Bionano Prep SP Cryopreserved Cell Tech Note

Document Number: TECHN-00001

Document Revision: 01

Disclaimer

This Bionano Prep SP Cryopreserved Cell Tech Note is not a fully validated protocol and has only been used to isolate ultra-high molecular weight (UHMW) DNA from cryopreserved lymphoblastoid cell lines, peripheral blood mononuclear cells (PBMC), and bone marrow mononuclear cells (BMNC) for the Bionano Saphyr system. For samples stored as frozen cell pellets, please refer to document 30398, Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol v2.

Table of Contents

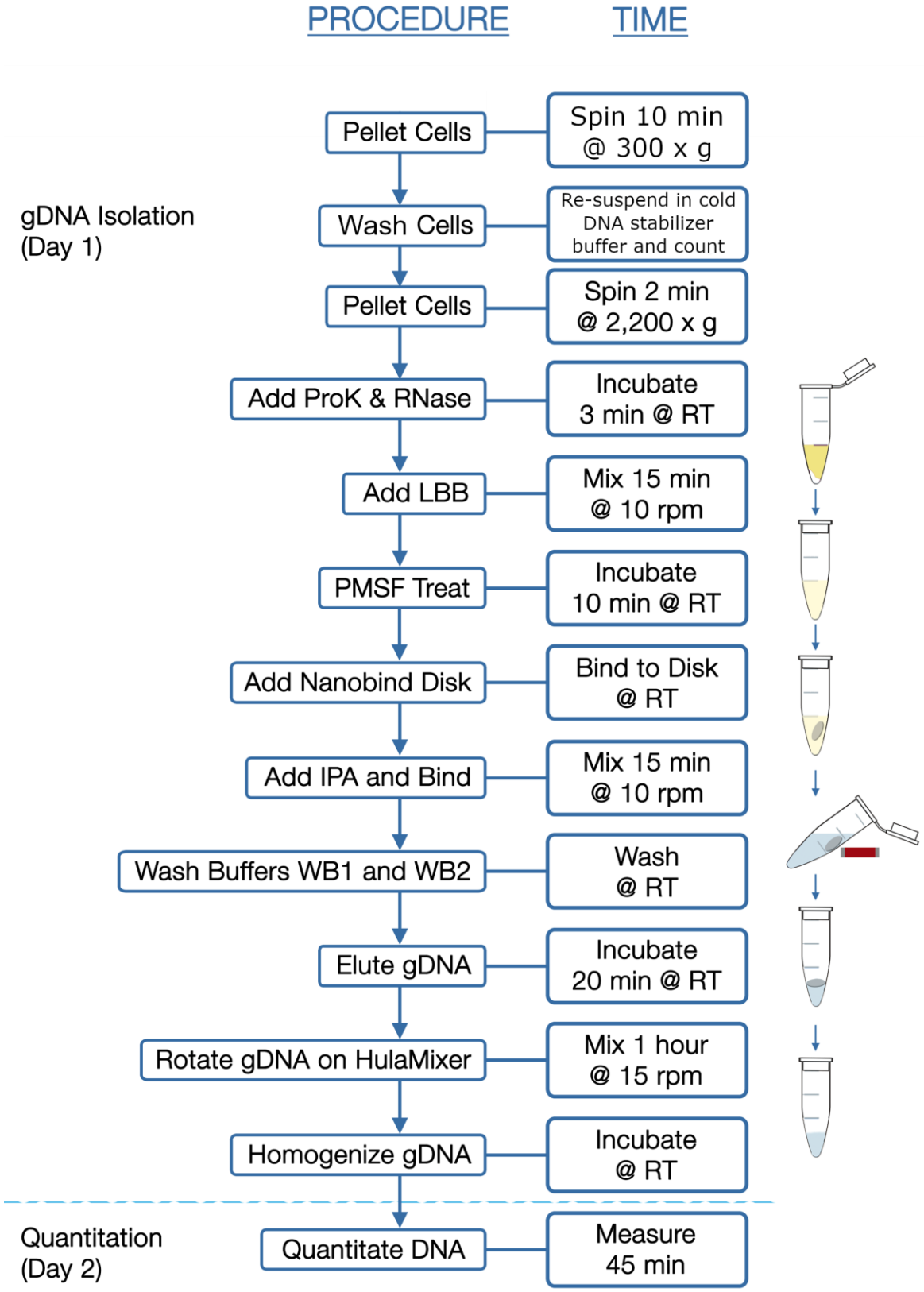
Legal Notice

For Research Use Only. Not for use in diagnostic procedures.

