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Native ChIP Using 10,000 Cells

I. Purpose

To provide details for immunoprecipitation reactions using 10,000 cells from fresh or frozen cells.

II. Scope

All procedures are applicable to the BCGSC Library Core and Library TechD groups.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001) Do not copy or alter this document To obtain a copy see a QS associate

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Production Coordinator to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Systems to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
ndChIP-seq	EGL017.07
Preparation and QC of SeraMag Bead Solution	EGL008.2

VI. Related Documents

Document Title	Document Number
Native ChIP Using 100,000 Cells	LIBPR.0138

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the safety data sheet (SDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number	Model or Catalogue #	
Small size safe touch nitrile gloves	Ultident	296359683		√
wet ice	In house	N/A	N/A	N/A
Ice bucket	Fisher	11-675-58		√
1.5 mL Microtubes	Diamed	PRE150-B		√
15 mL Conical Tubes	BD Falcon	352097		√
50 mL Conical Tubes	BD Falcon	352070		√
Gilson P10 pipetman	Mandel	GF-44802		√
Gilson P20 pipetman	Mandel	GF23600		√
Gilson P200 pipetman	Mandel	GF-23601		√
Gilson P1000 pipetman	Mandel	GF-23602		√
Diamond Filter tips DFL10 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171203		√
Diamond Filter tips DFL30 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171303		√
Diamond Filter tips DFL200 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171503		√
Diamond Filter tips DFL1000 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171703		√
Galaxy mini-centrifuge	VWR	37000-700		√
Large Kimwipes (Kimberly Clark/Kimtech)	Fisher Scientific	06-666-1A		√
Black ink permanent marker pen	VWR	52877-310		√
Small Autoclave waste bags 10”X15”	Fisher Scientific	01-826-4		√
Ultra Pure Water (RNase/DNase free)	Invitrogen	10977-023		√
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohols	People Soft ID: 23878		√
DNA Away	Molecular Bioproducts	7010		√
1M Tris Solution, pH 8.0	Ambion	AM9856		√
Triton® X-100, laboratory grade	Sigma	X100-100ML		√
20% SDS Solution	Ambion	AM9820		√
Centrifuge, Eppendorf 5417R, refrigerated high-speed, 115V	Fisher Scientific	5417 R	√	
VX-100 Vortex Mixer	Rose Scientific	S-0100	√	
Eppendorf Thermomixer 1.5 mL	Eppendorf	21516-166	√	
Parafilm	Fisher Scientific	13-374-12		√

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5M NaCl	Ambion	AM9760G		√
1M DTT	Invitrogen	P2325		√
1M Tris HCl pH 7.5	Invitrogen	15567-027		√
0.5M EDTA	Ambion	AM9260G		√
Deoxycholic Acid, Sodium Salt	Fisher Scientific	AC218590250		√
Sodium Bicarbonate	Sigma Aldrich	S5761-500G		√
0.2 mL Ultra Rigid Skirted 96 well PCR plate	Thermoscientific	FSSP9741450		√
Micrococcal Nuclease	NEB	M0247S		√
Nonstick 1.5 mL tubes	Ambion	AM12450		√
1M DTT	Invitrogen	P2325		√
RNase Away	Invitrogen	10328-011		√
Dynabeads Protein A,5 mL size	Invitrogen	100-02D		√
Protease Inhibitor Cocktail	Calbiochem	539134		√
LabQuake Shaker/Rotator with clips	Barnstead	415110	√	
Buffer G2	Qiagen	1014636		√
Qiagen Protease	Qiagen	19155		√
DynaMag 2 Magnet	Invitrogen	12321D		√
Disposable Trough	VWR	21007-972		√
Multi 12-channel Pipette P20	Rainin	17013803		√
Multi 12-channel Pipette P200	Rainin	17013805		√
EB Buffer	Qiagen	19086		√
96-well Plate Magnet	Alpaqua	002523		√
Tape Pads: Adhesive Plate Sealer	Qiagen	19570		√
Aluminum Foils	VWR	60941-126		√
Rainin 200µl barrier tips	Rainin	RT-L200F		√
Rainin 20µl barrier Tips	Rainin	RT-L20F		√
Sodium Butyrate	Millipore	19-137		√
Sodium Azide	Sigma-Aldrich	S2002-100G		√
H3K4me1 Antibody	Consult Supervisor	N/A	N/A	
H3K4me3 Antibody	Consult Supervisor	N/A	N/A	
H3K9me3 Antibody	Consult Supervisor	N/A	N/A	
H3K27me3 Antibody	Consult Supervisor	N/A	N/A	
H3K36me3 Antibody	Consult Supervisor	N/A	N/A	
H3K27ac Antibody	Consult Supervisor	N/A	N/A	
PCR tube strip, Domed 12 cap strip	Biorad	TCS 1201		√
Eppendorf Thermomixer C	Fisher Scientific	05-412-503		√
96 Well PCR plate Smartblock for Thermomixer C	Fisher Scientific	05-412-512		√
T36 Disinfex	VWR	CA11007-034		√
PEG-8000 (molecular biology grade)	Sigma-Aldrich	P5413-500G		√
Seramag speed beads	Fisher Scientific	09-981-123		√
Portable Pipett Aid, Multispeed XP, rechargeable	Fisher Scientific	13-681-15E		√
15 mL conical tube	VWR	CA21008-918		√

