Non Controlled Version

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

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Crosslinking Adherent Cell Lines

I. Purpose

To crosslink adherent cell lines using formaldehyde.

II. Scope

All procedures are applicable to the BCGSC FG-Library Construction Core.

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

SOP/Reference Title	SOP/Reference Number
N/A	N/A

VI. 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.

VII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
Fisherbrand Textured Nitrile gloves - large	Fisher Scientific	270-058-53		✓
Ice Chest	Igloo	PM PAL BLUE		✓
wet ice	In house	N/A	N/A	N/A
1.5 ml Eppendorf tube	Ambion	12400		✓
15ml Conical Tubes	VWR	CA21008-918		✓
50ml Conical Tubes	VWR	CA21008-940		✓

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 ul	CLP	Bt10XL		✓
Neptune barrier tips 20 ul	CLP	Bt20		✓
Neptune barrier tips 200 ul	CLP	Bt200		✓
Neptune barrier tips 1000 ul	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		✓
2ml serological pipettes	Fisher Scientific	CS004486		✓
5ml serological pipettes	Fisher Scientific	CS004487		✓
10ml serological pipettes	Fisher Scientific	CS004488		✓
25ml serological pipettes	Fisher Scientific	CS004489		✓
50ml serological pipettes	Fisher Scientific	CS004490		✓
Portable Pipet Aid, Multispeed XP, rechargeable	Fisher Scientific	13-681-15E		✓
Formaldehyde	Sigma	F8775-4X25ML		✓
D-MEM high glucose(1X), liquid w/sodium pyruvate, w/L-glutamine	Invitrogen	11995-073		✓
1x PBS, pH 7.4, 500mL	Invitrogen	10010-023		✓
Ultra Pure Water (Rnase/Dnase free)	Invitrogen	10977-023		✓
10X Glycine Buffer	Active Motif	ChIP-IT Kit		✓
100mM PMSF	Sigma-Aldrich	93482-50ML-F		✓
PIC	Active Motif	ChIP-IT Kit		✓
Microscope (Inverted)	VWR	782132	AE21	
Hemacytometer	Fisher Scientific	02-671-5		✓
15cm tissue culture dish	Fisher Scientific	08-772-24		✓
Trypsin-EDTA	StemCell	07901		✓
Trypan Blue Stain	Invitrogen	15250-061		✓
Cells scraper	VWR	CA15621-005		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓

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VIII. Procedure

Tissue Culture 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 DNAway.

1.3. Change gloves.

1.4. Retrieve fresh ice and all required reagents.

2. Preparing solutions for crosslinking

2.1. When cells are ready to be harvested, prepare fresh Glycine Stop-Fix Solution and Cell Scraping solution as follows.

Note: The following solutions are good for crosslinking **three** tissue culture dishes with **15cm diameter** each. Please adjust the volume based on the following ratio for different number of plates.

2.2. Glycine Stop-Fix Solution: Combine 1ml of stock Stop-Fix solution (2.5M glycine solution) with 9mL of 1X PBS. Mix well and leave at room temperature.

2.3. Cell Scraping Solution: Add 600µl 10X PBS to 5.4ml dH_2O, mix and place on ice. Just before use (in step 4.9 below), add 30µl of 100mM PMSF.

2.4. Place a 50ml tube of 1X PBS and Cell Scraping Solution and PMSF in an ice chest.

3. Reagents and equipment preparation

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

3.2 Wipe down the tissue culture hood, small equipment and microscope with 70% EtOH.

3.3 Prepare fresh Fixation Solution by adding 1.62ml of 37% formaldehyde to 60ml minimal cell culture medium and mix thoroughly. The final concentration of formaldehyde should be ~1%. Leave at room temperature.

3.4 Retrieve 15cm plates that are ready for harvesting (~70-90% confluency) from incubator.

Note: If a cell count is needed, proceed to Step3.5; otherwise, proceed to Section 4.

3.5 Do a cell count on a plate that won't be used in crosslinking later on by following step 3.6 to step 3.12.

3.6 Pick a plate which has the confluency that represents the average confluency of all plates that will be crosslinked.

3.7 Aspirate medium. Trypsinize cells with 1ml of trypsin to lift cells off of the plate, incubate at 37^oC if needed.

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3.8 Resuspend cells in 5ml of medium.

3.9 Take 20µl of samples and add 20ul of Trypan blue stain to the sample in a 0.5ml tube. Mix.

3.10 Add 10µl of the stained sample into the hemacytometer.

3.11 Count four 4x4 grids and take an average of one 4x4 grid.

3.12 Calculate cell counts by using the following formula:

Average of one 4x4grid x 2 x 10^4 cells/ml x 5ml = total number of cells on that plate

Note: 2 = the dilution factor
5ml is the volume that the cells were resuspended in

4. Crosslinking

4.1. Aspirate medium off the plates and add 20ml Fixation Solution to each plate.

4.2. Incubate for 10minutes at room temperature with occasional shaking.

4.3. Aspirate Fixation Solution off the plates and wash by adding 10ml of ice-cold PBS to each plate.

4.4. Rock the plate back and forth for 5 seconds and then aspirate off the PBS.

4.5. Stop the fixation reaction by adding 10ml Glycine Stop-Fix solution to each plate.

4.6. Swirling to cover the plate and then incubate at room temperature for 5 minutes with occasional shaking.

4.7. Aspirate the Glycine Stop-Fix solution off the plates and wash by adding 10ml of ice-cold PBS to each plate.

4.8. Rock the plate back and forth for 5 seconds and then aspirate off the PBS.

4.9. Add 30µl of 100mM PMSF to 6ml of Cell Scraping Solution. Adjust the volume of PMSF using the same ratio if different amount of Cell Scraping Solution was prepared. Mix by inverting several times.

4.10. Add 4ml of ice cold Cell Scraping Solution + PMSF to each plate and scrape cells with a rubber scraper. Hold the plate at an angle and scrape the cells down to collect them at the bottom edge of the plate.

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4.11. Use a 2ml pipette to transfer the cells to 15ml conical tubes on ice. Pool two to three plates of cells into one 15ml conical tube.

4.12. Pellet cells by centrifugation for 10minutes at 2500rpm at 4°C.

4.13. Aspirate off the supernatant. Put cells on ice in the ice-chest.

4.14. Wipe down tissue culture hood, small equipment and microscope with 70% EtOH.

4.15. Bag all tissue culture waste and bring them up to the 6th floor for autoclaving.

4.16. Retrieve PIC and PMSF from the $^{-}$20°C freezer. Add 1μl 100mM PMSF and 1μl PIC to each pellet. (1μl of each of PMSF and PIC is good for 4.5x10e7 cells, use more if there are more cells per 15ml conical tube).

4.17. Freeze cell pellets down at $^{-}$80°C on the 5th floor.

4.18. Wipe down work bench and small equipment with DNAway.

4.19. Record cell number on the “harvest” spreadsheet in the tissue culture folder.

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