Non Controlled Version

**Note: Controlled Versions of this document are subjected to change without notice*

Chromatin Immunoprecipitation (ChIP)

I. Purpose

To perform Chromatin Immunoprecipitation (ChIP) on crosslinked cell lines and tissues.

II. Scope

All procedures are applicable to the BCGSC Library Core group and Library Technology Development group.

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 QA associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Assurance Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
N/A	N/A

VI. Related Documents

Document Title	Document Number
Operation and Maintenance of the Agilent 2100 Bioanalyzer for DNA samples	LIBPR.0017
Quantifying DNA Samples using the Qubit Fluorometer	LIBPR.0030
qPCR of ChIP Samples	LIBPR.0075

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 material safety data

sheet (MSDS) for additional information. **Steps that involve the use of PCI should be performed in the fume hood.**

VIII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
Small size safetouch 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		✓
15mL Conical Tubes	BD Falcon	352097		✓
50mL 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		✓
10ml serological pipettes	Costar	CS004488		✓
Portable Pipet Aid, Multispeed XP, rechargeable	Fisher Scientific	13-681-15E		✓
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		✓
nProtein A Sepharose 4 Fast Flow	GE Healthcare	17-5280-01		✓
nProtein G Sepharose 4 Fast Flow	GE Healthcare	17-0618-01		✓
1M Tris Solution, pH 8.0	Ambion	AM9856		✓
Triton® X-100, laboratory grade	Sigma	X100-100ML		✓
Deoxycholic acid, sodium salt	Fisher Scientific	AC21859-0250		✓
20% SDS Solution	Ambion	AM9820		✓
5M Sodium Chloride	Ambion	AM9760G		✓
0.5M EDTA, pH8.0	Ambion	AM9260G		✓
Complete-Mini EDTA-free Protease Inhibitor Cocktail Tablet EasyPack	Roche Diagnostics	04 695 159 001		✓

Complete-Mini Protease Inhibitor Cocktail Tablet EasyPack	Roche Diagnostics	04 693 124 001		✓
Rnase-free Non-stick tubes, 1.5mL,	Ambion	AM12450		✓
Centrifuge, Eppendorf 5417R, refrigerated high-speed, 115V	Fisher Scientific	5417 R	✓	
Deoxyribonucleic acid, single stranded from salmon testes	Sigma	D7656-1ML		✓
Bovine Serum Albumin (BSA), Buffers, 10mg/ml	NEB	B9001S		✓
Non-stick 2ml tubes	Ambion	AM12475		✓
Syringe, 1ml, Gauge: 26	Fisher Scientific	333-309625		✓
Syringe,3ml,Gauge:21	Fisher Scientific	14-826-84		✓
LabQuake Shaker/Rotator with Clips	Barnstead	415110	✓	
Sonic Dismembrator 550 (cup horn)	Fisher Scientific	Discontinued	N/A	N/A
Foam tube holder	Ambion Sample	N/A	N/A	N/A
QIAquick PCR Purification Kit, 50rxn	Qiagen	28104		✓
Proteinase K (20µg/µL)	Ambion	AM2546		✓
2mL phase lock gel heavy tubes	Inter Medico	5P2302830		✓
Phenol/Chloroform/Isoamyl Alcohol	Fisher Scientific	BP1752-400		✓
Sodium Acetate, 3M, pH 5.5	Ambion	AM9740		✓
Distriman Pipettor	Mandel Scientific	GF-F164001		✓
96 Place Microfuge Tube Rack	WVR	CBGTR-096		✓
0.5mL Non-stick Microfuge Tube	Ambion	AM12350	N/A	N/A
VX-100 Vortex Mixer	Rose Scientific	S-0100	✓	
Sodium bicarbonate	Sigma	S7277-250G		✓
Rnase, Dnase-free, 500µg	Roche	11119915001		✓
Eppendorf Thermomixer 1.5 mL	Eppendorf	21516-166	✓	
Parafilm	Fisher Scientific	13-374-12		✓
Mussel Glycogen	Roche	10 901 393 001		✓
Spin-X Filter Tube	Fisher Scientific	CS008160		✓
Distriman Tips (12.5mL capacity)	Mandel Scientific	GF-F164150		✓
Liquid Nitrogen	Praxair	NI M-FILLTR		✓
H3K4me1	Diagenode	C15410037		✓
H3K4me3(C42D8) Rb mAb	Cell Signaling	9751S		✓
H3K9me3	Diagenode	C15410056		✓
H3K27me3	Diagenode	C15410195		✓
H3K36me3	Abcam	ab9050		✓
H3K27ac	Abcam	ab4729		✓
Closed System Tissue Grinder	Fisher Scientific	02-542-09		✓
1M DTT	Invitrogen	P2325		✓
Heated Lids	In House	n/a		