IX. Procedure

1. Retrieval of reagents and equipment preparation

1.1. Put on a lab coat and clean pair of gloves

1.2. Wipe down the assigned specific workstation, pipettes, and small equipment with DNA Away

1.3. Change gloves.

1.4. Wipe down everything with 70% ethanol to get rid of any residual DNA Away.

1.5. Fill ice bucket with ice.

1.6. Ensure all buffers, reagents, and antibodies (Ab) (see Appendix A for details for preparation of buffers) and enzymes have been validated using the Native ChIP production pipeline up to library qPCR QC before being released into production.

1.7. Make a copy of 'Worksheet for 10K Protocol' located in geneexplab\Library Core\Epigenomics\Native ChIP\Worksheets\Native ChIP Worksheet Template\10K. Save the copy in current year folder, adjust calculations keeping the below in mind. Print a copy.

- Number of IPs and what are the targets/antibodies to IP.
- Include positive control IPs (10K HL60 cells: include a whole histone modified panel of antibodies unless told otherwise by APC).

1.8. Dilute antibodies as required according to the worksheet. Keep on ice until ready to use. See also Appendix B and Step 2.18.

1.9. All reagents and buffers should be kept on ice unless otherwise noted.

1.10. HL60 cell line will be used as a control, either with 1 IP of H3K4me3 mark or whole panel. Consult with supervisor as to how many control IPs to be set up. The HL60 cells are located in the -80°C and are at a concentration of 100K cells/100 µL of 1x Lysis Buffer + PIC.

DAY 1

2. Preparation of Ab-Bead Complex

2.1. Aliquot 10 mL of IP Buffer into a 15 mL conical tube on ice.

2.2. Retrieve an aliquot of 500x Calbiochem Protease Inhibitor Cocktail (PIC) from the -20°C and thaw it at RT. Add 20 µL of the PIC to the IP Buffer aliquot. Mix well by inversion and keep on ice.

2.3. Retrieve 1M sodium butyrate from the 4°C fridge and add 100 µL to give a final concentration of 10 mM to the IP buffer + 1xPIC. Invert to mix and put on ice. Use this buffer for the entire IP panel as sodium butyrate is a histone deacetylase inhibitor.

2.4. Retrieve Dynabeads Protein A from the 4°C and mix the bottle very well to achieve a homogenous bead slurry. The beads can be vortexed gently.

2.5. Transfer the appropriate amount of Dynabeads Protein A into a new non-stick 1.5 mL tube by using the following formula:

(24 µL Protein A + dead volume*) x number of IPs = Amount of beads

*Dead volume is 3 reactions worth of beads.

Note: 12 µL of beads/well will be used for the Ab:bead complex plate
12 µL of beads/well will be used for preclearing plate

2.6. Place the bead tube on magnet stand and let separate until supernatant is clear. Remove and discard supernatant.

2.7. Remove the bead tube from magnet stand and place on ice.

2.8. Add an equal volume of ice cold IP Buffer + 1xPIC to beads (1:1 v/v bead : IP buffer)

2.9. Mix by pipetting up and down. Ensure that the beads are thoroughly mixed. **Do not vortex** the beads but mix by gently pipetting up and down **without** creating bubbles.

