volume to 20 µl (see Note 4) and then add 30 µl cold PBS.
4. Add 50 µl 2X lysis buffer (see Note 5), lyse cells by gently pipetting up and down 3 times and immediately transfer cell lysate to a 1.7 ml MR tube (see Note 6).
5. Sonicate the chromatin for 3 cycles (30 sec on, 30 sec off) using a Bioruptor Pico sonicator (see Note 7).
6. Spin 13,300 rpm for 10 min at 4°C to remove insoluble material and transfer the sonicated, cleared chromatin to a PCR tube (see Note 8).
7. Remove 5 µl of sonicated chromatin (2% of one ChIP sample) as input and transfer into a 1.7 ml MR tube containing 45 µl elution buffer.

3.4. Chromatin immunoprecipitation

1. Add 5 µl 1X lysis buffer to each tube to a final volume of 100 µl.
2. Add 10 µl (1/10 volume) of 10% Triton X-100
3. Spin down the bead/antibody complexes at 300xg for 5 sec to pellet the beads, place the tubes in a magnetic holder and allow the beads to be drawn to the magnet.
4. Remove the SN with a pipette.
5. Remove the tube from magnet, resuspend beads in 1 ml of antibody binding buffer and return to the magnet, turning the tube by 180° so that the any beads remaining on the side of the tube are pulled through the buffer.
6. Repeat wash twice more for a total of three washes, turning the tube each time.
7. Resuspend beads in a volume of antibody binding buffer identical to initial bead volume.

8. Add 10 µl bead/antibody complexes to sonicated chromatin.
9. Incubate overnight on an overhead shaker in the 4°C cold room.

3.5. Washes, tagmentation and reverse crosslinking

1. Spin PCR tubes at 300xg for 30 sec to pellet beads.
2. Place tubes in a pre-chilled PCR-tube sized magnetic holder, allow beads to be drawn to the magnet (see Note 9) and remove the SN (see Note 10).
3. Add 200 µl wash buffer I and return the tube to the magnet, turning by 180° so that the beads are pulled through the buffer.
4. Wash beads twice more with wash buffer I for a total of 3 washes.
5. Wash twice with wash buffer II.
6. During the second Tris wash, transfer the bead suspension into fresh PCR tubes (see Note 11).
7. Place the tubes into the magnetic holders, allow beads to be drawn to the magnet and remove the buffer.
8. Remove tubes from the magnet and place in a plastic rack on ice.
9. Resuspend beads in 25 µl Tagmentation reaction mix (see Note 12)
10. Incubate at 37°C for 10 min in a thermocycler. Resuspend beads after 5 min by pipetting up and down several times while leaving the tubes in the thermal cycler.
11. Immediately place tubes in a cold magnetic holder on ice and add 175 µl pre-chilled Tagmentation dilution buffer.
12. Remove diluted reaction mix and immediately add 200 µl Tagmentation dilution buffer.
13. Wash with Tagmentation dilution buffer one more time.

14. Wash twice with 200 µl low-salt TE buffer. Transfer beads to a new 1.7 ml MR tube during the second wash step.
15. Place the tubes into the magnetic holders, allow beads to be drawn to the magnet and remove as much buffer as possible.
16. Resuspend beads in 25 µl elution buffer
17. Incubate at 65°C for 1 hr in a thermo-shaker at 1,200 rpm. At this point also incubate the input samples saved from the previous day in a water bath at 65°C.
18. Spin 300xg for 5 sec to pellet beads, place the tube into a magnetic holder and allow beads to be drawn to the magnet.
19. Transfer DNA to a new 1.7 ml MR tube and incubate at 65°C in a water bath.
20. Add 25 µl fresh elution buffer to beads and incubate at 65°C for 30 min at 1,200 rpm in a thermo-shaker.
21. Combine both elutions in the same tube and incubate at 65°C in water bath for an additional 6 – 7 hrs.
22. Freeze tubes at -20°C.

3.6. RNaseA and Proteinase K treatment

1. Thaw tubes from 3.5, add 2 µl 10 µg/µl RNaseA and incubate at 37°C for 1 hr on a heat block
2. Add 1 µl 20 µg/µl proteinase K and incubate at 55°C for 4 hrs in a water-bath.
3. Either proceed directly to DNA purification or freeze at -20°C

3.7. DNA purification

1 Warm up SPRI beads for 30 min at RT before use.

2 Add 125 µl (2.5 volumes) of SPRI beads to the decrosslinked ChIP samples and input DNA (see Note 13). Vortex beads thoroughly before pipetting.

3 Briefly vortex at a low setting to mix and incubate at RT for 15 min.

4 Spin at 300xg for 5 sec, place the tube into the magnetic holder and allow the beads to be drawn to the magnet (5 min).

