Bionano Prep Methanol Glacial Acetic Acid Fixed Cell Preparation Technical Note

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Disclaimer

The Bionano Prep Methanol Glacial Acetic Acid Cell Preparation Tech Note is not a fully validated protocol. The technique has only been used to isolate UHMW gDNA from the Epstein-Barr virus (EBV) immortalized human lymphoblastoid cell line (GM12878) that has undergone a hypotonic swell and fixation, as described here, for the Bionano Saphyr system. For other cell types, please refer to the *Bionano Prep SP Fresh Cells DNA Isolation Protocol v2* (P/N 30396) or the *Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol v2* (P/N 30398).

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Legal Notice

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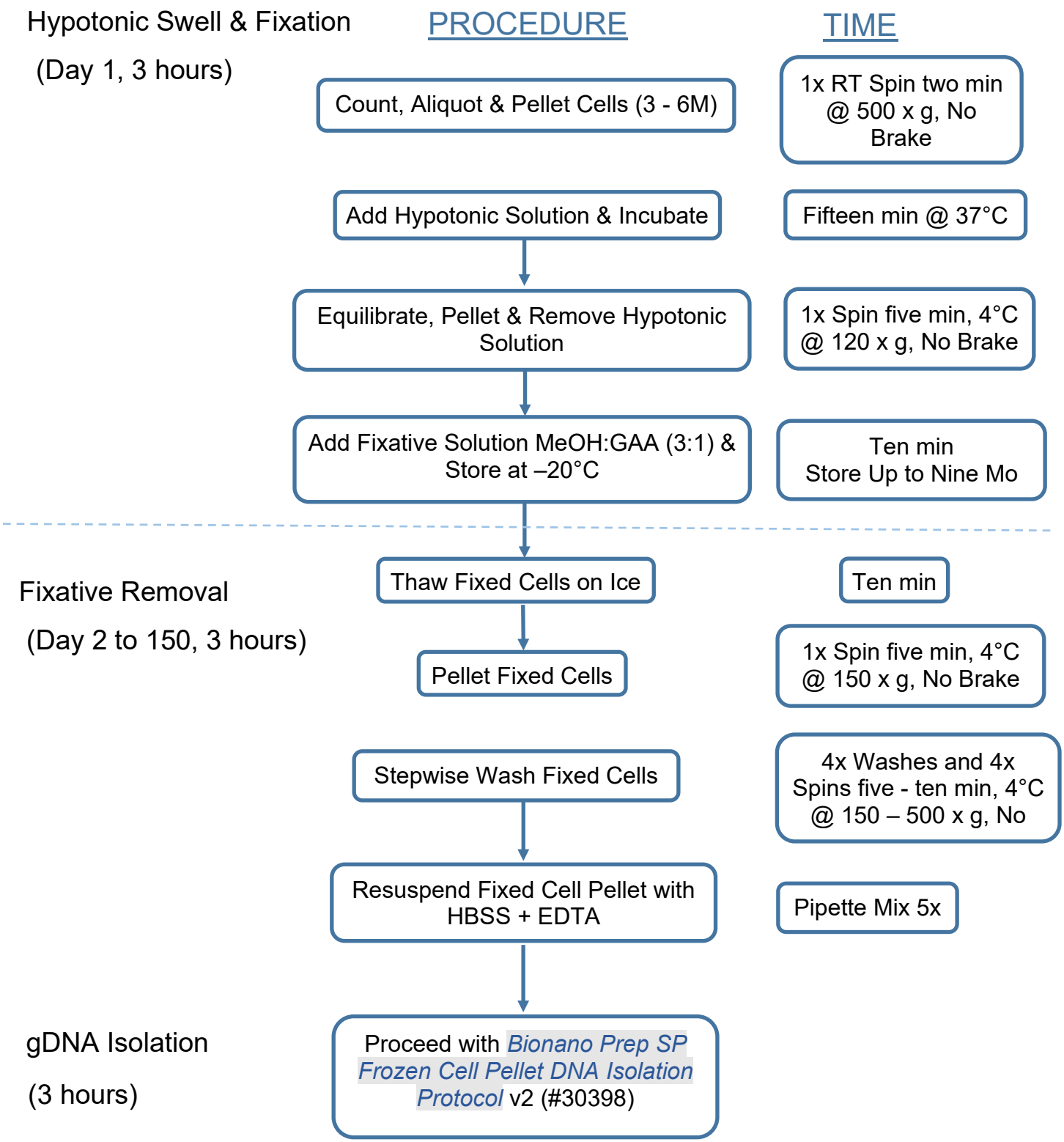
Revision History