2.10. Place the bead tube back on magnet stand and let separate. Remove the supernatant and discard.

2.11. Repeat the washes 2 more times with IP + 1xPIC buffer for a total of three washes.

2.12. After the final wash, resuspend the beads in an equal volume of IP Buffer + 1x PIC. Keep on ice.

2.13. On ice, aliquot 24 µL plus 5 µL of dead volume of the washed Dynabeads per well of sample into a new plate.

2.14. In a clean reagent trough, pour the remaining IP buffer containing 1xPIC. Keep on ice when not using.

2.15. Add 130 µL per well of IP buffer + 1xPIC per sample into each row of a new AB1000 plate. Label this plate as "Ab-Bead complex."

2.16. Mix the aliquoted beads (from Step 2.13) using a P20 multichannel pipette set to 20 µL. Ensure the suspension is homogenous as the beads settle quickly. Transfer 12 µL to each well containing the 130 µL of IP buffer + PIC plate. Mix by pipetting up and down with a P200 multichannel pipettor set at 110 µL to achieve a homogenous suspension.

2.17. Seal the remaining Dynabeads and keep on ice. This will be used for pre-clearing chromatin later in Section 4.

2.18. **Addition of Antibodies:** On ice, prepare Ab dilutions according to the Ab dilution calculator.

a. Label 0.5 mL non-binding tubes with Ab names
b. Retrieve stock Abs from -20°C & thaw on ice
c. Add appropriate amount of 1x Antibody Dilution Buffer to tube
d. Gently finger flick stock Ab & pulse spin
e. Slowly pipette appropriate amount of stock Ab to 0.5 mL tube.
Do not pipette <1 µL.
f. Pipette slowly up & down a few times to mix. Pulse spin. Keep on ice.

2.19. Add volume (µL) for the following amounts listed in Table 1 according to the plate layout. **Do not pipette <2 µL.**

Name	Amount (µg)
H3K4me1	0.25

H3K4me3	0.125
H3K9me3	0.125
H3K27me3	0.125
H3K36me3	0.25
H3K27ac	0.25

Table 1: Amount of each antibody to be used per IP reaction.

2.20. Set multi-channel P200 pipette to 100 µL and mix each row up and down 10 times. Change tips between each row.

2.21. Cover the Ab-bead complex plate with BioRad domed cap strips securely. Ensure the beads are mixed thoroughly and no air pockets are visible in the wells. If there are air pockets then gently flick the lid(s) of the well(s) to disrupt them. Air pockets will prevent the contents of the wells to mix well during the preceding incubation.

2.22. Incubate the plate at 4°C on a rotating platform for a minimum of 2.0 hours or until pre-clearing is done.

2.23. Proceed to Step 5 after incubation.

3. Cell Lysis and MNase I Digestion of Chromatin

*** Turn on thermomixer and set to 25°C***

3.1. Retrieve 10x lysis buffer from 4°C and place on ice.

3.2. Prepare 1 mL of fresh 1x Lysis buffer + 1xPIC (this is sufficient for 4 templates). If you have more than for 4 templates, scale up the volume, accordingly. To 1 mL of 1x Lysis buffer, add 2 µL of 500xPIC and 10 µL of 1M Sodium Butyrate as described in Appendix A. Mix well by pulse vortexing. Pulse spin and keep on ice.

3.3. Prepare 1 mL of MNase I Dilution Buffer in a fresh 1.5 mL non-stick tube as shown in Table 2. Cut off the end of a 1 mL pipette tip with a clean sterile scalpel before pipetting the glycerol, as it is quite viscous. Vortex and pulse spin to ensure that all the glycerol goes into solution and place the MNase 1 dilution buffer on ice. **Make fresh for every use**.

Reagent	Amount (µL)
1M Tris, pH 7.5	10
5M NaCl	10
0.5M EDTA	2
Glycerol	500
Ultrapure Water	478

Table 2: MNase I Dilution Buffer

3.4. In a new 96 well plate, aliquot 5.5 µL of 250 µM EDTA per each row of samples plus 5 µL of dead volume. Keep at RT.

Example: For 2 rows the volume should be (5.5 µL x 2) + 5 µL = 16 µL/well x # of IPs

3.5. Aliquot 10x Lysis buffer (1% Triton-X100 containing 1% DOC) into a new 96-well plate. Label the plate. Aliquot 5.5 µL per each row of samples plus 5 µL of dead volume. Keep on ice.

Example: For 2 rows the volume should be (5.5 µL x 2) +5 µL=16 µL/well x # of IPs.