IX. Procedure

6th floor ChIP Room

1. Retrieval of reagents, sample(s) and equipment preparation (Day 1)

***** Consult with supervisor on the status of the sample. If working with cells it is important to know the cell species, amount of cells, vessel the cells are in, if the cells are crosslinked or not, and how long the cells were crosslinked for if they already are. If working with tissue, it is important to know the tissue species, approximate weight of tissue, if the tissue has been crosslinked already and if so, how long the tissue was crosslinked for. Also regardless of the sample type, it is important to clarify as to approximately how many IPs are to be set up and with which antibodies. This helps determine how many beads to block.*****

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

1.2. Wipe down the work bench, small equipment, and ice bucket with DNA Away, dH_2O and 70% Ethanol.

1.3. Change gloves.

1.4. Retrieve fresh ice and all required reagents.

1.5. Print out the ChIP worksheets located at R:\Library Core\Work Sheets and Calculators\Epigenomics\ChIP

2. Lysing of cells or tissue

2.1. Dissolve 1 Complete-Mini PIC tablet in 10mL of ChIP Lysis Buffer and mix by inverting. Keep the mix at room temperature.

2.2. If the sample(s) is a cell line or primary cells, proceed to Step 2.3. If the sample(s) is tissue, proceed to Step 2.4.

2.3. Based on the cell count, add the following amounts of ChIP Lysis Buffer + PIC to the cell pellet to resuspend. Do not invert the tube when resuspending the pellet. Proceed to Step 2.5.

Cell Count	**ChIP Lysis Buffer + PIC to add**
$<1x10^6$ cells	500µL
$1x10^6$ - $10x10^7$ cells	1mL
$\geq 10x10^7$ cells	2mL

2.4. For tissue that was crosslinked by the collaborator, it is important to physically breakdown the tissue a bit before and during lysis. After adding lysis buffer (1-2mL) to the tissue, transfer the sample to the closed system tissue grinder tube. Consult with supervisor if unsure what volume of lysis buffer to add. The tissue sample should appear as a 'slurry' in lysis buffer. Securely close the lid. Ensure not to touch the tip of the grinder to prevent any contamination of the sample. Twist the handle of the grinder, to grind and break up the tissue along the sides of the tube. DO NOT plunge the handle up and down. The lysis buffer contains SDS and foam will generate. Once the tissue looks fully broken down, open the system and aspirate the sample out using a wide bore P1000 tip. Consult with supervisor if unclear. Proceed to Step 2.5.

2.5. Incubate on ice for 30 minutes. Beads can be prepped during this incubation (See Step 3).
2.6. Transfer a maximum of 1mL of lysed sample into a 2mL non-stick conical tube. Use more tubes if there is more than 1mL of sample.

2.7. Pass the sample through a chilled 1mL syringe (with 26 gauge) 5-6 times. If the sample is tissue, it is sometimes difficult to pass the lysed material through a 26 gauge needle. Pass through a 21 gauge needle.

2.8. Spin at 5000rpm for 10 minutes at 4^{o}C.

2.9. Carefully and slowly remove supernatant as the pellet can be aspirated quite easily. The pellet will appear almost jelly-like.

2.10. Resuspend each pellet with ChIP Lysis Buffer + PIC to a final volume of 250uL per pellet. If the chromatin pellet is very large, the volume can be increased up to 300uL with ChIP Lysis Buffer + PIC.

2.11. Resuspend the pellet by finger vortexing and pulse spinning.

2.12. Proceed to Step 4.

3. Preparation of Beads

3.1. Calculate the amount of beads (1:1 mixture of Protein A Sepharose and Protein G Sepharose beads) required for IP set-up and preclearing the chromatin.