Revision	**Release Date**	Notes
A	01/27/2023	Tech Note Release

Workflow Overview



Bionano Prep Methanol Glacial Acetic Acid Reagents and User-Supplied Materials

Table 1: Bionano Prep Methanol Glacial Acetic Acid Cell Preparation uses Bionano Prep SP Blood and Cell DNA Isolation Kit v2 reagents (Part # 80042, ten preps)

Item	Amount	Part Number	Storage
4 mm Nanobind Disks	10 disks	20402	Room Temp (15-25°C)
Protein LoBind Microcentrifuge Tubes, 1.5 ml	10 tubes	20422	Room Temp (15-25°C)
Protein LoBind Microcentrifuge Tubes, 0.5 ml	10 tubes	20421	Room Temp (15-25°C)
RNase A Enzyme	200 µl	20373	Refrigerate (2-8°C)
DNA Stabilizer	350 µl	20423	Room Temp (15-25°C)
Standard Microfuge Tubes, 2.0 ml	10 tubes	20396	Room Temp (15-25°C)
Cell Buffer	50 ml	20374	Room Temp (15-30°C)
Proteinase K Enzyme	0.5 ml	20372	Room Temp (15-25°C)
Lysis and Binding Buffer (LBB)*	2.5 ml	20375	Room Temp (15-25°C)
Wash Buffer 1 Concentrate (2.5X) (WB1)*	3.25 ml	20376	Room Temp (15-25°C)
Wash Buffer 2 Concentrate (2.5X) (WB2)	5 ml	20377	Room Temp (15-25°C)
Elution Buffer (EB)	1.1 ml	20378	Room Temp (15-25°C)
Magnetic Disk Retriever Plastic Sheath	10 each	20381	Room Temp (15-25°C)

* See Important Notes section for hazardous waste information

Table 2: User-Supplied Materials

Item	Supplier	Catalog #
Day 1 – Hypotonic Swell and Fixation		
Biosafety Cabinet	General Lab Supplier	
Hemocytometer and Microscope, or Automated Cell Counter	General Lab Supplier	
2M KCl	Invitrogen	AM9640G
ddH_2O	General Lab Supplier	
Water bath	General Lab Supplier	
Methanol, HPLC Grade	Sigma Aldrich or Equivalent	34860
Glacial Acetic Acid, ASC Grade	Thermo Fisher or Equivalent	S25118
Refrigerated Centrifuge, Swinging Bucket Rotor for 15 ml Conical Tubes	General Lab Supplier	
Disposable Transfer Pipettes	Thermo Fisher or Equivalent	13-711-9AM
Conical Centrifuge Tubes, 15 ml, PP	Thermo Fisher or Equivalent	05-539-12
Serological Pipette Controller and Serological Pipettes	General Lab Supplier	
Ice bucket and ice	General Lab Supplier	
Fume Hood	General Lab Supplier	
10% Bleach	General Lab Supplier	
Disinfectant Concentrate, TexQ TX651	Texwipe	TX651
Liquid Hazardous Waste Container	General Lab Supplier	
Day 2 to 150 – Fixative Removal, Pelleting, gDNA Isolation and Homogenization		
Hank's Buffered Salt Solution (HBSS), no calcium, no magnesium	Thermo Fisher or Equivalent	14170-112
Fetal Bovine Serum, heat inactivated, certified, One Shot™	Thermo Fisher or Equivalent	A3840001
0.5M Ethylenediaminetetraacetic acid (EDTA) pH 8.0, Molecular Biology Grade	General Lab Supplier	
Disposable Transfer Pipettes	Thermo Fisher or Equivalent	13-711-9AM
Serological Pipette Controller and Serological Pipettes	General Lab Supplier	
Bionano Prep SP Magnetic Retriever (2 pack)	Bionano Genomics	80031
FNalgene™ Rapid-Flow™ Sterile Single Use Vacuum Filter Units (optional)	Thermo Fisher	564-0020
DynaMag-2 Magnetic Tube Rack	Thermo Fisher	12321D