3.6. Pull out the cell pellets from the -80°C freezer. Thaw the cells in the palm of your hand for a few seconds.

3.7. To each cell pellet immediately add, 20 µL of 1x Lysis buffer + 1xPIC to a final concentration of 10,000 cells/20 µL. For example for 100K cells, the total final volume of buffer that should be added is 200 µL. Pipette the cells up and down at least 10 times. **Avoid** generating bubbles during the pipetting process, whilst making sure that **the cells are not clumped together**.

3.8. Retrieve HL60 cells, already prepared at 100,000 cells/100 uL lysis buffer + PIC and sample cells.

3.9. Make sure the concentration of the cells is equal to or slightly more than 10,000 cells/20 µL. Digestion will work best if the cells are in the range 10,000-15,000 cells/20 µL.

3.10. Thaw gently with gloved hands. Add 100 µL of 1x Lysis Buffer + PIC to HL60 cell control and enough 1x Lysis Buffer + PIC ensuring the correct concentration range as in Step. 3.9.

3.11. Aliquot 20 µL of the cells in each well into a 96- well plate according to the plate layout. Cover the plate with a plastic seal and allow the plate to sit on ice for 20 minutes to allow lysis to come to a total completion.

3.12. While the cells are lysing, prepare MNase I digestion Master Mix (minus the Mnase) according to the calculator in worksheet.

3.13. Once the 20 minute lysis on ice is complete, remove the plate from ice and place on the thermoblock.

3.14. Dilute the MNase 1 Stock enzyme from 2000 U/µL to 20 U/µL using a 2 serial dilution as in Table 3 below. Ensure to mix thoroughly by gentle pipetting and followed by a pulse spin. Keep dilution on ice

Dilution No.	Dilution Factor	Volume of MNase 1 Dilution Buffer (µL)	Amt Stock/diluted Enzyme (µL)
1	1/10 (200 U/µL)	18	2 µL of stock enzyme
2	1/10 of 200 U/µL (20 U/µL)	18	2 µL of Dilution No.1

Table 3: Serial Dilution of MNase 1 Stock Enzyme

3.15. Add the 1:100 diluted enzyme last to the Digestion Master Mix (Step 3.12). Mix the brew very well by pipetting the contents up and down gently.

3.16. In a new 96 well plate, aliquot, on ice block, 20 µL of the Mnase Digestion Master Mix per each row of samples plus 5 µL of dead volume.

Example: For 2 rows the volume should be (20 µL x 2) + 5 µL = 45 µL/well x # of IPs

3.17. Add 20 µL MNase 1 Digestion Mix to the first 2 rows of the cell lysis plate (on the thermomixer). Mix the first row by pipetting up and down 15x quickly. Start the timer for 5 minutes after the first row addition. Change tips between rows.

Note: Do not process more than 2 rows at a time.

3.18. Cover the plate with a plastic seal.

3.19. Set the P20 multichannel pipette to 20 µL. Halfway through the incubation (at approximately 2.5 minutes), mix the contents of the wells by pipetting up and down 10 more times. Cover the plate again with a plastic seal and allow the incubation to continue for another 2.5 minutes for a total of 5 minutes.

3.20. Immediately add 5.5 µL of 250 µM EDTA to stop the MNase 1 digestion. Change the multichannel pipettor volume to 20 µL. Pipette the contents up and down for 15 times at room temperature, changing tips between rows.

3.21. Aliquot 5.5 µL of 10X lysis buffer (1% Triton containing 1% DOC). Change the multichannel pipettor volume to 20 µL. Pipette the contents up and down for 10 times, changing tip between rows.

3.22. Cover the plate with a plastic seal.

3.23. Spin down the plate at 200g, for 1 minute, at 4°C and incubate on ice for 15 minutes. The total volume in each well should be 51 µL.

3.24. Proceed to Step 4.1 during incubation time.

3.25. If processing more than two rows of samples (more than 4 templates), repeat the process of MNase I digestion+EDTA quenching two rows at a time (use separate plate for each round). You can re-use all of the already prepared solutions except the Mnase I 20 U/µL enzyme dilution and Mnase I Master mix. Those two solutions need to be prepared again.

4. Input Separation and Preclearing

4.1. While the MNase I digestion plate is incubating, aliquot 130 µL of IP Buffer +1x PIC into 1 row of a new 96-well plate, if working with just one row (i.e. 2 templates only) or two rows (if working with 4 templates). Seal plate with plastic cover and keep on ice.

4.2. After the 15 minute incubation, using a P200 pipette, pool all wells for the same cell template into a non-stick 1.5 mL tube. If working with more than 1 template, make sure the tubes are clearly labeled to avoid mix ups. Mix thoroughly without foaming. Keep on ice.