5 Discard the SN and add 650 µl 80% ethanol, keeping the tubes in the magnet.

6 Remove ethanol with a pipette, placing the tip against the tube wall opposite the beads (see Note 14).

7 Wash beads with ethanol once more and then remove all drops of ethanol with a pipette.

8 Place the magnetic holder with the tubes on top of a 37°C heat block for 15 – 30 min until the beads are dry. Then remove the tubes from the magnet and place in a rack.

9 Add 10 mM Tris pH 8.0 (see Note 15) to beads and gently flick several times to resuspend beads. Incubate at RT for 2 min.

10 Spin at 300xg for 5 sec, place the tube into the magnetic holder and allow the beads to be drawn to the magnet (5 min).

11 Transfer eluted DNA into new 1.7 ml maximum recovery tube. Set the pipette to 2 µl less than the initial volume in which the beads were resuspended to avoid taking up beads.

12 Store purified tagmentation DNA at -20°C.

3.8 Determine optimal cycle number for library amplification

1. Aliquot KAPA HiFi HotStart Real-Time PCR Ready Mix into PCR tubes (see Note 16) and incubate at 98°C for 45 sec.

2. Immediately place tubes in an ice-water-bath until use.
3. Setup quantitative PCR reactions in duplicate:

2X KAPA HiFi HotStart Real-Time PCR Ready Mix		5 µl
Tagmentation DNA		1 µl
Nextera universal primer 10 µM Ad1_noMX	(see Table 1)	0.75 µl (750 nM final)
Nextera index primer 10 µM Ad2.X	(see Table 1)	0.75 µl (750 nM final)
Nuclease-free dH_2O		2.5 µl

Cycling conditions:

a. 72°C 5 min
b. 98°C 30 sec
c. 25 cycles of:
    i. 98°C 10 sec
    ii. 63°C 30 sec
    iii. 72°C 30 sec + fluorescence read
d. 72°C 1 min

4. Plot the linear Rn (fluorescent signal at each cycle) versus cycle number.
5. Draw a line from the mid-point between the baseline and the plateau down to the x-axis. The rounded-up quantification cycle is the number of cycles for which the rest of the tagmentation DNA should be amplified.

3.9 Library amplification

For instructions of how to avoid DNA contamination at this step please see Note 17.

2X KAPA HiFi HotStart Ready Mix (preheated at 98°C)	25 µl
Tagmentation DNA	14 µl
Nextera universal primer 10 µM Ad1_noMX (see Table 1)	3.75 µl (750 nM final)
Nextera index primer 10 µM Ad2.X (see Note 18 and Table 1)	3.75 µl (750 nM final)
Nuclease-free dH_2O	3.5 µl

Cycling conditions:

a. 72°C 5 min
b. 98°C 30 sec
c. x cycles as determined in step 2.8:
    i. 98°C 10 sec
    ii. 63°C 30 sec
    iii. 72°C 30 sec
d. 72°C 1 min

3.10 Library purification and size selection

1. Warm up the SPRI beads at RT for 30 min before use.
2. Add 25 µl (see Note 19) SPRI beads to each tube of amplified library. Vortex beads thoroughly before pipetting.
3. Briefly vortex at a low setting to mix and incubate at RT for 10 min.

4. Spin a 300xg for 5 sec, place the tube into the magnetic holder and allow the beads to be drawn to the magnet (5 min).
5. Transfer 75 µl supernatant to new PCR tubes and add 25 µl beads (see Note 20) to the transferred supernatant.
6. Briefly vortex at a low setting to mix and incubate at RT for 10 mins.
7. Spin at 300xg for 5 sec, place the tube into the magnetic holder and allow the beads to be drawn to the magnet (5 min).
8. Remove and discard SN and then add 200 µl 80% ethanol, keeping the tubes in the magnet and placing the pipette tip against the tube wall opposite to the beads.
9. Remove ethanol with a pipette as above.
10. Wash beads with ethanol one more time and then carefully remove all ethanol with a pipette.
11. Place magnet with tubes on top of 37°C heat block for 15 – 30 min until the beads are dry.
12. Remove tubes from magnet, add 20 µl 10 mM Tris pH 8.0 and gently flick tubes several times to resuspend the beads. Incubate at RT for 10 min.
13. Spin at 300xg for 5 sec, place the tube into the magnetic holder and allow the beads to be drawn to the magnet (2 min).
14. Transfer 18 µl eluted DNA into a new 1.7 ml maximum recovery tube and store library at -20°C.