Revision History

Revision	Effective	Notes
01	04/06/2022	Initial Release

Workflow Overview



Bionano Prep SP DNA Isolation Kit and User-Supplied Materials

Bionano Prep SP Blood and Cell DNA Isolation Kit v2 Contents (Part # 80042, 10 preps)

Item	Amount	Part Number	Storage
4 mm Nanobind Disks	10 disks	20402	Room Temp (18-25°C)
Protein LoBind Microcentrifuge Tubes, 1.5 ml	10 tubes	20422	Room Temp (18-25°C)
Protein LoBind Microcentrifuge Tubes, 0.5 ml	10 tubes	20421	Room Temp (18-25°C)
RNase A Enzyme	200 µl	20373	Refrigerate (4°C)
DNA Stabilizer**	350 µl	20423	Room Temp (18-25°C)
Standard Microfuge Tubes, 2.0 ml	10 tubes	20396	Room Temp (18-25°C)
Cell Buffer	50 ml	20374	Room Temp (18-25°C)
Proteinase K Enzyme	0.5 ml	20372	Room Temp (18-25°C)
Lysis and Binding Buffer (LBB)*	2.5 ml	20375	Room Temp (18-25°C)
Wash Buffer 1 Concentrate (2.5X) (WB1)*	3.25 ml	20376	Room Temp (18-25°C)
Wash Buffer 2 Concentrate (2.5X) (WB2)	5 ml	20377	Room Temp (18-25°C)
Elution Buffer (EB)	1.1 ml	20378	Room Temp (18-25°C)
Magnetic Disk Retriever Plastic Sheath	10	20381	Room Temp (18-25°C)

* See Important Notes Section for hazardous waste information.
** DNA Stabilizer is provided with the kit, but additional DNA Stabilizer is needed for this protocol. See part number 80036 below.

Bionano Prep DNA Stabilizer (Part # 80036, ≥ 40 preps)

Item	Amount	Part Number	Storage
DNA Stabililzer	4 ml	20398	Room Temp (18-25°C)

Bionano Prep DNA Stabilizer must be ordered separately.

User-Supplied Materials

Item	Supplier	Catalog #
Day 1 – Counting, Washing Pelleting, gDNA Isolation and Homogenization		
Bionano Prep SP Magnetic Retriever (2 pack)	Bionano Genomics	80031
Hemocytometer & Phase Contrast Microscope or Automated Cell Counter	DeNovix	CellDrop BF-UNLTD
DynaMag-2 Magnetic Tube Rack	Thermo Fisher	12321D
HulaMixer Sample Mixer	Thermo Fisher	15920D
Microcentrifuge Tubes, 1.5 ml, Nuclease Free	VWR	87003-294
Phenylmethylsulphonyl 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
Bleach for Cell Media Disposal	General Lab Supplier	
Conical Centrifuge Tubes, 50 ml, PP	Thermo Fisher or Equivalent	14-432-22
Conical Centrifuge Tubes, 15 ml, PP	Fisher Scientific	05-539-12
Mini Benchtop centrifuge with 1.5 ml tube rotor (2,200 x g spin)	General Lab Supplier	
Refrigerated centrifuge with 1.5 ml Tube Rotor	General Lab Supplier	
Ice Bucket and Ice	General Lab Supplier	
Sterile 5- and 10-ml Disposable Pipettes (TD+)	General Lab Supplier	
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 Rainin Equivalent	16466-008