Item	Supplier	Catalog #
HulaMixer Sample Mixer	Thermo Fisher	15920D
Biosafety Cabinet	General Lab Supplier	
Fume Hood	General Lab Supplier	
Phenylmethylsulfonyl Fluoride Solution (PMSF),100 mM	Sigma-Aldrich	93482
Ethanol, 200 Proof, Molecular Biology Grade	Sigma-Aldrich	E7023
Isopropanol (IPA), ≥ 99.5%, Molecular Biology Grade	Fisher Scientific	A461-212
Disinfectant Concentrate, TexQ TX651	Texwipe	TX651
Conical Centrifuge Tubes, 50 ml, Polypropylene	Thermo Fisher or Equivalent	14-432-22
Conical Centrifuge Tubes, 15 ml, Polypropylene	Thermo Fisher or Equivalent	05-539-12
Refrigerated Centrifuge, Swinging Bucket Rotor for 15 ml Conical Tubes	General Lab Supplier	
Ice Bucket and Ice	General Lab Supplier	
Sterile 5- and 10-ml Disposable Pipettes (TD+)	General Lab Supplier	
Mini Benchtop Microcentrifuge (2,000 x g spin)	Cole-Parmer or Equivalent	EW-17701-11
Pointed Forceps	Electron Microscopy Sciences, or Equivalent	78141-01
Wide-Bore Pipette Tips, Filtered, Aerosol, 200 µl	VWR or Rainin Equivalent	46620-642
Extra Long 1000 µl Tips, Sterile	VWR or Equivalent	76322-154
Pipettes (10, 20, 200, and 1,000 µl) and Nuclease Free, Filtered Pipette Tips	General Lab Supplier	

Introduction and Important Notes

1. INTRODUCTION

1.1. The purpose of this document is to provide a method for preparing methanol fixed cells for isolation of ultra-high molecular weight (UHMW) gDNA. This technique can be used for 3 – 6 million (M), 3:1 Methanol:Glacial Acetic Acid (MeOH:GAA) fixed cells that have been immediately stored at -20°C after fixation for up to nine months. It is intended for extraction in batches of up to six samples (three samples for novice users) within six hours. This tech note is different from the current *Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol* (P/N 30398), which does not support UHMW gDNA isolation from MeOH:GAA fixed cells. Following a recommended fixation protocol consistent with techniques used in various cytogenetic labs, this tech note utilizes a stepwise washing method for fixative removal prior to a lysis, bind, wash, and elute procedure common in silica-based gDNA extraction technologies in combination with a novel paramagnetic disk. Unlike magnetic beads and silica spin columns, which shear large gDNA, the Nanobind Disk binds and releases gDNA with significantly less fragmentation, resulting in UHMW gDNA. High gDNA binding capacity is the result of novel nano-structured silica on the outside of the thermoplastic paramagnetic disk. This procedure has been used to process the Epstein-Barr virus (EBV) immortalized human lymphoblastoid cell line (GM12878) that grows in suspension culture after undergoing the process of hypotonic swell and fixation, and the gDNA prepared has been evaluated with the Bionano Direct Label and Stain (DLS) labeling kit. The results demonstrated improved quality compared to the current *SP Frozen Cell Pellet DNA Isolation* protocol.

1.2. See the VIDEO and SP gDNA Isolation Training Video for critical steps and troubleshooting; the steps mentioned in the latter video correspond to the *Bionano Prep SP Frozen Human Blood DNA Isolation Protocol* (P/N 30246), but are the same processes as described here.

2. OVERVIEW

2.1. MeOH:GAA fixed cells have been widely used in karyotyping, fluorescence in situ hybridization (FISH), and chromosomal microarray (CMA) assays. Unlike these traditional methods of analysis, optical genome mapping (OGM) on the Saphyr system utilizes ultra-long DNA molecules for high resolution structural variation and copy number analysis. As such, OGM has the potential to completely replace time consuming and labor-intensive karyotyping and FISH assays. The workflow depicted here can generate OGM data from MeOH:GAA fixed cells archived from previous karyotyping and FISH analysis. To achieve desired OGM results, samples used in traditional experiments can be fixed using a hypotonic swell and MeOH:GAA fixation process performed on 3 – 6M live cells per sample, as described here. After a fixative removal/pH neutralization process using a balanced salt solution is performed, cell lysis and an enzymatic (Bionano Proteinase K) digestion is performed in a chaotropic buffer and the released gDNA binds to the Nanobind Disk upon the addition of isopropanol. After three wash steps, the disk is transferred to a fresh tube and the gDNA is eluted from the disk. The recovered UHMW gDNA is subjected to limited shearing for more UHMW gDNA homogeneity. The gDNA is then equilibrated at room temperature (RT) for a minimum of two days to facilitate DNA homogeneity before its concentration is determined.

