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Chromatin Immunoprecipitation (ChIP)

I. Purpose

To perform Chromatin Immunoprecipitation (ChIP) on crosslinked cell line.

II. Scope

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

III. Policy

All production procedures shall be documented and controlled by approved systems.

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 Assurance Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Reference Title	Reference Number
Nanodrop ND-1000 Full Spectrum UV/Vis Spectrophotometer Users Manual	IM.0136

VI. Related Documents

Document Title	Document Number
Operation and Maintenance of the Agilent 2100 Bioanalyzer for DNA samples	LIBPR.0017

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.**

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VIII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
Fisherbrand Textured Nitrile gloves - large	Fisher Scientific	270-058-53		✓
wet ice	In house	N/A	N/A	N/A
1.5 ml Eppendorf tube	Ambion	12400		✓
15ml Conical Tubes	VWR	CA21008-918		✓
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P20 pipetman	Mandel	GF23600		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Neptune barrier tips 10 µl	CLP	Bt10XL		✓
Neptune barrier tips 20 µl	CLP	Bt20		✓
Neptune barrier tips 200 µl	CLP	Bt200		✓
Neptune barrier tips 1000 µl	CLP	Bt1000		✓
Galaxy mini-centrifuge	VWR	37000-700		✓
Large Kimwipes	Fisher Scientific	06-666-117		✓
Black ink permanent marker pen	VWR	52877-310		✓
Small Autoclave waste bags 10”X15”	Fisher Scientific	01-826-4		✓
10ml serological pipettes	Fisher Scientific	CS004488		✓
Portable Pipet Aid, Multispeed XP, rechargeable	Fisher Scientific	13-681-15E		✓
DEPC water	Ambion	AM9924		✓
Ultra Pure Water (Rnase/Dnase free)	Invitrogen	10977-023		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓
DNA away	Molecular Bioproducts	7010		✓
nProtein A Sepharose 4 Fast Flow	Amersham	17-5280-01		✓
nProtein G Sepharose 4 Fast Flow	Amersham	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		✓
Sodium Chloride, Biological Grade	Fisher Scientific	S-6713		✓
EDTA, Disodium salt,	Fisher Scientific	BP120-1		✓
Complete-Mini EDTA-free Protease Inhibitor Cocktail Tablet	Roche Diagnostics	04 695 159 001		✓
Complete-Mini Protease Inhibitor Cocktail Tablet	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		✓
2ml tubes	Diamed	PRE-2000N		✓
Syringe, 1ml, Gauge: 26	Fisher Scientific	14-823-2E		✓
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)	Invitrogen	2553049		✓
2ml phase lock gel heavy tubes	Eppendorf	E 32005.152		✓
Phenol/Chloroform/Isoamyl Alcohol	Fisher Scientific	BP1752-100		✓
Sodium Acetate, 3M, pH 5.5	Ambion	9740		✓
NanoDrop ND-1000 Spectrophotometer	NanoDrop Tech.	ND-1000	✓	
0.6ml tubes	Active Motif	ChIP-IT Kit	N/A	N/A
VX-100 Vortex Mixer	Rose Scientific	S-0100	✓	
NaHCO3	Sigma	S7277		✓
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		✓

IX. Procedure

6th floor ChIP Room

1. Retrieval of reagents and equipment preparation (Day 1)

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 DNAway (Thermo Fisher Scientific – Molecular BioProducts, USA) and Ethanol.

1.3. Change gloves.

1.4. Retrieve fresh ice and all required reagents.

2. Preparation of Beads

2.1. Resuspend the stock of Protein G Sepharose and Protein A Sepharose beads fully by inverting the bottles several times.

2.2. Amount of beads to take out from the Protein G Sepharose and Protein A Sepharose stock bottle in total:

40µl of beads (20µl of each bead type) per 1x10e8 cells (for pre-clearing) + 20µl of beads (10µl of each bead type) x number of IPs

ie: If the starting material is 2x10e8 cells and the number of IPs in this experiment = 4, take out 80µl of beads for pre-clearing and 80µl of beads for IP, a total of 160µl of beads should be taken out. (Always take out a bit more than is needed). If 160µl of beads in

total is needed, take out ~90µl from each of the Protein G Sepharose and the Protein A Sepharose stock bottle.

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.