Pipettes (10, 20, 200, and 1,000 µl) and Nuclease Free, Filtered Pipette Tips	General Lab Supplier	
Laboratory task wipes	Kimberly-Clark	34155
Day 2 - Quantitation		
Benchtop Vortexer	General Lab Supplier	
Bath Sonicator (recommended)	Branson or Equivalent	CPX 952-119R
15 ml Conical Tube	Fisher Scientific	05-539-12
Fluorometer, Qubit®	Thermo Fisher or Equivalent	Q33216
Qubit® BR (Broad Range) dsDNA Assay Kit	Thermo Fisher or Equivalent	Q32853
Qubit® Assay Tubes	Thermo Fisher	Q32856
Positive-Displacement Pipette MR-10 (optional)	Rainin or Equivalent	17008575
Pipette Tips, 10 µl, C-10 for Pos. Displ. Pipette (optional)	Rainin or Equivalent	17008604

Introduction and Important Notes

Introduction

This Bionano Prep Cryopreserved Cells Tech Note can provide ultra-high molecular weight (UHMW) gDNA in less than 4 hours from 1.5 million mammalian cells. It utilizes a lyse, bind, wash, and elute procedure that is common for 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 a novel nano structured silica on the outside of the thermoplastic paramagnetic disk. This protocol was tested using an Epstein-Barr Virus (EBV) immortalized human lymphoblastoid cell line (GM12878) that grows in suspension culture, as well as with Ficoll-separated PBMCs and BMNCs. gDNA prepared using this protocol has been tested only with DLS labeling. See Training Video for technically critical steps and troubleshooting; the steps mentioned in the video correspond to the Frozen Blood Protocol, but are the same processes as presented here.

Overview

Cell lysis and Proteinase K digestion occur in a chaotropic buffer and 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 to ensure homogeneity. The gDNA is then mixed and equilibrated overnight at room temperature to facilitate DNA homogeneity and the concentration is determined. Typical gDNA size range is from 50 Kbp to ≥ 1 Mbp.

Important Notes

Additional Reagent Required

This protocol requires both the Bionano Prep SP Blood and Cell DNA Isolation Kit v2 Contents (Part# 80042, 10 preps) and additional DNA Stabilizer (Part# 80036, ≥40 preps).

DNA Homogeneity

Recovered gDNA is subjected to pipette mixing with a 200 µl standard pipet tip to increase homogeneity, ensuring consistent DNA sampling for labeling.

gDNA Quantitation

gDNA quantitation is used to measure concentration and serves as a gauge of UHMW gDNA homogeneity. Qubit quantitation is preferred over other quantitation methods since it can also be used for measuring gDNA concentration of the labeling reaction. The Qubit Broad Range (BR) dsDNA Assay measures gDNA concentration after isolation, while the High Sensitivity (HS) dsDNA Assay measures gDNA concentration after labeling.

To gauge gDNA homogeneity, it is essential to measure the concentration of gDNA at multiple positions in the solution. Since viscous gDNA is difficult to pipet, follow guidelines in the Important Notes and gDNA Quantitation sections below for accurate pipetting. Standard assays for quantification of gDNA concentration will not provide accurate measurements of long gDNA due to its viscous nature.

- Effective fragmentation of sampled gDNA via sonication or extensive vortexing is necessary for accurate quantitation.
- The coefficient of variation (CV) from three unique samplings should be less than 0.30.
- Typical gDNA concentration is 50-120 ng/µl.

Pipetting Viscous Genomic DNA (gDNA)

To draw viscous gDNA, hold the stock tube for close-up visualization, depress the pipette plunger until the first stop, submerge the pipette tip, and carefully and slowly release the plunger to start drawing the viscous gDNA into the tip while carefully monitoring uptake. Keep the tip submerged even after the viscous solution stops moving upward and levels off. Viscous gDNA can take a few seconds to fill up to 2 µl. Releasing the plunger too fast can produce a bubble in the tip leading to under-sampling and the user should start over if this occurs. After the solution in the tip has leveled off and while the tip is still submerged in the gDNA solution, scrape the tip against the bottom of the tube 3-5 times using a circular motion. Remove the tip from the gDNA solution and visually inspect to confirm that it is filled to 2 µl. Removing the pipette tip from the gDNA solution too early, or ineffectively scraping the tip to break gDNA strands from the tip, can produce a bubble at the opening of the pipette tip indicating under-sampling. Again, the user should start over if this happens.

gDNA Handling

- Mixing of recovered gDNA is always carried out with a wide bore pipette tip to prevent shearing.
- Recovered gDNA should never be frozen or vortexed.
- Pipetting of recovered gDNA for accurate sampling is always carried out with a standard tip or positive displacement pipette.