3. IMPORTANT NOTES

Before beginning, review the tech note in its entirety, including Tables 1 and 2.

Suggested Input (Cell Count) and Fixed Cell Storage Temperature

- It is recommended to start the fixation process with 3 – 6M live cells with a viability of no less than 70%.
- For best results, storing fixed cells at -20°C immediately following fixation is highly recommended. Fixed cells can be stored for up to nine months at this temperature.
- Fixed cells that have been stored at 4°C should not be used for UHMW gDNA isolation for Bionano OGM analysis.

Batch Size

- Processing up to six samples at a time (up to three at a time for novice users) is recommended.

Hazardous Waste Disposal

All biohazardous waste, including plasticware, should be disposed of in accordance with local regulations.

Buffers LBB and WB1 contain guanidine hydrochloride (GuHCl). GuHCl is harmful if swallowed or inhaled and causes skin and eye irritation. **WARNING:** DO NOT mix with bleach or acidic reagents. Liquid waste containing GuHCl should be safely decontaminated with a quaternary ammonium disinfectant before disposal in a hazardous waste stream. Bleach for decontamination of pellet supernatant and TexQ for decontamination of all solutions mixed with GuHCl is recommended. This conforms to disposal requirements in the state of California, US, but may vary by location. Please consult local requirements for decontamination and disposal.

4. PREPARATION FOR 75 MM POTASSIUM CHLORIDE (KCL) SWELL AND MEOH:GAA (3:1) FIXATION

Note: While standard practice is likely to be adequate, for best results, preparing fixed cells as described below is encouraged.

Before First Use

- Verify access to refrigerated centrifuge with swinging bucket rotor that can accommodate 15 ml polypropylene conical tubes to pellet the fixed cells.
- Verify access to a water bath set to 37°C. Ensure water level reaches the 5 ml mark of a 15 ml conical tube.

Set Up

4.1. Prepare 75 mM KCl from 2M stock. Then incubate at 37°C for a minimum of ten minutes before use.

4.1.1. Each sample receives a total of 5 ml of pre-warmed (37°C) 75 mM KCl. Prepare 7 ml of 75 mM KCl for each sample.

4.2. In a fume hood, prepare 3:1 MeOH:GAA fixative. Place fixative on ice and leave in fume hood.

4.2.1. This should be made fresh at the beginning of the experiment.

4.2.2. Each sample receives a total of 5 ml of cold fixative.

4.3. Set refrigerated swinging bucket centrifuge to RT, 500 x g, for two minutes with brake set to 0.

4.4. For biological waste disposal, prepare a 10% bleach waste container and place in a biosafety cabinet.

4.5. For hazardous waste disposal, add 1 ml of TexQ to a waste container and place in fume hood.

4.6. For each sample, label one 15 ml conical tube.

4.6.1. For ease of use, mark the 200 µl level on each tube.

4.7. Notes on processing more than three samples

4.7.1. When processing more than three samples at a time (e.g., six samples), it is recommended to work in batches of three for solution addition Steps 5 through 7, Steps 8 and 9, Steps 15 and 16, and Steps 17 and 18 to ensure delicate handling and maintain appropriate timing of the hypotonic swell and fixation across all samples.

4.7.2. For Steps 5 through 7: resuspension, followed by the addition of 3 ml of pre-warmed (37°C) 75 mM KCl and gentle mixing is conducted for the first three samples before performing the same steps on the next three samples. This ensures that the cells are fully resuspended prior to

hypotonic swell and that the variation of the time cells are exposed to the hypotonic solution across samples is kept at a minimum.

4.7.3. For Steps 8 and 9, 15 and 16, 17 and 18: the addition of solutions and gentle mixing is conducted for the first three samples before performing the same steps on the next three samples. This falls in line with the staggering of the previous steps.

5. KCL SWELL AND MEOH:GAA (3:1) FIXATION - THREE HOURS

5.1. Using a hemocytometer and microscope, or automated cell counter, determine concentration of cell suspension (minimum of 70% viability).