4.3. Using a P200 pipette to measure the volume of each pool. Note the volume. Transfer 3% v/v into a new labeled fresh non-stick 1.5 µL tube. This will be the 'Input DNA' for that pooled sample. Store at 4°C until the following day. Consult supervisor if unsure.

4.4. Distribute the sample equally (amongst number of IPs to set up) into a new 96 well plate. Put plate on ice. (See worksheet for plate layout).

4.5. Add 100-110 µL 1xIP Buffer + 1xPIC (from Step 2.14) to each sample well and mix 10 times by pipetting up and down. Make sure that each well is well mixed before proceeding to the next step. Keep plate on ice.

4.6. Retrieve the pre-washed and aliquoted Dynabeads that were kept on ice from Step 2.17.

4.7. Using a P20 multichannel pipette, mix the beads by pipetting up and down a few times making sure the bead slurry is homogenous. Try not to introduce bubbles.

4.8. Using a P20 multichannel pipette, transfer 12 µL of beads to the wells. After addition of the beads, mix by gently pipetting up and down a few times. Use fresh tips for each row.

To make sure that the samples are well mixed, use a P200 multichannel pipette set to 100 µL and mix each row 10 times by pipetting up and down. Change tips between rows.

4.9. Seal the plate very well with Biorad domed cap strip. Ensure there are no air bubbles present in the wells. If there are, gently flick the well to pop them.

4.10. Incubate on a rotating platform at 4°C for at least 1.5 hours.

5. Immunoprecipitation Reaction

5.1. Before starting this step, make sure that the Ab-bead complex incubation has occurred for at least 2 hours (step 2.22) and the pre-clearing incubation has gone on for at least 1.5 hours (from step 4.10).

5.2. Quick spin down both plates at 200g, for 1 minute, at 4°C.

5.3. Place the Ab-Bead complex plate (from Step 2.22) on a magnet and wait for the beads to separate.

5.4. Carefully remove and discard the supernatant. Make sure not to disturb the beads.

5.5. Remove the plate from the magnet and put the plate on ice.

5.6. Place the pre-clearing reaction plate (from Step 4.10) on a magnet and wait for the beads to separate.

5.7. Set the P200 multichannel pipette to 200 µL and carefully transfer the supernatant from the pre-clearing reaction plate to the Ab-bead complex plate kept on ice. Ensure that no beads are transferred.

5.8. Gently mix by pipetting up and down 15 times. Ensure no air bubbles are present in the wells. Gently flick the plate if there are any, to pop them.

5.9. Seal the plate well with domed cap strip and incubate at 4°C on a rotating platform over night.

DAY 2

6. Washes and Elution

6.1. Put on a clean lab coat, fresh gloves, wipe down bench and pipettes with DNA Away solution.

6.2. Change gloves.

6.3. Wipe down everything with 70% ethanol to get rid any residual DNA Away.

6.4. Turn on the thermomixer and set temperature at 65ºC.

6.5. Retrieve ice and place Low Salt Wash Buffer and High Salt Buffer on it.

6.6. In a clean plastic trough, pour 5 mL of the Low Salt Wash Buffer.

6.7. In another clean plastic trough, pour 5 mL of the High Salt Wash Buffer. Keep both clearly labeled troughs on ice.

6.8. Spin down the IP reaction plate (from Step 5.9) at 200g, for 1 minute, at 4ºC.

6.9. Place the IP reaction plate on a magnet and wait for the beads to separate. Carefully remove and discard the supernatant.

6.10. Using a multichannel pipettor, add 120 µL of ice cold Low Salt Wash buffer per well to the IP reaction plate.

6.11. Slowly mix 10 times to resuspend the beads. Change tips between rows.

6.12. Place the IP reaction plate back on the magnet.

6.13. Set the multichannel pipette at 120 µL and discard the supernatant. Make sure not to remove any beads.

6.14. Repeat the Low Salt buffer wash for a total of 2 washes.

6.15. Place the IP reaction plate on ice.

6.16. Using a multichannel pipettor, add 120 µL of High Salt Wash buffer per well to the IP reaction plate.

6.17. Slowly mix 10 times to resuspend the beads. Change tips between rows.

6.18. Place the IP reaction plate on a magnet.

6.19. Set the multichannel pipette at 160 µL and discard the supernatant. Make sure not to remove any beads.

6.20. Remove the IP reaction plate from the magnet and place it on ice.

6.21. Add 120 µL of High Salt Wash buffer to each well of the IP reaction plate. Perform the high salt buffer wash for a total of two times as well.