40µL of beads per sample (for preclearing) + (20µL of beads x number of IPs)

e.g. 2 samples and 16 IPs

(40µL x 2 samples) + (20µL of beads x 16 IPs) = 400µL of A/G Sepharose Bead Mix

Note: When pipetting out beads, use a p200 tip that has the end of the tip cut off for a wider opening. All subsequent handling of beads should be performed with p200 tips that have the end of the tip cut off.

3.2. Resuspend the stock of Protein G Sepharose Beads and Protein A Sepharose Beads by inverting the bottles several times.

3.3. Add Protein A Sepharose Beads and Protein G Sepharose beads together in a 1:1 ratio into a 1.5mL non-stick tube. See below using the example in Step 2.1.

400µL of A/G Sepharose Bead Mix = 200µL of Protein A Sepharose Beads + 200µL of Protein G Sepharose Beads

3.4. Dissolve 1 tablet of Complete-Mini EDTA-free PIC into 10ml of IP Buffer and mix by inverting. Do not vortex the buffer. Keep the mix at 4°C or on ice.

3.5. Add 1mL of the IP Buffer and PIC mix to beads and wash by inverting several times.

3.6. Pellet the beads by centrifuging for 2 minutes at 4000rpm at 4^{o}C.

3.7. Place beads on a rack and allow 30 seconds for the beads to fully settle.

3.8. Remove the supernatant with a 200µL pipette. Avoid disturbing the beads when discarding supernatant.

3.9. Repeat wash with 1ml of the IP Buffer and PIC mix.

3.10. Pellet beads by centrifuging for 2 minutes at 4000rpm at 4^{o}C.

3.11. Place beads on a rack and allow 30 seconds for the beads to fully settle.

3.12. Remove the supernatant with a 200uL pipette. Avoid disturbing the beads when discarding supernatant.

3.13. Add 1mL IP Buffer and PIC to the beads and then add:

5µL of 10mg/mL Salmon Sperm DNA/100µL bead mix
13µL of 10mg/mL BSA/100µL bead mix

Adjust based on the same ratio if different volume of beads is used.

3.14. Parafilm the tube and rotate on rotator for 3hrs at 4°C.

3.15. Pellet beads as described above in Step 3.10-3.12.

3.16. Resuspend beads 1:1 with IP Buffer and PIC mix to make a 50% slurry.

3.17. Store slurry on ice until ready to use.

4. Sonication

Note: When performing sonication, wear protective ear phones.

4.1 Connect either end of the coaxial High Frequency Cable to the SHV connector on to the rear panel of generator and connect other end of cable to the SHV connector on the convector. Push the connectors on and turn the chrome rings ¼ turn to secure the connectors.

4.2 Mount the convertor and the cup horn onto a laboratory stand with large clamps. Do not hold or clamp the convertor by the black portion or by the horn itself, only support the convertor by clamping around the broad (chrome-plated) section.

4.3 Put the two black screws into the cup horn.

4.4 Turn the generator on by pressing "I" on Power Switch. The switch will illuminate when the power is on.

4.5 Adjust the amplitude by turning the dial to "7".

4.6 Press CLEAR to select Programmed Mode.

4.7 Press PROG/DATA to select programmed mode. The display will show the program screen and memory location.

4.8 Press PROG key a second time to begin programming the selected memory location.

4.9 Enter the process time: 00:10:00 (10 minutes) and press enter.

Note: Sonication time needed might be different for different samples, optimization might be needed. Consult supervisor.

4.10 Enter pulse "ON" time: 00:30:00 (30 seconds) and press enter.

4.11 Enter pulse "OFF" time: 00:30:00 (30 seconds) and press enter.

4.12 Secure sample (2.0mL tube) in the cup horn by placing the tube in an adapter manufactured by the GSC Engineering Group. Ensure the bottom of the tube is directly over the hole on the radiating surface of the cup horn.

4.13 Fill the cup horn with water and a little bit of ice until the water level is level with the sample in the 2mL tube.

4.14 Press start to begin sonicating the sample.

4.15 Set a timer to count up. Sonication runs for 20 minutes.

4.16 When the sonicator is in the pulse "OFF" time, aspirate water out and replenish with more ice to maintain the water level as in Step 4.13.

4.17 Once the program finishes, it will stop on its own. The program timer will automatically reset itself.

4.18 Sonicate the remaining samples as described above.

4.19 Once sonication is done, turn the amplitude dial to “0”. Press “O” on the Power Switch to turn the generator off.

4.20 Disconnect the cable from SHV connector on the convector.

4.21 Spin the samples at 14,000rpm for 12 min at 4^{o}C.

4.22 Transfer the supernatant to a fresh 1.5mL non-stick tube labeled as sonicated chromatin. Pool the supernatant from the same crosslinked sample together.