5.2. Calculate and transfer a total of 3 – 6M live cells per sample into a pre-labeled 15 ml conical tube.

5.3. Centrifuge balanced tubes using a swinging bucket rotor at RT, 500 x g, for two minutes with brake set to 0. **Note:** Following this step, it is recommended to set the centrifuge to 4°C, 120 x g, for five minutes with brake set to 0 for Step 12. Turn on the Fast Temp function if available (or start the centrifuge) to quickly cool down the chamber to 4°C.

5.4. Using a P1000 ul pipette, remove all but 200 µl of the supernatant (use transfer volume in Step 2 to subtract 200). Discard aspirated supernatant into 10% bleach container.

5.5. Resuspend the pellet with a 200 µl standard bore tip by pipetting up and down five to ten times. Ensure that the cells are fully resuspended. **Note:** If processing more than three samples work in batches of three for Steps 5 through 7. For details see “Notes on processing more than three samples” in the prior section.

5.6. Using a P1000 pipette or a serological pipette and pipette aid set to slow, slowly add a total volume of 3 ml of pre-warmed (37°C) 75 mM potassium chloride (KCl) hypotonic solution to the tube wall while slowly rotating the tube. **Note:** Adding the hypotonic solution slowly to the cell suspension will prevent hypotonic shock and maintain the viability of the cells.

5.7. Cap the tube then gently tilt the tube to a near horizontal position and slowly rotate 360 degrees to mix. Prevent the liquid from touching the tube cap. **Note:** Please refer to Campos P.B., Sartore R.C., Abdalla S.N., Rehen S.K. (2009). Chromosomal Spread Preparation of Human Embryonic Stem Cells for Karyotyping. JoVE. 31. http://www.jove.com/details.php?id=1512, doi: 10.3791/1512. (Step 6 on page 1).

5.8. Slowly add another 2 ml of pre-warmed (37°C) 75 mM KCl hypotonic solution to the tube wall while slowly rotating the tube. **Note:** If processing more than three samples work in batches of three for Steps 8 and 9. For details see “Notes on Processing More Than Three Samples” in the prior section.

5.9. Cap the tube then gently tilt the tube to a near horizontal position and slowly rotate 360 degrees to mix. Prevent the liquid from touching the tube cap.

5.10. Incubate each sample in a water bath set to 37°C for fifteen minutes. **Note**: Do not exceed fifteen minutes. If left longer than fifteen minutes in the hypotonic solution, cells will start to burst. This will lead to reduced DNA yield after fixative removal and SP DNA isolation.

5.11. **Working in a fume hood**, slowly add three drops of cold fixative solution (3:1 MeOH:GAA), cap the tube, and then place on ice for two minutes.

5.12. Centrifuge balanced tubes using a swinging bucket rotor at 4°C, 120 x g, for five minutes with brake set to 0.

5.13. Place samples back on ice and bring to the fume hood.

5.14. Using a disposable transfer pipette, gently remove all but 200 µl of supernatant. Do not disturb the pellet. Discard the supernatant into the TexQ waste container.

5.15. Using a P1000 pipette or a serological pipette set to slow, slowly add a total volume of 3 ml of cold fixative solution to the tube wall while slowly rotating the tube. Do not disturb the pellet. **Note:** If processing more than three samples work in batches of three for Steps 15 and 16. For details see "Notes on processing more than three samples" in the prior section.

5.16. Cap the tube then gently tilt the tube to a near horizontal position and slowly rotate 360 degrees to mix. Prevent the liquid from touching the tube cap.

5.17. Add another 2 ml of cold fixative solution to the tube wall while slowly rotating the tube. **Note:** If processing more than three samples work in batches of three for Steps 17 and 18. For details see "Notes on processing more than three samples" in the prior section.

5.18. Cap the tube then gently tilt it to a near horizontal position and slowly rotate 360 degrees to mix. Prevent the liquid from touching the tube cap. Then place samples back on ice. **Note:** Only proceed to the next step once all samples have the total 5 ml of cold fixative and have been gently mixed.

5.19. Transfer to and store the fixed cells at -20°C for up to nine months.

6. PREPARATION FOR FIXATIVE REMOVAL

Note: For best results, washing and neutralizing the fixed cells prior to using the *Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol* v2 (P/N 30398) as described below, is encouraged.

