Crosslinking of Frozen Cell Pellet

I. Purpose

To crosslink cell pellets with formaldehyde immediately prior to a ChIP experiment.

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
N/A	N/A

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 Sigma Ultra >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	N/A	N/A
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓

IX. Procedure

ChIP Room

1. Retrieval of reagents and equipment preparation

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 and 80% Ethanol.

1.3. Change gloves.

1.4. Retrieve fresh ice and all required reagents.

1.5. Fast cool the 4ºC centrifuge.

2. Preparing solutions for crosslinking

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

2.2. 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.

2.3. 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 up to the 6th floor ChIP room. Scale up depending on the number of samples. 5mL of FIX is required per sample.

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

3. Cell Transfer

3.1 Quickly transport the vial of cells from the -80ºC freezer on the 5th floor to the 6th floor ChIP room. The cells should be contained in a 15mL tube.

4. Crosslinking

4.1. Add 5mL of FIX Solution immediately to the cell pellet.

4.2. Incubate for 10 minutes at room temperature with gentle inversion of the tube.

4.3. Stop the fixation reaction by adding 5mL of Stop-Fix solution to the tube.

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

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

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

4.7. Add 5mL of ice cold 1X PBS to the tube.

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

4.9. Pellet the cells by centrifugation for 5 minutes at 4000rpm at 4°C.

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

4.11. Add ChIP Lysis buffer and PIC solution made in-house and pipette up and down to dislodge the pellet.

4.12. Proceed with the ChIP SOP LIBPR.0015.

4.13. 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 is located in: R:\Library Core\Work Sheets and Calculators\ChIP\IP\ChIP Worksheet.

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