6.22. Slowly mix 10 times to resuspend the beads and put the plate back on the magnet.

6.23. Once the bead suspension has started to clear, carefully remove the supernatant and discard making sure not to remove any beads and changing tips between rows.

6.24. Take the plate off the magnet and keep it at room temperature.

6.25. In a clean trough, pour 5 mL of ChIP Elution Buffer and keep at room temperature.

6.26. Add 30 µL of ChIP Elution Buffer to each well and mix slowly to resuspend the beads.

6.27. Seal the plate with domed cap strip and incubate at 65°C, for 1.5 hours, with mixing at a speed of 1350 rpm.

6.28. Check the plate after 45 minutes to ensure that plate is still well sealed.

6.29. While the plate is incubating, start preparations for the protein digestion and DNA purification steps as described in the sections 7 and 8 below.

6.30. After 1.5 hours, spin down the plate at 200g, for 1 minute, at **room temperature**.

6.31. Place the elution plate on a magnet and wait for the solution to clear.

6.32. Using P200 multichannel transfer all of the supernatant to a new 96 well plate. Make sure not to transfer any beads.

6.33. Cover the IP plate and keep at room temperature and proceed to the next step.

7. Protein Digestion

7.1. Retrieve SeraMag beads (30% PEG/1M NaCl) from 4°C fridge and incubate at room temperature in the dark for at least 30 minutes before use. It is **critical that the beads are at room temperature as a cooler solution will yield lower product**. Mix the beads well immediately before use. Due to the high percentage of PEG, the solution is quite viscous. Vortexing the tube is required to reach a homogenous suspension.

7.2. In addition, retrieve a new fresh aliquot of 70% EtOH and keep at room temperature for at least 30 minutes before use.

7.3. Retrieve the Input DNA sample set aside, from Section 4.3, from the 4°C fridge.

7.4. Spin down the plate from step 6.33 at 200g, for 1minute, at **room temperature** and allow to sit at room temperature.

7.5. Top up the Input DNA Samples to 30 µL with Qiagen EB buffer to each Input DNA sample and mix slowly by pipetting up and down. Pulse spin.

7.6. Using a single channel P200 pipette, transfer the Input DNAs to the IP plate according to the plate layout worksheet. Cover the plate with a plastic seal.

7.7. On ice, prepare the DNA Purification Master Mix. Scale up the DNA Purification Reaction Mix as shown in Table 4 depending on the number of IPs/Input DNA wells that you have.

Reagent	Volume (µL) per reaction
Qiagen EB	31
Qiagen G2 buffer	8
Qiagen Protease	1.75
Total	**40.75**

Table 4: DNA Purification Master Mix

7.8. On ice, aliquot 40 µL of DNA Purification Master Mix per row of samples plus 5 µL dead volume into a new 96 well plate (45 µL in total).

7.9. Using a P200 multichannel, transfer 40 µL of DNA Purification Master Mix to IP plate (including to the Input DNA wells). Mix slowing by pipetting up and down 15 times. Change tips between each row.

7.10. Seal the plate with Bio-Rad domed cap strips and incubate in a thermomixer at 50°C for 30 minutes, with mixing at a speed of 500 rpm (volume in each well should be 70 µL).

7.11. After incubation, spin plate at **room temperature,** at 200g, for 1 minute.

8. DNA Purification

8.1. Pour 70% EtOH into a clean plastic trough and keep at room temperature and cover for later use.

8.2. In a new plate, add 35 µL of Qiagen EB buffer plus 5 µL of dead volume per sample well. Cover and leave at room temperature for later use.

8.3. To the IP plate samples from Section 7.11, add an equal volume of beads (70 µL). As long as the bead volume is equal or just a little over, this is sufficient.

8.4. Mix by pipetting up and down gently 10 times.

8.5. Cover the plate and incubate at room temperature for 10-12 minutes.

8.6. Put the plate on the magnet and let the solution become clear (approximately 10 minutes)

8.7. Remove and discard the supernatant. Leave plate on magnet.

8.8. Add 150 µL of 70% EtOH to each sample well.

8.9. Do not mix the beads and 70% EtOH as this is only a wash. Remove the supernatant.

8.10. Repeat the 70% EtOH wash step.

8.11. Take the plate off of the magnet.

8.12. Allow the beads to dry which takes a few minutes.(If the beads pellet appears cracked, then they are too dry).

8.13. Add 35 µL of Qiagen EB buffer to the sample wells. Thoroughly mix by pipetting up and down at least 10 times or until the mixture appears homogenous. Cover the plate.