Set Up

6.1. Set refrigerated swinging bucket centrifuge to 4°C, 120 x g, five minutes with brake set to 0.

6.2. Prepare fresh HBSS washing solutions.

- **HBSS-1** = HBSS + 5% Heat-Inactivated FBS + 5 mM EDTA (pH 8.0): Total of 12 ml per sample. Optional step of sterile filtration.
- **HBSS-2** = HBSS + 5 mM EDTA (pH 8.0): Total of 35 ml per sample.

6.3. Pre-chill HBSS washing solutions on ice or at 4°C.

6.4. Prepare empty liquid and solid chemical hazardous waste containers to be used for MeOH:GAA liquid waste and solid waste. Place both containers in fume hood.

6.5. Notes on processing more than three samples

6.5.1. All samples should be processed at once for centrifugation and supernatant removal, regardless of batch size.

6.5.2. When processing more than three samples at a time (e.g., six samples), working in batches of three for the stepwise wash and fixative removal process to ensure delicate handling and maintain appropriate timing of pH neutralization across all samples is recommended.

Example: after centrifugation of and removal of the bulk of the supernatant from all samples, the addition of HBSS washing solution and gentle mixing would be conducted on the first three samples before performing the same steps on the next three samples.

7. STEPWISE WASH FOR FIXATIVE REMOVAL (THREE HOURS)

Note: Keep samples on ice when not being processed. A training video is available for the fixative removal technique described in steps 3, 4, 5, 8 , 9, 13, 19-23: Tutorial for Stepwise Wash for Fixative Removal.

7.1. Retrieve the 15 ml conical tube containing MeOH:GAA fixed cells from -20°C and place the tube on ice for ten minutes.

7.2. Centrifuge balanced tubes using a swinging bucket rotor at 4°C, 120 x g, five minutes with brake set to 0. Proceed to the next step as soon as the centrifuge stops.

7.3. Remove each tube from the centrifuge and place on ice. **Working in a fume hood**, carefully remove supernatant with a transfer pipette, leaving behind approximately 200 µl. Discard supernatant in chemical hazardous waste container. Discard the used transfer pipette in the solid hazardous waste container. Place samples on ice.

7.4. With the tube tilted at a 45-degree angle and using a 10 ml serological pipette gently add 10 ml cold HBSS-1. Dispense the buffer at the top of the tube against the wall using a flowrate of approximately 3 ml per minute. **Note:** Pellet will be extremely delicate at this step. It is normal to see a small portion of the pellet resuspend, but this should not exceed a third of the pellet being disrupted. Dispense liquid *slowly* to prevent disturbing the pellet. Always observe the pellet while dispensing liquid to avoid disruption.

7.5. Cap the tube and gently tilt the tube to a horizontal position, allowing the liquid at the narrow portion of the tube to slosh toward the cap briefly, then turn the tube a full 360 degrees to mix the sample. Place samples on ice. **Note:** The color of the solution should change from dark pink to yellow at this point, indicating the presence of the acidic fixative.

7.6. Centrifuge the tube using a swinging bucket rotor at 4°C, 120 x g, for five minutes with brake set to 0. Proceed to the next step immediately after the centrifuge stops.

7.7. Remove each tube from the centrifuge and place on ice. Working in a fume hood, carefully remove the supernatant with a new transfer pipette, leaving behind approximately 200 µl. Discard supernatant in the chemical hazardous waste container. Discard the used transfer pipette in the solid hazardous waste container.

7.8. With the tube tilted at a 45-degree angle, and using a 10 ml serological pipette, gently add 10 ml cold HBSS-2 at the top of the tube against the wall using a flowrate of approximately 7.5 ml per minute. **Note:** At this step, the pellet should be tight. Always observe the pellet while dispensing liquid to avoid disruption. Immediately decrease the flowrate if the pellet begins to break apart.

7.9. Ensure cap is fully sealed then tilt the tube to a horizontal position and flick the narrow end when a small amount of liquid is near the pellet to resuspend. **Note:** If a pellet does not resuspend immediately, tilt the tube to an upright position and repeat the resuspension process until the pellet is fully resuspended.

7.10. Centrifuge the tube using a swinging bucket rotor at 4°C, 120 x g, for ten minutes with brake set to 0. Proceed to the next step immediately after the centrifuge stops.