4.23 Aliquot 5uL of chromatin into a new 1.5mL microfuge tube with 195uL of Ultrapure water for reverse-crosslinking QC.

4.24 Store the remainder of the sonicated chromatin at 4^{o}C.

5 Reverse-Crosslinking for sonicated chromatin QC check

5.1 Reverse crosslink by adding 8µL of 5M NaCl and 10µL of 0.5µg/µL RNase (Roche).

5.2 Incubate at 68°C for 30 minutes on the thermomixer.

5.3 Briefly spin down the tube and let cool to room temperature. Change the temperature setting on the thermomixer to 42°C.

5.4 Add 1uL of Proteinase K (20µg/µL).

5.5 Incubate at 42°C for 30 minutes on the thermomixer.

5.6 Spin down 2mL phase lock gel tubes at 14,000rpm for 1 min at room temperature.

5.7 Add sample (~200µL) to the 2mL phase lock gel tube and add 200µL PCI to the sample. Mix by inverting.

5.8 Centrifuge at 14,000rpm for 5 min at room temperature.

5.9 Transfer the supernatant to a fresh 1.5mL tube. Add 1uL of 10mg/mL mussel glycogen, 20µL of 3M NaOAc pH5.5, and 500µL of ice cold 100% EtOH. Vortex to mix completely and place at -20°C for at least 20 minutes.

5.10 Centrifuge at 14,000rpm for at least 30 minutes at 4°C.

5.11 Carefully remove and discard supernatant. Do not disturb pellet.

5.12 Wash pellet with 1mL 70% EtOH.

5.13 Spin at 14,000rpm for 5 min at 4°C.

5.14 Remove and discard supernatant.

5.15 Repeat Steps 5.12 to Step 5.14.

5.16 Allow pellet to dry.

5.17 Resuspend pellet in 5µL of ultrapure water.

5.18 Make a 1:3 dilution of the reverse crosslinked sample.

5.19 Run the sample and the 1:3 dilution on an Agilent DNA 7500 assay to check fragment sizes. If no fragment is between 100-300bp, discuss with supervisor for further sonication.

5.20 Run a BR Qubit assay to determine the concentration.

5.21 Send the Agilent profile and Qubit quant to the supervisor to confirm there is enough chromatin to proceed with IPs. If there is not enough chromatin available to set-up IPs, proceed with Step 6.1-6.3 and then snap freeze the precleared chromatin in liquid nitrogen as per Appendix B.

6 Preclearing (Day 2)

6.1. Add 40µL of the blocked bead mix slurry to the pooled chromatin fraction, parafilm the tube and rotate at 4°C for 2 hrs.

Note: 40µL of blocked beads are sufficient for pre-clearing chromatin from 1x10e8 cells, adjust the amount of beads needed if different amount of cell number were used.

6.2. Spin at 4,000rpm for 2 min at 4°C. Transfer supernatant carefully into new 1.5mL non-stick tube labeled as precleared chromatin.

6.3. Spin the supernatant at 4,000rpm for 2 min at 4°C to ensure all beads have been removed.

7. Immunoprecipitation (IP) Set Up

7.1 Set up IPs in non-stick 0.6mL tubes. Use 5µg of chromatin/IP. Determine the volume of chromatin to use based on the Qubit concentration. Consult supervisor if not enough chromatin is present to set up the full panel of IPs or if the volume of 5µg of chromatin exceeds 30µL.

7.2 Fill out the ChIP Sample Summary Sheet found in :\Library Core\Epigenomics\ChIP Sample Summary_121128-Present with the sample name, antibody details and Qubit quant of the chromatin to determine the amount of precleared chromatin and IP buffer + PIC to add to each IP.

7.3 Aliquot out the amount of precleared chromatin and IP buffer + PIC indicated on the ChIP Sample Summary Sheet.

7.4 Add the corresponding antibody following the parameters listed on the table below (Table1). Parafilm the tubes and rotate at 4°C for 1 hr.