Characteristics of High Quality gDNA for Bionano Mapping

- A clear gDNA solution is ideal, but an unclear solution does not always correlate with poor sample quality.
- Recovered gDNA in solution is viscous.
- Presence of mega base size gDNA is measured by pulsed field gel electrophoresis (PFGE).
- Recovered gDNA is homogenous as measured with the Qubit gDNA quantitation assay with a CV < 0.30.

Using the Bionano Prep SP Magnetic Retriever

a. Hold a plastic sheath on the sides near the top and insert the Bionano Prep SP Magnetic Retriever into the sheath, positioning it such that it is sitting at the bottom of the sheath.
b. Insert the sheathed retriever into the Protein LoBind microfuge tube to attract the Nanobind Disk to the retriever in the sheath.
c. Carefully lift the sheathed retriever with the bound disk out of the tube and insert the sheathed retriever into a new Protein LoBind microfuge tube.
d. Holding the sheath on the side near the top, pull the retriever up until the Nanobind Disk disassociates from the sheath and drops into the new tube.
e. Change the sheath for each new sample.

Batch Size

- We recommend processing up to 6 samples at a time.

Hazardous Waste Disposal

Buffers LBB and WB1 contain guanidine hydrochloride (GuHCl). GuHCl is harmful if swallowed or inhaled and causes skin and eye irritation. 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. We recommend bleach for decontamination of pellet supernatant and TexQ for decontamination of all solutions mixed with GuHCl. While conforming to disposal requirements in the state of California, US, requirements may be different in other locations. Please consult local requirements for decontamination and disposal.

Bionano Prep SP Cryopreserved Cell Tech Note

Preparation for gDNA Isolation from cryopreserved cells

Note: See Appendix for instructions on cryopreserving cells.

Before First Use

- Verify refrigerated centrifuge spin speed is 300 x g and set to a temperature of 4°C.
- If using a separate benchtop mini centrifuge, verify that the spin speed is 2,200 x g.
- PMSF decomposes rapidly in aqueous solutions. Create aliquots of 120 µl in 1.5 ml screw cap tubes and store stock and aliquots at 4°C. Each aliquot will be sufficient for ten gDNA isolations.
- Add 100% Ethanol to Wash Buffers (WB1 and WB2) and mix thoroughly:
    - Add 5 ml of 100% Ethanol to Wash Buffer 1 (WB1) for a final volume of 8.25 ml.
    - Add 7.5 ml of 100% Ethanol to Wash Buffer 2 (WB2) for a final volume of 12.5 ml.
- Prepare Stabilizing Buffer by mixing Cell Buffer (Bionano) with DNA Stabilizer (Bionano) in a DNAse-free 50mL conical vial. Then, remove 500 µl Stabilizing Buffer and place in a 1.7 ml DNAse-free microcentrifuge tube. Stabilizing Buffer is stable at 4°C and at room temperature, and can be made in advance; however, for convenience, it is recommended to store the larger volume (~49.5 ml aliquot) at 4°C.
    - 50 ml Stabilizing Buffer = 49 ml Cell Buffer + 1 ml DNA Stabilizer, vortex to mix and pulse spin.
    - Final volume is 49.5 ml Stabilizing Buffer in one 50 ml conical vial, and 0.5 ml Stabilizing Buffer in one 1.7 ml microcentrifuge tube.

Set Up

- Gather materials (see “User Supplied Material” section above).
- Verify refrigerated centrifuge has been set to 4°C with maximum acceleration and no deceleration.
- Prepare 37°C water bath or heat block to thaw frozen cell pellets. Verify temperature with thermometer.
- Pre-chill the 50 ml conical vial containing Stabilizing Buffer on ice. Keep the 1.7 ml microcentrifuge tube containing 0.5 ml Stabilizing Buffer at room temperature.
- For waste disposal, prepare:
    - One 50 ml conical with 5 ml bleach + 20 ml water; invert several times to mix.
    - One 50 ml conical with 100 µl TexQ decontaminant per sample (to be disposed as hazardous waste).
- For each sample, label one 0.5ml Protein LoBind Tube (Bionano), one 1.5ml Protein LoBind Tube (Bionano) and one 2.0 ml microfuge tube (Bionano).

