Non Controlled Version

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

Crosslinking of Frozen or Fresh Tissue

I. Purpose

To crosslink small pieces of frozen or fresh tissue with formaldehyde immediately prior to a ChIP experiment.

II. Scope

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

Document Title	Document Number
N/A	N/A

VI. Related Documents

Document Title	Document Number
Chromatin Immunoprecipitation (ChIP) Validation of Antibodies for ChIP	LIBPR.0015 LIBPR_Work Inst.0024

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.

VIII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
Fisherbrand Textured Nitrile gloves	Fisher Scientific	270-058-53		✓
Ice Chest	Igloo	PM PAL BLUE		✓
Wet ice	In house	N/A	N/A	N/A
DNA away	Molecular Bioproducts	7010		✓
15ml Conical Tubes	VWR	CA21008-918		✓
50ml Conical Tubes	VWR	CA21008-940		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Diamond Filter Tips 1000ul	Mandel	GF-F171703		✓
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		✓
Centrifuge, Eppendorf 5417R, refrigerated high-speed, 115V	Fisher Scientific	5417R	✓	
SERUM-FREE tissue culture media	GIBCO	11995-065		✓
5ml serological pipettes	Fisher Scientific	CS004487		✓
10ml serological pipettes	Fisher Scientific	CS004488		✓
25ml serological pipettes	Fisher Scientific	CS004489		✓
50ml serological pipettes	Fisher Scientific	CS004490		✓
37% Formaldehyde	Sigma	F8775-4X25ML		✓
1x PBS, pH 7.4, 500mL	Invitrogen	10010-023		✓
UltraPure Water (Rnase/Dnase free)	Invitrogen	10977-023		✓
Glycine SigmaUltra, >99% titration (250g)	Sigma-Aldrich	G7403-250G		✓
Complete-Mini EDTA-free Protease Inhibitor Cocktail Tablet	Roche Diagnostics	04 693 124 001		✓
ChIP Lysis Buffer	In-House	N/A		
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓
100x15mm Fisherbrand Petri Dishes with Clear Lids	Fisher Scientific	08-757-12		✓
Single Edge Industrial Razor Blades	VWR	55411-050		✓
T36 Disinfex Disinfectant Cleaner Spray	VWR	CA26200-152		✓
Closed Tissue Grinder System 15mL	Fisher Scientific	0254209		✓

IX. Procedure

1. Blood Room Preparation

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

1.2. Turn on the blower in the BSC (Biological Safety Cabinent) and allow the air to circulate for 15-20 minutes.

1.3. Spray and wipe down the entire hood with T36 Disinfectant.

ChIP Room

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

1.5. Wipe down the work bench, small equipment, and ice bucket with DNA away and 80% Ethanol.

1.6. Change gloves.

1.7. Retrieve fresh ice and all required reagents.

1.8. Fast cool the 4ºC centrifuge.

2. Preparing solutions for cross-linking

Prepare fresh Stop Fix (1% of 125mM glycine solution) and FIX (1% formaldehyde) as follows:

2.1. Stop-Fix (250mM): Prepare in the ChIP room. Combine 1mL of stock Stop-Fix solution (2.5M glycine solution) with 9mL of 1X PBS. Mix well and leave at room temperature. Scale up depending on the number of samples to be processed. 5mL of STOP-FIX is needed per sample.

2.2. FIX (1% formaldehyde): Prepare in the fumehood in the 5th floor RNA area. Aliquot 405µL of 37% formaldehyde to 14.6mL of SERUM-FREE tissue culture media in a 15mL tube. Mix by inverting the tube and bring the tube to the 6th floor Blood Room BSC. Spray and wipe down the FIX tube with 70% EtOH before bringing the FIX tube into the BSC. Scale up depending on the number of samples. 5mL of FIX is required per sample.

2.3. Place a 50mL tube of 1x PBS on ice in the ChIP room.

3. Cross-linking of Tissue

3.1. Ensure that the BSC has been completely wiped down before samples are retrieved. Pre-label 15mL Tissue Grinder conical tubes with sample ID, petri dishes and transfer a few new sterile razor blades into the hood.

3.2. Retrieve the tissue and quickly transport the tissue on dry ice to the BSC.

3.3. Transfer the piece of tissue to a small sterile petri dish. Immediately add enough FIX to coat the piece of tissue. Record the amount of FIX added to the tissue. Ideally 3mL should be enough FIX to cover the piece of tissue.

3.4. Set timer for 10 minute incubation. While incubating, use a disposable, sterile razor blade to chop up the piece of tissue as much as possible, as quickly as possible. Top up with remaining 2mL of FIX.

3.5. Transfer the minced tissue to the prelabeled Tissue Grinder tube using p1000 pipettor with a cut tip.

3.6. Cap the tube with the grinder and gently grind the tissue by twisting and turning the grinder handle. Grind the tissue until the suspension appears cloudy and the tissue is not breaking up anymore. Discard the grinder in the biohazard waste. Secure the regular cap on the tube.

3.7. Invert the FIX and tissue mixture until the 10 minute incubation is complete. It is now safe to take the tube out of the BSC and complete the remaining of the protocol on the ChIP bench.

3.8. Stop the fixation reaction by adding the same volume of Stop-Fix solution as FIX solution to the tube. That is add 1:1 v/v FIX: Stop-Fix. The final concentration of the Stop-Fix in the tube should be 125mM.

3.9. Incubate for 5 minutes at room temperature with gentle inversion of the tube.

3.10. Pellet the cells by centrifugation for 5 minutes at 4000rpm at 4ºC.

3.11. Decant the supernatant into the formaldehyde waste bucket located in the fumehood of the Library Construction room and invert the tube on to a kimwipe. Ensure the pellet does not become dislodged while decanting.

3.12. Add 5mL of ice-cold PBS to the tube.

3.13. Dislodge the cell pellet by finger flicking to thoroughly wash the pellet.

3.14. Pellet the cells by centrifugation for 5 minutes at 4000rpm at 4^{o}C.

3.15. Decant the supernatant and invert the tube on to a kimwipe. Ensure the pellet does not become dislodged while decanting.

3.16. Add the appropriate amount of ChIP Lysis buffer and PIC and pipette up and down to dislodge the pellet.

3.17. Proceed with the ChIP SOP LIBPR.0015.

3.18. Record the sample ID and the crosslinking time on the ChIP Crosslinking worksheet tab, located in R:\Library Core\Work Sheets and Calculators\ChIP\IP\ChIP_Worksheet.

Appendix

1. The ChIP Solutions are prepared in batches. Once one solution from the batch is empty, a new batch of solutions is prepared and validated by ChIP. The stock stop-fix solution is a part of the batch solutions. The recipes for the solutions are located in: R:\Library Core\Work Sheets and Calculators\ChIP\IP\ChIP Worksheet.

2. Stock Stop-Fix (2.5M glycine solution): Prepare in the chemical prep room. Combine 46.9 grams of Glycine Buffer and 250ml ultrapure in a 500mL bottle. Filter sterilize. Mix well and leave at room temperature.