Antibody Name	Company Name	Catalog #	ug or uL /IP
H3K4me1	Diagnode	pAb-037-050 (C15410037)	5ug
H3K4me3	Cell Signaling	9751S	1ug
H3K9me3	Diagenode	pAb-056-050 (C15410056)	5ug
H3K27me3	Diagenode	pAb-069-050 (C15410069)	5ug
H3K36me3	Abcam	ab9050	2.5ug
H3K27ac	Abcam	ab4729	2.5ug

Table 1

Note: Antibody to be used in IPs might be different from those listed above. Refer to work request details from supervisor.

7.5 Pulse spin IP tubes.

7.6 Resuspend the Protein A/G prepared slurry by finger vortexing.

7.7 Add 20µL of Protein A/G prepared slurry to each IP using a wide bore P200 tip. Pipet up and down after adding beads to rinse the tip.

7.8 Parafilm tubes and rotate at 4°C overnight.

8. Washing (Day 3)

8.1 Place ChIP Wash buffer and Final ChIP Wash buffer on ice.

8.2 Remove the parafilm and pulse spin the IPs.

8.3 Using a p200 tip with the end cut off, transfer the IPs to a Spin-X column.

8.4 Centrifuge the Spin-X columns at 1,000rcf for 1 min at 4°C. Discard the flow-through.

8.5 Add 400µL of ChIP Wash buffer to the Spin-X columns.

8.6 Rotate the beads at 4°C for 3 min.

8.7 Centrifuge the Spin-X column at 1,000rcf for 1 min at 4°C. Discard the flow-through.

8.8 Repeat Steps 8.5-8.7 with 400µL of ChIP Wash Buffer and then once with 400µL of Final ChIP Wash Buffer. Ensure there is no liquid on the beads after the last spin. If there is, repeat Step 8.7 until there is no liquid present on the beads.

8.9 Transfer the column to a new labeled 1.5mL non-stick tube.

9. Elution and Reverse Crosslinking

9.1 Pre-heat the thermomixer to 68°C.

9.2 Retrieve a 10uL aliquot of DTT from the -20°C freezer. Each aliquot is enough to make 2ml of the ChIP elution buffer + DTT mix. Scale up or down the number of DTT aliquots depending on the number of IPs set up. Store on ice until ready to add to the columns.

9.3 Add 2mL of ChIP Elution to each pre-aliquoted tube of DTT and warm up the solution on a thermomixer at 68°C.

9.4 Add 125 µL of pre-heated elution buffer + DTT to the columns.

9.5 Incubate the columns for 15 min at 68°C. Set the thermomixer to shake on intervals at 1,000rpm for 30 seconds, every 1 minute. Firmly attach the lid to the block. **DO NOT switch on the heat for the lid component**. Note the equipment ID of the thermomixer and the lid used.

9.6 Centrifuge the columns at 1,000 rcf for 1 min at room temperature. Ensure all liquid has spun through. Spin again if the liquid has not completely spun through the column. Do not change the collection tube.

9.7 Add another 125 µL of pre-heated ChIP elution buffer + DTT to the column. Incubate for 15 min at 68°C with shaking. Ensure the lid is on securely. **DO NOT switch on the heat for the lid component**.

9.8 Centrifuge the columns at 1,000 rcf for 1 min at room temperature. Ensure all liquid has spun through. Spin again if the liquid has not completely spun through the column. There should be 250 µL of ChIP DNA. Detach the lid and discard the column.

9.9 Aliquot out 100ng for Input fraction into a non-stick 1.5mL tube. Store Input fraction at 4°C.

9.10 Add 250µL of elution buffer + DTT to the input sample (From Step 9.9).

9.11 Add 10µL of RNase A to all the IPs and input samples.

9.12 Invert tubes to mix and pulse spin.

9.13 Incubate the samples at 68°C on the thermomixer with the lid. **Switch 'ON' the heating component of the lid with NO SHAKING**. The lid is preset to 78°C. Ensure the lid is attached securely. Incubate the tubes for 5 hours or overnight.

9.14 Snap freeze the remaining precleared chromatin. See Appendix B.

10 Purification of DNA

10.1 Purify the DNA with Qiaquick columns using the QIAquick PCR Purification Kit. Use a 12.5mL Distriman pipettor for the distribution of the PBI and PE buffers.

10.2 Add 1250µL PBI to the 250µL sample and pipet up and down to mix. Add approximately 750µL max of the sample to the Qiaquick column.