- Invert tubes of PMSF, RNase A (Bionano) and Proteinase K (Bionano) three times to mix, pulse spin briefly. Place PMSF and RNase A on ice.

gDNA Isolation (3 hours)

Thaw cryopreserved cells, wash in Stabilizing Buffer, and pellet in Protein LoBind Tubes

Note: For instructions on preparing cryopreserved cells, please refer to the Appendix. For optimal data quality, it is recommended to use cells with ≥ 80% viability post-thaw. Trypan Blue Exclusion methods for cell counting and viability are recommended (e.g., manual counting with a hemacytometer or automatic counting with a DeNovix CellDrop BF-UNLTD or equivalent device). PBMCs and BMNCs may be counted using a HemoCue WBC Analyzer (catalog # 22-601-017).

1. Before thawing the cells, estimate the volume of each frozen sample needed to obtain 1.5 million live cells for downstream DNA isolation. If cell count is unknown, determine an approximate volume containing at least 1.5 million live cells.
    a. 1.5 million cells * [volume of cryopreserved cells] / [total # of cryopreserved cells in vial] = [volume cells for DNA isolation]
    b. For a vial of 2 million cells/mL: 1.5 million cells * 1mL / 2 million cells = 0.75 ml cells for DNA isolation
2. Using the volumes of frozen sample determined in step 1, calculate the required volume of cold Stabilizing Buffer needed to top-off each sample to 1.5 mL.
    a. 1.5 ml total volume – [volume for DNA isolation] = [volume of Stabilizing Buffer to add]
    b. For a vial of 2 million cells/mL: 1.5 ml total volume – 0.75 ml cells for DNA isolation = 0.75 ml Stabilizing Buffer to add
3. For each sample, add the volume of cold Stabilizing Buffer calculated in step 2 to a pre-labeled 1.7 ml protein LoBind tube. Keep the protein LoBind tubes on ice.
4. Place the cryopreserved cell vials in a 37°C water bath. Continuously swirl the vials in the water bath, checking every 20 seconds to see if any vials have fully thawed.
5. Once a vial of cells has thawed, immediately remove it from water bath, dry it, and place on ice.

    Note: Depending on the volume in each vial, some vials may thaw sooner than others. If a vial has thawed, but others are still frozen, place the thawed vial on ice and continue swirling the remaining vials in the 37C water bath.
6. Using a P1000 pipet, gently mix the first vial of cryopreserved cells and transfer the pre-determined volume containing 1.5 million cells (calculated in step 1) into the corresponding 1.7 ml protein LoBind tube with Stabilizing Buffer (prepared in step 3). Repeat for each of the samples.
7. Invert cells three times and centrifuge at 4°C for 10 minutes at 300 x g.

    Note: it is helpful to orient all the tubes in the same direction, with the opening of the caps facing the center of the centrifuge rotor. This makes the resulting pellets easier to find.