8.14. Incubate the plate at room temperature for 3 minutes. Label a new 96 well plate with plate ID, date and initials and cover.

8.15. Place plate back on magnet and let sit for 2-3 minutes.

8.16. Carefully transfer the supernatant to the new 96 well plate.

8.17. Seal the plate with a foil cover and store at 4°C overnight or at -20°C for long term storage.

Appendix A - Buffer Preparation

10% Triton X-100

Ingredient	Volume (mL)
Triton X-100	1
Ultrapure water	9

Allow to mix on shaker/mixer at room temperature for 1 hour at room temperature or until totally dissolved and store at 4°C. Valid for **2 months.**

10% SDS

Ingredient	Volume (mL)
20% SDS	5
Ultrapure water	5

Mix well and store at room temperature. Valid for **2 months.**

1M $NaHCO_3$

Ingredient	Volume (mL)
1M $NaHCO_3$	0.840g

Add ultrapure water to make up to 10 mL. Mix well ensuring all the powder has dissolved. Store at room temperature. Valid for **2 month**.

Immunoprecipitation (IP) Buffer

Ingredient	per 15 mL
1M Tris HCl (pH7.5)	0.30
0.5M EDTA	0.06
5M NaCl	0.45
1% Triton X-100 +1% DOC	1.50
1M Sodium Butyrate	0.15
Ultrapure water	12.54

Prepare fresh for every time.

10x Lysis Buffer

Ingredient	per 10 mL
1% Triton X-100	1 mL
Ultrapure water	5 mL
1% Sodium Deoxycholate (DOC)	100 mg

Mix well making ensuring the powder is completely dissolved. Top up with ultrapure water to 10 mL. Store at 4°C. Valid for **2 months.**

1x Lysis Buffer

Ingredient	per 1.0 mL
1% Triton X-100 + 1%DOC	0.10
Ultrapure water	0.89
1M Sodium Butyrate	0.01

Prepare fresh for every use

Low Salt Buffer

Ingredient	per 10 mL
1M Tris HCl (pH8.0)	0.20
0.5M EDTA	0.04
5M NaCl	0.30
10% Triton X-100	1.00
10% SDS	0.10
Ultrapure water	8.36

Mix well and store at 4°C. Valid for **2 months.**

High Salt Buffer

Ingredient	**per 10 mL**
1M Tris HCl (pH8.0)	0.20
0.5M EDTA	0.04
5M NaCl	1.00
10% Triton X-100	1.00
10% SDS	0.10
Ultrapure water	7.66

Mix well and store at 4°C. Valid for **2 months.**

ChIP Elution Buffer

Ingredient	**per 5 mL**
1M $NaHCO_3$	0.50
10% SDS	0.50
Ultrapure water	4.00

Prepare fresh for every use

10x Antibody Dilution buffer

Ingredient	**Volume (mL)**
Sodium Azide	0.25g (250 mg)
1xPBS	20

Add 1xPBS to make 50 mL. Mix well ensuring all powder has dissolved.
Store at 4˚C. Valid for **2 years** at 4°C.

1x Antibody Dilution buffer

Ingredient	**Volume (mL)**
10xAntibody Dilution buffer	1
1xPBS	9

Mix well. Store at 4◦C.Valid for **1 year** at 4°C.

Note: All buffers, enzymes will need to be validated using the Native ChIP production pipeline up to library qPCR QC before being released for production use.

Appendix B

Antibody Dilution Calculator

Located in: R:\\Library Core\Epigenomics\Native ChIP\10K ChIP\Antibody Dilutions\Antibody Dilution Calculator

Antibody Name	Antibody Stock Concentration (ug/ul)	Volume of Stock to Dilute (uL)	Amt Diluted Ab/IP (ug)	Volume of Diluted Ab/IP (ul)	Final Concentration of Diluted Ab/IP (ug/ul)	Volume of Dilution Buffer (ul)
H3K4me1	2.9	2	0.25	2	0.125	44.4
H3K4me3	0.25	2	0.125	2	0.0625	6
H3K9me3	1.52	2	0.125	2	0.0625	46.64
H3K27me3	1.9	2	0.125	2	0.0625	58.8
H3K36me3	0.4	2	0.25	2	0.125	4.4
H3K27ac	1	2	0.25	2	0.125	14

Note: the above table is an example of the antibody dilution calculator. Information is subject to change depending on the antibody concentration. Refer to the 10K ChIP worksheet for the most updated calculator.