3.11 Tagmentation of input samples

1. Setup in PCR tube:

Decrosslinked and purified input DNA	2.5 ng
5X Tagmentation buffer	1 µl

1:10 diluted TDE1 Tagment DNA Enzyme	1 µl
Nuclease-free dH_2O	Add to 1 µl final volume

2. Incubate in a thermal cycler at 55°C for 5 min.
3. Add 1 µl of 0.6% SDS, mix and incubate at RT for 5 min to stop the tagmentation reaction.
4. Add the following PCR reagents to the tube with the tagmented input DNA:

2X KAPA HiFi HotStart Ready Mix (preheated at 98°C)		25 µl
Tagmentation DNA		6 µl
Nextera universal primer 10 µM Ad1_noMX	(see Table 1)	3.75 µl (750 nM final)
Nextera index primer 10 µM Ad2.X	(see Note 18 and Table 1)	3.75 µl (750 nM final)
Nuclease-free dH_2O		11.5 µl

Cycling conditions:

d. 72°C 5 min

e. 98°C 30 sec

f. 12 cycles:

iv. 98°C 10 sec

v. 63°C 30 sec

vi. 72°C 30 sec

e. 72°C 1 min

5. Purify amplified input library as described in step 3.10.

3.12 Library QC and pooling

1. Measure DNA concentration of 1 µl of each library using the Qubit dsDNA High Sensitivity assay kit.
2. Determine average fragment length of each library with an Agilent Bioanalyzer DNA 1000. An example of a library Bionalyzer profile with an ideal DNA fragment distribution is shown in Fig. 2.
3. Calculate molarity of the library using the DNA concentration and average fragment size determined in step 1 and 2.
4. Dilute an aliquot of each library to 15 nM in 10 mM Tris pH 8.0 (15 µl final volume).
5. Prepare serial dilution of 10 nM library (1:1000, 1:2,000 and 1:4,000) with 15 µl volume per dilution.
6. Quantify serial dilutions with NEBNext Library Quant Kit for Illumina and calculate molarity of the libraries by adjusting for fragment length as determined in step 2 (DNA standard is 399 bp).
7. Dilute an aliquot of all libraries intended for the same pool to the same concentrations (see Note 21). If the concentration of all libraries is above 10 nM, adjust the concentration of each library to 10 nM. If the concentration of some libraries is below 10 nM, adjust the concentrations to the concentration of the library with the lowest concentration. The concentration of the library pool can be as low as 2 nM.
8. Prepare the library pool by combining equal volumes of each library in a tube.
9. Prepare serial dilutions of the pool (1:400, 1:800 and 1:1,600) and quantify serial dilutions with NEBNext Library Quant Kit for Illumina as described in steps 5 – 6.

10. Sequence the library pool using a single-end read and at least 50 bp read-length. The adapter index sequences are provided in Table 1.

Notes

1. This protocol is optimised for cell numbers between 10^5 and $5x10^6$. Choose a culture volume appropriate for the cell numbers used and then adjust the volumes of fixation solution and quenching solution accordingly.
2. For cell numbers up to $5x10^6$ we recommend using 10 µl magnetic beads. Up to 300 µl protein G magnetic beads can be washed in one tube. If larger bead volumes are required, prepare several tubes in parallel.
3. The optimal amount of antibody used per cell number should be experimentally titrated for each individual antibody. As a guideline we recommend using 2 µg antibody per ChIP reaction.
4. Considering the small cell numbers used in this protocol we don't recommend removing all buffer from the tube in order to avoid cell loss.
5. Up to $5x10^6$ cells can be sonicated in a volume of 100 µl. If higher cell numbers are used, split the sample into several tubes and pool again after sonication. In contrast to other protocols, the lysis buffer used in this protocol does not contain SDS and therefore doesn't require dilution after sonication. In our experience the low sample volume during immunoprecipitation increase the enrichment compared to ChIP conducted with diluted SDS buffers.
6. Diagenode recommends using their proprietary tubes made from harder plastics to increase sonication efficiency. Although these tubes do produce more efficient DNA sonication we found that efficient sonication of the chromatin into small fragments doesn't necessarily translate into better enrichment. The Bioruptor Pico is more powerful compared to other

sonicators and we don't find it necessary to increase the sonication efficiency even further. See Note 7 for further discussion of this issue.

7. We found that although more sonication cycles increase the amount of small DNA fragments, it results in poorer enrichment. We assume that the sonication denatures the chromatin and therefore impairs the binding of the antibody to the epitope. We recommend conducting a pilot experiment to determine which sonication cycle number produces the best enrichment.
8. We recommend using 8-tube PCR strips so that the samples can be processed using multichannel pipettes. This considerably reduces the processing time in the subsequent steps.
9. Pre-chill the magnet and keep the magnet on ice during all washing and tagmentation steps.
10. Use a multi-channel pipette for the processing steps in this section. Alternatively, the beads can be transferred to a 1.7 ml MR tube and then each tube processed individually.
11. Moving the samples into new tubes at this point is crucial. It separates the bead-bound chromatin from chromatin that is unspecifically bound to the tube plastic. Therefore, this step is important to reduce the background signal.
12. The signal-to-noise ratio is dependent on the ratio between amount of tagmentation enzyme and cell number. As a guide we suggest to use 0.5 µl tagmentation enzyme for up to $1x10^6$ cells and 1 µl for $5x10^6$ cells. If required, the optimal tagmentation enzyme concentration and tagmentation time can be titrated in a pilot experiment.
13. The SPRI bead to sample ratio is dependent on the SPRI manufacturer. At this step, the aim is to purify all DNA from the sample. If using other SPRI beads, use bead-to-sample ratios as specified by the manufacturer.