8. For each sample, remove as much supernatant as possible without disturbing the pellet. Discard supernatant in bleach waste.
9. Add an additional 1 ml cold Stabilizing Buffer and re-suspend the cells with a p1000 pipet.
10. Gently mix the re-suspended cells 3X using a p1000 pipet set to 900 µl.
11. If the initial cell counts are unknown, count the cells immediately after mixing with the p1000 pipet. Because cells settle rapidly, it is better to mix each cell suspension immediately before counting instead of mixing all the cells at once.
    a. **Note**: for purified primary blood cells, a HemoCue WBC Analyzer (catalog # 22-601-017) may be used, as described in the Bionano Prep SP Frozen Human Blood DNA Isolation Protocol (#30246). For other cell types, a method that assesses both cell counts and viability, such as Trypan Blue Exclusion, is recommended.
    b. If a cell count is above 1.5 million live cells per tube, then discard excess cells and replace any discarded volumes of cell suspension with cold Stabilizing Buffer. Each cell suspension should contain ~1.5 million live cells in 1 ml Stabilizing Buffer.
    c. If a cell count is below 1 million live cells, then do not proceed with DNA isolation for that sample. Repeat steps 1-11 with increased input later.
12. Add another 500 µl cold Stabilizing Buffer to each cell suspension.
13. Invert cells three times and centrifuge at 4°C for 2 minutes at 2200 x g.
14. For each sample, remove as much supernatant as possible without disturbing the pellet. Discard supernatant in bleach waste.
15. Transfer tubes to room temperature benchtop and add 40 µl of room temperature Stabilizing Buffer on the top of each pellet.
16. Disrupt the pellet with a 200 µl wide bore tip, then continue to resuspend the pellet by pipetting up and down 10 times. Transfer the entire volume of suspension (>40 µl) into a previously labeled Protein LoBind tube with a standard 200 µl tip.

Lyse and Digest Cells

17. Add 50 µl of Proteinase K and 20 µl of RNase A to each of the Protein LoBind tubes containing resuspended cells. **DO NOT PIPET MIX.**
18. Incubate at room temperature for 3 minutes.
19. Add 225 µl Buffer LBB to sample with a standard 1,000 µl tip. Cap and invert tube 15 times to mix.

Note: Buffer LBB is a viscous and foamy solution which will adhere to the pipette tip. Dispense slowly and change tips between dispensing to ensure accuracy of dispensed volume.

20. Rotate sample on HulaMixer for 15 minutes at room temperature at 10 rpm, ensuring that no shaking/vibration occurs.
21. Pulse spin tube for 2 seconds to collect liquid at the bottom of the tube.
22. Add 10 µl of 100 mM PMSF into the liquid portion of the tube. Cap and invert tube 5 times to mix, then pulse spin the tube for 2 seconds to collect liquid at the bottom of the tube.
23. Incubate at room temperature for 10 minutes.

gDNA Bind, Wash and Elute

24. Using forceps, carefully transfer a single Nanobind Disk to the lysate.

    Note: Disks can sometimes stick together.

25. Add 340 µl 100% isopropanol to all tubes. Cap and invert tubes 5 times to mix.
26. Rotate sample on a HulaMixer for 15 minutes at room temperature at 10 rpm, ensuring that no shaking/vibration occurs.

    Note: Ensure that the Nanobind Disk does not remain in the lid of the tube during initial rotations. If it does, turn off the rotator and invert the microfuge tube until the Nanobind Disk goes back into the solution. Replace the tube on the HulaMixer and resume mixing.

27. Examine gDNA association with the Nanobind Disk and invert to increase binding (See Training Video, 0:25):
    a. Place sample tubes into a clear Dynamag tube rack and visually inspect all tubes in the rack to ensure that gDNA is tethered to the Nanobind Disk.
    b. If gDNA strands are visibly hanging low, quickly invert 180° to bring the gDNA into closer association with the Nanobind Disk.
    c. 180° inversions can be done many times until the gDNA association with the Nanobind Disk appears unchanged.

28. Combine the clear rack with the magnetic base as outlined below, making sure the Nanobind Disk is secured by the magnet near the top of the liquid level. If this is not the case, re-rack (See Training Video, 0:50).

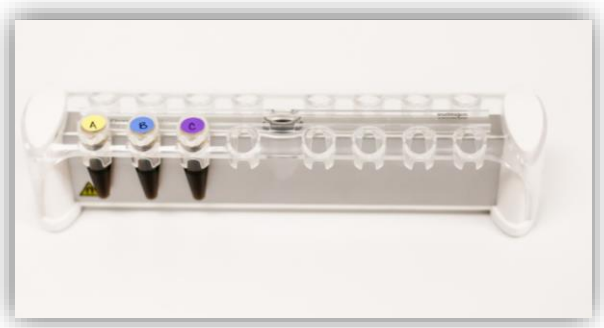
    Note: The color of liquid in the pictures below was modified for illustrative purposes.

    a. Invert clear Dynamag tube rack and place upside down with sample lids touching the work surface. The tubes will be on the same row of the rack, and in the row furthest from the user.