2.3. Mix the beads together into a 1.5ml non-stick tube.

2.4. Dissolve 1 tablet of Complete-Mini EDTA-free PIC into 10ml of IP Buffer and mix.

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

2.6. Pellet the beads by centrifuging for 2 minutes at 4000rpm at 4°C.

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

2.8. Remove the supernatant with a 200µl pipette. Avoid disturbing the beads when discarding supernatant.

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

2.10. Pellet beads by centrifuging for 2 minutes at 4000rpm at 4°C.

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

2.12. Remove the supernatant with a 200ul pipette. Avoid disturbing the beads when discarding supernatant.

2.13. To the beads, add 1ml IP Buffer and PIC mix gently,

7.5µl of 10mg/ml Salmon Sperm DNA/160µl bead mix
20µl of 10mg/ml BSA/160µl bead mix

Adjust based on the same ratio if different volume of beads is used.
Rotate on rotator for 3hrs at 4°C.

Note: During the 3hrs of blocking of beads, continue with lysing and sonication of cells as described in step 3.

2.14. Pellet beads as described above.

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

2.16. Store slurry on ice until ready to use.

3. Lysing of cells

3.1 Dissolve 1 Complete-Mini PIC tablet in 10ml of ChIP Lysis Buffer and mix.

3.2 Thaw out cell pellet (~1x10e8 cells are good for ~ 7-8 IPs if each IP is with 100µg chromatin). The following procedure is based on using 1x10e8 cells. For different number of cells, adjust ratio of reagents needed.

3.3 Add 2ml of ChIP Lysis Buffer + PIC and pipette up and down to mix.

3.4 Incubate on ice for 30minutes.

3.5 Transfer cells into two 2ml tubes. (1ml of sample into one 2ml tube, use more tubes if there are more than 2ml of samples)

3.6 Pass cells through 1ml syringe (with 26 gauge) 5-6 times and spin at 5000rpm for 10minutes at 4^{o}C.

3.7 Carefully remove supernatant and resuspend each pellet with ChIP Lysis Buffer + PIC to a final volume of 250µl per pellet.

4. Sonication

4.1. Connect either end of coaxial High Frequency Cable to SHV connector on rear panel of generator, and connect other end of cable to 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. Fill the cup with water and a little bit of ice to the black mark on the cup horn.

4.5. Turn generator on by pressing "I" on Power Switch, switch will illuminate when power is on.

4.6. Press CLEAR to select Programmed Mode

4.7. Press PROG?DATA to select programmed mode; Display will show 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 (10minutes) and press enter.

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

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

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

4.12. Secure sample (2.0ml tube) in the cup horn by using a floating tube holder. Sit the sample right on top of the hole on the radiating surface of the cup horn.

4.13. Press start to begin processing sample as programmed. Adjust amplitude setting to "7".

4.14. Aspirate water out and replenish more ice to maintain water level at the black mark during the process when the sonicator is in the pulse off time. Ensure that the sample remains in upright position above the hole on the radiating surface all the time.

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

4.16. Return amplitude setting to "0".

4.17. Sonicate the rest of the samples as described above.

4.18. Once sonication is done, return amplitude setting to "0". Press "O" on the Power Switch to turn generator off.

4.19. Disconnect cable from SHV connector on the convector.

4.20. Spin samples at 14000rpm for 12min at 4^{o}C.

4.21. Transfer supernatant to fresh 1.5ml non-stick tube. Pool supernatant from the same crosslinked sample together.

4.22. Aliquot 5ul of chromatin into 195ul of Ultrapure water for reverse-crosslinking QC check of the quantity and size of the sonicated chromatin. Proceed to section 5.

4.23. Store the remaining of the sample 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 30minutes on the thermomixer.

5.3. Briefly spin down tube and let cool to room temperature.

5.4. Add 1ul of Proteinase K (20µg/µl).

5.5. Incubate at 42°C for 30minutes.

5.6. Spin down 2ml phase lock gel tubes at 14000rpm for 1min 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 14000rpm for 5min at room temperature.

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

5.10. Centrifuge at 14000rpm for at least 20minutes at 4°C.

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

5.12. Wash pellet with 1ml 70% EtOH. Spin at 14000rpm for 5min at 4°C.

5.13. Remove and discard supernatant.

5.14. Repeat 70% EtOH wash one more time.

5.15. Allow pellet to dry.

5.16. Resuspend pellet in 20µl of DEPC water.

5.17. Run on Agilent DNA 1000 assay to check fragment sizes. If no fragment is between 100-300bp, discuss with supervisor for further sonication.

5.18. Run on nanodrop to determine concentration.

6. Preclearing (Day 2)

6.1. Add 40µl of the blocked bead mix slurry to the pooled chromatin fraction 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 4000rpm for 2min at 4°C. Transfer supernatant carefully into new 1.5ml non-stick tube.

6.3. Spin the supernatant at 4000rpm for 2min at 4°C to ensure all beads have been removed.

6.4. Aliquot out 20µl for Input fraction into a 1.5ml tube. Store Input fraction at 4°C.

7. IP

7.1 Based on the nanodrop quant, set up IPs in non-stick 0.6ml tubes.

7.2 Aliquot out the amount of pre-cleared chromatin needed based on the nanodrop quant into non-stick 0.6ml tubes. Top up to 200ul final volume with IP buffer + PIC.