7.11. Remove each tube from the centrifuge and place on ice. Working in a fume hood, carefully remove the supernatant with a new transfer pipette, leaving behind approximately 200 µl. Discard supernatant in chemical hazardous waste container. Discard the used transfer pipette to the solid hazardous waste container.

7.12. With the tube tilted at a 45-degree angle, and using a 10 ml serological pipette, gently add 10 ml cold HBSS-2 at the top of the tube against the wall.

7.13. Gently tilt the tube to a horizontal position, allowing the liquid at the narrow portion of the tube to slosh toward the cap briefly, then turn the tube a full 360 degrees to mix the sample. **Note:** At this step, the color of the solution should remain dark pink indicating that the acidic fixative is mostly removed.

7.14. Centrifuge balanced tubes using a swinging bucket rotor at 4°C, 120 x g, for five minutes with brake set to 0. Proceed to the next step immediately after the centrifuge stops.

7.15. Remove each tube from the centrifuge and place on ice. Working in a fume hood, carefully remove the supernatant with a new transfer pipette, leaving behind approximately 200 µl. Discard supernatant in chemical hazardous waste container. Discard the used transfer pipette to the solid hazardous waste container.

7.16. Using a P1000 pipette, add fresh 1.0 ml cold HBSS-2 at the top of the tube against the wall.

7.17. Gently tilt the tube to a horizontal position, allowing the liquid at the narrow portion of the tube to slosh toward the cap briefly, then turn the tube a full 360 degrees to mix the sample.

7.18. Centrifuge samples using a swinging bucket rotor at 4°C, 500 x g, for five minutes with brake set to 0. Proceed to the next step immediately after the centrifuge stops. Place the sample back on ice.

7.19. Remove each tube from the centrifuge and place on ice. Working in a fume hood, carefully remove the supernatant with a new transfer pipette, leaving behind approximately 20 µl (remove 1.18 ml). Discard supernatant in chemical hazardous waste container.

7.20. Move samples to a biosafety cabinet.

7.21. Using a 200 µl wide-bore pipette tip and P200 pipettor set to 20 µl, gently pipette up and down twice then measure the exact cell suspension volume.

7.22. Using a 200 µl standard pipette tip, add cold HBSS-2 to target SP starting volume of 40 µl.

7.23. Using a 200 µl wide-bore pipette tip and P200 pipettor set to 40 µl, gently pipette up and down five times then transfer the entire suspension to a pre-labeled, pre-chilled 1.5 ml Protein LoBind tube.

7.24. Proceed to the *Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol* v2 (P/N 30398), Step 4. **Note:** This tech note was developed without the use of the end-over-end homogenization described in Step 33 of the *Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol* v2 (P/N 30398). Skipping this step for MeOH:GAA fixed cells is recommended.

Frequently Asked Questions and Troubleshooting

What cell types are compatible with this tech note procedure?

This technique has been used to process EBV immortalized human lymphoblastoid cell line (GM12878) that have been fixed using a hypotonic swell followed by MeOH:GAA (3:1) fixation process as described here.

How many samples can be processed?

This tech note can be used for batch sizes of up to six samples. It will take up to three hours for hypotonic swell and fixation, and up to six hours for fixative removal and gDNA isolation for a batch of this size.

Why is my gDNA quality low?

The following may negatively affect gDNA quality:

- Low viability cells (<70%) were used for MeOH:GAA fixation.
- High percentage of MeOH and GAA residual after fixative removal procedure.
- Fixed cells were not stored at -20°C immediately after MeOH:GAA fixation.
- Storage temperature fluctuations.
- Long term storage at -20°C.
- Using fixed cells that have been stored at 4°C, rather than the recommended temperature.

Why is my gDNA yield low?

- Starting the fixation process with fewer than three million live cells. For instance, inaccurate live cell count may result in low gDNA yield.
- Using a fixed-angle rotor during centrifugation steps.
- The duration of the hypotonic swell preceding fixation and handling during fixation will influence gDNA yield. Dispensing hypotonic solution onto cells too quickly or incubation at 37°C for longer than fifteen minutes may result in cell burst and therefore reduce the gDNA yield.
- Improper storage and handling of fixed cells during fixative removal will result in low gDNA yield.
- Cell input post-fixation may vary between operators. Smaller initial pellet sizes upon the initial centrifugation step of the fixative removal process may result in lower gDNA yields.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

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