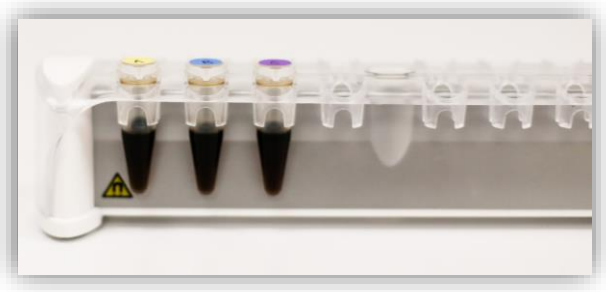
    b. Invert Dynamag magnetic base and lower onto clear rack.

    c. Tilt combined apparatus slowly 90° towards the front while it continues to rest on the surface. The tubes will now be horizontal and visible.

    d. Tilt combined apparatus slowly 90° towards the front while it continues to rest on the surface, so that it stands fully upright, and tubes are facing up.



e. Make sure Nanobind Disk is held to the magnet near the top of the liquid level.



29. Set one 1,000 µl pipette to 1,000 µl and a second to 700 µl.
30. Remove supernatant as outlined below, being careful not to aspirate the gDNA (See Training Video, 1:15):
    a. Angle entire rack at a 45° angle by holding in one hand, grasping the entire apparatus from below with tubes visible and the lids facing up.
    b. Wait 2 seconds for gDNA to lay on the Nanobind Disk.
    c. Slowly remove all liquid with a 1,000 µl extra-long tip angled away from the Nanobind Disk and/or gDNA to avoid disruption.
    d. Dispense supernatant into conical tube containing TexQ.

⚠ Ensure that the gDNA is not removed by visually inspecting the tip containing buffer before discarding. If gDNA is accidentally aspirated or becomes unbound from the disk, refer to the Troubleshooting section below.

31. Perform Wash WB1 (see Training Video, 2:21):
    a. Dispense 700 µl of Buffer WB1 directly onto the disks in the tubes and cap tubes.
    b. Lift clear tube rack to separate from magnetic base.
    c. Invert clear rack with tubes 180°, 4 times, to wash.
    d. Re-rack clear tube rack and tubes with magnetic base as described in step 15.
    e. Remove supernatant as described in step 17.

⚠ Ensure that the gDNA is not removed by visually inspecting the tip containing buffer before discarding. If gDNA is accidentally aspirated or becomes unbound from the disk refer to the Troubleshooting section below.

32. Set the second pipette to 500 µl (previously at 700 µl).
33. Perform Wash WB2 (See Training Video, 4:10):
    a. Dispense 500 µl of Buffer WB2 directly onto the disks in the tubes, and cap.
    b. Lift clear rack to separate from magnetic base.

c. Invert clear rack 180°, 10 times, to wash.
d. Re-rack clear tube rack and tubes with magnetic base as described in step 15.
e. Remove supernatant as described in step 17.

⚠ Ensure that the gDNA is not removed by visually inspecting the tip containing buffer before discarding. If gDNA is accidentally aspirated or becomes unbound from the disk refer to the Troubleshooting section below.

34. Repeat Wash WB2, step 20 (See Training Video, 5:50).

    Note: Remove buffer from 2 or 3 tubes at a time and process through Buffer EB incubation step in small batches to prevent the disk/gDNA from drying out.

35. Open tube lid fully (parallel to lab bench) and lift each tube apart from the base.
36. Near to a 0.5ml Protein LoBind tube, transfer the Nanobind Disk to the 0.5ml Protein LoBind tube using the Bionano Prep SP Magnetic Retriever (see Important Notes section for proper usage). Cap the tube to prevent the disk drying out (See Training Video, 7:30).
37. Spin the Protein LoBind tube in a benchtop microcentrifuge for 5 seconds.
38. Remove all residual liquid at the bottom of the tube using a 10 µl standard tip.

    Note: It is necessary to displace the Nanobind Disk using the tip to reach the liquid at the bottom of the tube. Move the tip around with a small circular motion to remove all residual liquid.