10.3 Spin at 13,000rpm for 1 min. Discard the flow-through.

10.4 Add the remaining sample to the column.

10.5 Spin at 13,000rpm for 1 min.

10.6 Add 750µL of PE buffer.

10.7 Spin at 13,000rpm for 1 min. Discard the flow-through.

10.8 Spin column one additional time for 2 minutes at 13,000rpm.

10.9 Use p200 tip to aspirate any addition ethanol trapped on the rim in the Qiagen column.

10.10 Transfer the column to a new labeled 1.5mL non-stick tube.

10.11 Heat the EB buffer (Qiagen) to 50°C.

10.12 Let the column air dry for 1 minute with the lid open before adding EB.

10.13 Add 30 µL of EB to the centre of the column and let sit for 1 minute.

10.14 Spin down at 13,000rpm for 1 minute. Discard columns.

10.15 Wipe down work bench and small equipment with DNAway.

10.16 QC the ChIP samples by qPCR. Refer to LIBPR.0075 qPCR of ChIP Samples to set up qPCR.

10.17 Update the ChIP Sample Summary Sheet (see Step 7.2 for location).

10.18 Email the qPCR results to supervisors. Once the IPs and Input Control samples are approved for library construction proceed to Step 10.19.

10.19 Top up each IP and Input Control sample to 35µl with EB buffer.

Appendix A

Reagent	Stock Concentration	Volume	Final Concentration
ChIP Lysis Buffer			
Tris-HCl (pH 8.0)	1M	25mL	50mM
SDS	20%	25mL	1%
EDTA (lab stock)	0.5M	10mL	10mM
Ultrapure Water		Up to 500mL	
Complete-Mini PIC tablet			Refer to protocol

IP Buffer			
Tris-HCl (pH 8.0)	1M	5mL	10mM
Triton X-100	100%	5mL	1%
Deoxycholate*	10%*	5mL	0.1%
SDS	20%	2.5mL	0.1%
NaCl	5M	9ml	90mM
EDTA (lab stock)	0.5M	2mL	2mM
Ultrapure Water		Up to 500mL	
Complete-Mini PIC tablet			Refer to protocol

ChIP Wash Buffer			
Tris-HCl (pH 8.0)	1M	10mL	20mM
SDS	20%	2.5mL	0.1%
Triton X-100	100%	5mL	1%
EDTA (lab stock)	0.5M	2mL	2mM
NaCl	5M	15mL	150mM
Ultrapure Water		Up to 500mL	

ChIP Final Wash Buffer			
Tris-HCl (pH 8.0)	1M	10mL	20mM
SDS	20%	2.5mL	0.1%
Triton X-100	100%	5mL	1%
EDTA (lab stock)	0.5M	2mL	2mM
NaCl***	5M***	50mL	500mM
Ultrapure Water		Up to 500mL	

ChIP Elution Buffer			
$NaHCO_3$****	1M****	5mL	100mM
SDS	20%	2.5mL	1%
Ultrapure Water		Up to 500mL	

*10% Deoxycholate stock Make a 10% stock by adding 1g of powder and top up to 10mL with water in a 50mL falcon tube

****NaHCO3	Make a 1M stock by adding 8.4g of powder and top up to 100mL with water in sterile bottle

Appendix B

Snap Freezing Precleared Chromatin

1. Record the concentration of the precleared chromatin from the qubit quant in Step 5.18 on to the precleared chromatin tube.

2. Measure the remaining volume of precleared chromatin and enter the volume in the ChIP Sample Summary Sheet (File location in Step 7.2).

3. Put the tubes into an empty pipette tip box and transport the tubes to the 5th floor tissue culture room.

4. With proper personal protective gear, including face shield, remove one of the cell storage chambers from the liquid nitrogen cryo tank.

5. Place the tubes into the chamber and lower the chamber into the liquid nitrogen cryo tank.

6. The tubes should snap freeze immediately. If unsure, leave the tubes in the tank for a minimum of 1 minute.

7. Transfer the tubes to an appropriate box in the -80°C located on the 5th floor. Record the rack location and enter it into the ChIP Sample Summary Sheet.

Appendix C

LIMS for ChIP

1. SLX-ChIP: CHP_version # pipeline (eg. CHP_2.1)
2. Update antibody volume for control in LIMS under the "Antibody_Inventory_Tracking" view.