Non Controlled Version

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Harvesting Cell Lines for DNA/RNA Extraction and ChIP

I. Purpose

To provide details regarding the preparation and storage of harvested cells for DNA, RNA extractions and Chromatin Immunoprecipitation (ChIP) protocols.

II. Scope

All procedures are applicable to the BCGSC Library Core Group and Library Construction TechD 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 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

Reference Title	Reference Number
N/A	N/A

VI. Related Documents

Document Title	Document Number
N/A	N/A

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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 - large	Fisher Scientific	270-058-53		✓
wet ice	In house	N/A	N/A	N/A
Ice bucket	Fisher	11-675-58		✓
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		✓
Diamond Filter Tips 10µl	Mandel	GF-F171203		✓
Diamond Filter Tips 30µl	Mandel	GF-F171303		✓
Diamond Filter Tips 200µl	Mandel	GF-F171503		✓
Diamond Filter Tips 1000µl	Mandel	GF-F171703		✓
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		✓
2ml tubes	Diamed	PRE-2000N		
Ultra Pure Water (Rnase/Dnase free)	Invitrogen	10977-023		✓
Protease Inhibitor Cocktail	Active Motif	37490		✓
Rnase-free Non-stick tubes, 1.5ml,	Ambion	AM12450		✓
VX-100 Vortex Mixer	Rose Scientific	S-0100	✓	
Liquid Nitrogen	Praxair	NI M-FILLTR		
Phosphate Buffered Saline (PBS)	Invitrogen	10010-023		✓
100mM PMSF	Sigma-Aldrich	93482-50ML-F		✓

Microscope(Inverted)	VWR	782132	✓	
Hemacytometer	Fisher Scientific	02-671-5		✓
Trypan Blue Stain	Invitrogen	15250-061		✓
Trypsin-EDTA	Stem Cell Technologies	07901		✓
Cell Scraper	VWR	CA15621-005		✓

IX. Procedure

1. Preparation of Buffers/Reagents

1.1. Wash buffer is prepared fresh and kept on ice for every harvest. Discard after use.

1.2. Scale up recipe depending on the number of 15mL conical tubes to be harvested. Follow the recipe listed in Table 1.

Table 1.: Preparation of Wash Buffer for 1x 15mL Conical tube

Reagent	Volume to Add
1x PBS	5mL
100mM PMSF	25ul

2. Count Cells

2.1. Following standard tissue culture practices and harvesting guidelines of specific cell line, harvest 1 plate worth of cells for a cell count. If working with an adherent cell line, it is ok to trypsinize the cells for the count. DO NOT use these cells for purposes of this SOP.

2.2. Record the cell count (cells/mL) and total amount of cells per tissue culture vessel.

2.3. Determine the number of flasks/plates needed to harvest for extractions as per Table 2.

2.4. If working with a suspension cell line, proceed to Step 4.

Table 2: Guidelines for Harvesting Cell Lines for Extractions and ChIP

Extraction Type	Number of Cells Required	Storage Vessel Type	Storage Medium
DNA	10 million	15mL conical	PIC+PMSF

RNA	10 million	15mL conical	PIC+PMSF
ChIP (chromatin)	20 million	15mL conical	PIC+PMSF
Back Up	10 million	15mL conical	PIC+PMSF

3. Harvesting Adherent Cell Lines

*******It is important to proceed quickly yet thoroughly*******

3.1. Cells that adhere to the bottom of the tissue culture vessel need to be physically removed off of the bottom of the vessel using a cell scraper. It is easier to scrap cells thoroughly and efficiently from a plate rather than a flask in this case.

3.2. Remove the media off of the cells (this ensures all non viable cells are not harvested) and add ~4-5mL of cell line specific media to the vessel.

3.3. Using a cell scraper, scrape the plate bottom until all cells have been removed. Transfer the cell/media slurry to a 15mL conical tube.

3.4. Rinse and wash the tissue culture vessel with 3-4mL of media to collect remaining cells. Ensure the number of cells/conical tube is reflective of the guidelines listed on Table 2. Adjust cell+media transfer if necessary.

3.5. Note the cell count on the 15mL conical tube.

3.6. Keep tube on ice if harvesting multiple tubes.

3.7. Proceed to Step 5.

4. Harvesting Suspension Cell Lines

4.1. Transfer cell suspension to a 15mL conical tube. Ensure the number of cells/conical tube is reflective of the guidelines listed on Table 2. Adjust cell+media transfer if necessary.

4.2. Note the cell count on the 15mL conical tube.

4.3. Keep tube on ice if harvesting multiple tubes.

5. Washing and Pelleting of Cells

*******It is important to proceed quickly yet thoroughly*******

5.1. Once cells have been transferred to 15mL conical tubes, spin down at 4000rpm for 5min at 4°C.

5.2. It is now ok to work outside of the BSC.

5.3. Decant the media as much as possible.

5.4. Add Wash Buffer (1X PBS + PMSF) to the conical tube. Invert tube a few times to ensure complete washing of the pellet.

5.5. Spin down at 4000 rpm for 5min at 4°C.

5.6. Decant Wash Buffer as much as possible.

5.7. To the pellet add 1ul of PIC (Protease Inhibitor Cocktail) and 1ul of 100mM PMSF.

5.8. Keep tubes on ice in preparation for snap freezing.

6. Snap Freezing of Cell Pellets and Storage

6.1. Wear the proper protective gear including a full face shield and gloves to handle the liquid nitrogen carefully.

6.2. Transfer a small amount of liquid nitrogen to a Styrofoam or any other liquid nitrogen safe container. Ensure there is enough liquid nitrogen to submerge at least the bottom ¼ of a 15mL conical tube. It is up to the discretion of the technician to handle 1 tube at a time or multiple.

6.3. Submerge the bottom of the 15mL conical tube into the liquid nitrogen. Snap freezing should be instantaneous but 'incubate' the tube in the liquid nitrogen approximately 20-30 seconds to ensure complete snap freezing.

6.4. Immediately transfer the tube to a -80°C freezer for long term storage.