Appendix C-Preparation of 30% PEG SeraMAG Beads

1. 30% PEG in 1M NaCL buffer preparation

1.1. Place a new, sterile 50 mL falcon tube on the analytical scale plate (use a styrofoam rack) and zero it. Accurately weigh 15.0 g of PEG-8000 directly into the 50 mL tube. Repeat when preparing multiple tubes.
1.2. Add 20 mL of ultrapure water (DNase/RNase free distilled water). Put on the lid on the tube and mix by inverting.
1.3. Add 10 mL of 5M NaCL.
1.4. Add 500 µL of 1M Tris-HCL (pH8.0).
1.5. Add 100 µL of 0.5M EDTA.
1.6. Mix by inverting until the PEG goes into the solution and all of the components are uniformly dispersed. The solution should become completely clear. Use a nutating mixer if preparing more than one tube at a time (recommended).
1.7. Label the tube with a permanent marker and keep the mixture at room temperature while washing the beads.

2. Bead Washes

2.1. Remove SeraMag beads from its 4°C storage, mix very well to make sure that the beads are evenly dispersed, aliquot 1 mL into a new 1.5 mL non-stick tube.
2.2. Place the tube in the DynaMag magnet stand and incubate for 2 minutes. Make sure all the beads are drawn to the sides and the solution is completely clear.
2.3. Remove the supernatant and discard. Be very careful not to remove any beads. Take the tube off the magnet stand.
2.4. Add 1 mL of TE buffer to the beads, close the tube and mix by gentle, repeated pulse-vortexing.
2.5. Place the tube back on the magnet and incubate for 2 minutes. Make sure that all of the beads separate to the side and the solution becomes completely clear.
2.6. Remove and discard the supernatant. Be careful not to remove any beads.
2.7. Take the tube off the magnet and repeat TE buffer wash two more times (for a total of 3 washes).
2.8. After the last wash resuspend the beads in 1 mL of TE buffer and keep it at room temperature.

3. SeraMag Bead Stock Preparation

3.1. Aliquot 1 mL of the washed SeraMag beads to the 30% PEG, 1M NaCl solution. Resuspend the beads by inversion.

3.2. Top up the solution to 50 mL with ultrapure water, close the lid and mix by inverting and *gentle*, repeated, pulse vortexing until uniformly brown.
3.3. When preparing multiple 50 mL conical tubes of beads at a time, mix all vials together in a pre-sterilized glass bottle to create one batch solution. This reduces the number of QC tests.
3.4. Label the tube (or the glass bottle) as "Do not use. QC in progress."
3.5. Remove an aliquot of the freshly prepared SeraMag bead solution for QC testing. Store the rest at 4°C, **protected from light**.

4. Quality Control Testing

4.1. Test every new batch of beads prepared by performing Native ChIP using 10,000 HL60 cells. IP whole histone modification panel.
4.2. Perform library construction including Quant-iT QC for product amount and Caliper for size distribution.
4.3. Perform qPCR using histone modification target specific primers and calculate fold enrichment.
4.4. Compile all results and confer with supervisor.
4.5. If the bead batch passes all QC gates proceed to Step.5. If batch production was unsuccessful prepare a fresh batch.

5. Aliquot Beads

5.1. After the new batch passes QC, retrieve the newly prepared SeraMag bead working stock from its designated 4°C storage. Make sure the stock fully reaches room temperature (for larger volumes this can take 1 hour or more).
5.2. Mix the SeraMag bead stock very well by inverting and gentle, repeated, pulse-vortexing. Make sure that the beads are uniformly dispersed in the supernatant and that the solution is uniform in color.
5.3. Aliquot 1 mL of beads to 1.5 mL non-stick pre-labelled tubes.
5.4. Store aliquots at 4°C, **protected from light**. Make sure to label and date each new batch on the tubes and on the storage box.
5.5. Bead solution stored at 4°C and protected from light should be stable for at least 6 months. After 6 months re-test monthly for performance.

Appendix D:

LIMS:

1. Native ChIP

2. Native ChIP qPCR (to be done after library construction and qPCR QC)