14. Process up to eight samples in one batch. Add ethanol to all eight tubes and then remove the ethanol from all tubes starting from first to ensure each tube is incubated for the same length of time. Then repeat the wash.
15. Resuspend beads in 20 – 40 µl 10 mM Tris pH 8.0 depending on the number of cells used per ChIP reaction.
16. This is necessary to activate the KAPA hotstart polymerase so that it can carry out nick translation from the first PCR step. Do not pre-activate more than 100 µl of KAPA master mix per tube as most thermal cyclers can only efficiently incubate samples up to this volume.
17. During library amplification, it is important to avoid contamination with previously prepared libraries. Therefore, we recommend to spatially separate the handling steps before and after the PCR amplification and use dedicated equipment and consumables for each step. In particular, the libraries should be prepared with a specific set of pipettes and consumables at one bench. If available, this step can be carried out in a laminar flow cabinet. After PCR amplification, the libraries should be purified with another set of pipettes and consumables at a separate bench. Furthermore, once the libraries have been purified all further procedures such as quantification and pooling should be carried out at the post-PCR bench with the corresponding equipment and consumables. As an additional precaution to minimise DNA contamination, we recommend treating equipment and working surfaces with DNA decontamination reagents such as DNA AWAY from Molecular BioProducts.
18. Use a different index primer for each ChIP sample.
19. 0.5X bead-to-sample ratio removes fragments above 1,000 bp.

20. 1X bead-to-sample ratio removes fragments below 150 bp (25 µl beads from the first step + 25 µl beads at this step = 50 µl beads in total in relation to the initial 50 µl volume of the PCR-amplified library).
21. The optimum number of reads depends on the estimated number of binding sites for the protein in question. For site-specific transcription factors we aim for 2 – 3 $x10^7$ aligned reads per library which roughly equates to eight libraries per lane of a HiSeq 2500 Rapid Run. Adjust the number of libraries per pool accordingly if another instrumentation and/or sequencing type is used.

5 References

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Table 1: Library amplification primers. Order as standard desalted oligonucleotides.

Oligo Name	Index Sequence	Oligo Sequence
Ad1_noMX	No index	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1	TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2	CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3	AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4	TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5	GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6	TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7	CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8	CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9	GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10	CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11	AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12	GTAGAGGA	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13	GTCGTGAT	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14	ACCACTGT	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15	TGGATCTG	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16	CCGTTTGT	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.17	TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
Ad2.18	GAGGGGTT	CAAGCAGAAGACGGCATACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
Ad2.19	AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT
Ad2.20	GTGTGGTG	CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGT
Ad2.21	TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
Ad2.22	TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23	TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT
Ad2.24	CCACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTGGGTCTCGTGGGCTCGGAGATGT

Figures

Fig. 1 Comparison of ChIP-seq enrichment profiles from sequencing libraries prepared by standard ChIP-seq and by ChIPmentation.

ChIP-seq binding profiles for T-bet at the murine *Ifng* locus in *in vitro* polarised Th1 cells. The number of sequencing reads from ChIP-enriched DNA are plotted per million background-subtracted total reads and aligned with the mouse genome. ChIPmentation and standard ChIP samples were carried out with identical samples of 10^6 cells and processed in parallel using the protocol described here, except that the bead/chromatin complexes of the standard ChIP-seq samples were washed seven times with wash buffer and one time with low-salt TE. Sequencing libraries of the standard ChIP-seq samples were prepared by standard Illumina protocols, except that DNA in the range 150-350 bp was gel-purified after PCR-amplification. T-bet ChIP-seq data for standard ChIP with 10^8 cells was reported previously ***(4)***.

Fig. 2 Agilent Bionalayzer profile of a ChIPmentation library.

DNA fragment size distribution of a ChIPmentation sequencing library measured with an Agilent Bionalyzer High Sensitivity DNA kit. The library DNA size range should ideally range between 150 bp and 450 bp. DNA fragments below this range indicate adapter contamination and fragments above this range indicate over-amplification. Peaks at 35 bp and 10,380 bp denote DNA ladder positions. FU = Fluorescence units.