7.3 Add the corresponding antibody. Parafilm tubes and rotate at 4°C for 1 hr.

7.4 Add 20µl of ProteinA/G prepared slurry to each IP. Parafilm tubes and rotate at 4°C overnight.

8. Washing (Day 3)

8.1 Centrifuge samples at 4000rpm for 2min at 4°C. Let beads fully settle by placing on a rack for 30seconds and remove supernatant with P200 pipette. Avoid disturbing beads.

Note: All subsequent washes should be performed as described above.

8.2 Add 400µl of ChIP Wash buffer and vortex **gently** for 10sec. Rotate beads at 4°C for 3min.

8.3 Wash one more time with ChIP Wash Buffer and then once with Final ChIP Wash Buffer.

8.4 Before centrifuging samples in Final ChIP Wash Buffer, transfer IPs to new 1.5ml non-stick tubes. Centrifuge and remove supernatant as described above.

9. Elution and Reverse Crosslinking

9.1 Retrieve Input DNA from -20°C freezer. Add 180µl of Elution Buffer to Input DNA to bring up to 200µl final volume.

9.2 Add 100µl Elution Buffer to IP samples (which have just the beads).

9.3 Add 10µl of 0.5µg/µl RNase (Roche) to all samples, including the Input DNA. Incubate all samples including the input DNA in a thermomixer at 68°C for 2hrs with mixing set at 1000rpm, mixing on for 30secs and off for 1min.

9.4 For the IP samples (not the Input DNA sample), spin down at 4000rpm for 2min. Let beads fully settle by placing on a rack for 30seconds. Transfer supernatant into 1.5ml tubes.

9.5 Repeat elution of beads by adding another 100µl of Elution Buffer to the beads. Incubate at 68°C for 5 min.

9.6 Remove all samples (including the Input DNA) from the thermomixer. Pool supernatant of the same sample together (ie. from the second round of elution with the first round of elution). All samples should be around 200µl each (including the Input DNA sample). Discard tubes with bead mix.

10. Purification of DNA

10.1 Purify DNA on Qiaquick columns using the QIAquick PCR Purification Kit.

10.2 Follow the Qiagen protocol with the changes listed below.

10.3 Perform the second spin for 2 minutes instead of one after the PE wash.

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

10.5 Let the column air dry for 1 minute before adding buffer EB (Qiagen).

10.6 Heat the elution buffer to 50 degrees.

10.7 Add 30 µl of EB to the centre of the column and let sit for 1 minute prior to centrifugation.

10.8 Wipe down work bench and small equipment with DNAway.

Note: QC check such as Qubit quant and/or qPCR will need to be done on samples.

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Appendix A

Reagent	Final Concentration
ChIP Lysis Buffer	
Tris-HCl (pH 8.0)	50mM
SDS	1%
EDTA	10mM
Complete-Mini PIC tablet from Roche	Refer to protocol
IP Buffer	
Tris-HCl (pH 8.0)	10mM
Triton X-100	1%
Deoxycholate	0.1%
SDS	0.1%
NaCl	90mM
EDTA	2mM
Complete-Mini PIC tablet (EDTA Free) from Roche	Refer to protocol
ChIP Wash Buffer	
Tris-HCl (pH 8.0)	20mM
SDS	0.1%
Triton X-100	1%
EDTA	2mM
NaCl	150mM
ChIP Final Wash Buffer	
Tris-HCl (pH 8.0)	20mM
SDS	0.1%
Triton X-100	1%
EDTA	2mM
NaCl	500mM
Elution Buffer	
$NaHCO_3$	100mM
SDS	1%

Appendix B

LIMS for ChIP

1. Scan in SLX-gDNA plate #.
2. Save as new tube set.
3. Click on Start Protocol, choose SLX-ChIP and click on "continue prep".
4. Enter "SLX-ChIP" as pipeline. Transfer 1.5mL tube.
5. Enter the Equ ID of the instrument that was used for sonication (Sonicator = Equ206) and the sonication time.
6. Skip the "Micrococcal Nuclease Digestion" step.
7. Enter all applicable antibody solution number and attribute fields.
8. Enter the following information if known: Amount of chromatin used per IP, Name of the ChIP protocol, Number of IPs pooled, Fold Enrichment, DNA concentration (ng/ul), Number of Cells per IP, Bead Type, Bead Amount and Sonicator Type.
9. Decant to set volume of ChIP sample to "0".
10. Enter the solution number and the volume of EB that the ChIP sample was eluted in. Scan ChIP sample into a rack.