39. Add 65 µl of Buffer EB to the Protein LoBind tube.
40. Spin the tube on a benchtop microcentrifuge for 5 seconds.
41. Using a 10 µl standard tip, gently nudge the Nanobind Disk towards the bottom of the tube, making sure that it is fully submerged in liquid. The disk should remain parallel to the bench surface (see Training Video, 8:20).
42. Incubate submerged Nanobind Disk in Buffer EB at room temperature for 20 minutes.
43. Collect extracted gDNA by transferring the eluate to a previously labeled 2.0 ml microfuge tube with a 200 µl standard tip.
44. Spin the tube with the Nanobind Disk on a benchtop microcentrifuge for 5 seconds and transfer all the remaining eluate containing viscous gDNA to the same standard 2.0 ml microfuge tube, as in the previous step, with a standard 200 µl tip. The disk may be removed before aspirating the remaining elution buffer.

    Note: Almost all the viscous gDNA will come off the Nanobind Disk during the spin.

Homogenization of gDNA Solution (70 minutes)

Homogenization of gDNA Solution

45. Slowly pipette the entire gDNA volume into a standard bore 200 µl tip, then slowly dispense the gDNA. Avoid creating bubbles.
    - Repeat this process 3 times for a total of 4 strokes: (1 stroke = 1 aspiration and 1 dispense).

    Note: If gDNA uptake stalls due to high viscosity, it may be necessary to stir gently while slowly releasing the plunger to withdraw the gDNA.
46. Place standard 2.0 ml microfuge tube containing gDNA in the Hula Mixer Sample Mixer and rotate at room temperature for 1 hour at 15 rpm.

    Note: During initial rotations, ensure that the gDNA gets drawn from the bottom of the microfuge tube to reside in the lid of the tube during rotations. If the DNA solution remains in the bottom of the tube during initial rotations, turn off the Hula Mixer and position the rack so the microfuge tube is oriented upside down. Gently flick the bottom of the microfuge tube until the gDNA is drawn into the lid and resume mixing.
47. Remove microfuge tube from the Hula Mixer and spin the tube on a benchtop microcentrifuge for 2 seconds to bring the gDNA to the bottom of the tube. Allow the gDNA to equilibrate overnight at room temperature (25°C) to homogenize.

    Note: Most samples will become homogenous by the third day of the beginning of the protocol, but samples may be labeled as soon as they become homogenous.

gDNA Quantitation (45 minutes)

Qubit Quantitation - BR dsDNA Assay

Refer to the Qubit dsDNA BR Assay Kit user manual for kit details and follow the methods described in the "Pipetting Viscous Genomic DNA" section, to ensure accurate pipetting of viscous gDNA.

1. Equilibrate the Qubit BR Assay Kit Standards to room temperature.

   Note: If the gDNA has been stored at 4ºC, equilibrate at room temperature before moving to the next step.
2. Add Qubit BR Buffer to 0.5 ml Qubit Assay Tubes:
   a. For each sample, add 18 µl of Qubit BR Buffer to three separate Qubit Assay Tubes.
   b. For the Qubit Standards, add 10 µl Qubit BR Buffer to two separate Qubit Assay Tubes.
3. Using a 200 µl pipette with a wide bore tip, gently mix the entire gDNA sample volume by pipetting up and down 5 times, being careful not to generate bubbles.
4. Using a fresh standard bore pipette tip or positive displacement pipette tip for each draw:

Remove 2 µl aliquots from the left side, middle, and right side of each sample and dispense into the BR Buffer of corresponding Qubit Assay Tube, rinsing the tip when dispensing. Place the Assay Tubes in a floating rack and sonicate for 10 minutes. Perform steps 5 and 6 during sonication.

Note: If a bath sonicator is not available, vortex for at least 30 seconds at maximum speed, then spin down briefly for 2 seconds.

5. Prepare Working Solution by diluting the Dye Assay Reagent into BR Dilution Buffer (1:200):
    a. 200 µl Working Solution for each of the two standards (400 µl total).
    b. 200 µl Working Solution for each sample aliquot (600 µl for each sample).
6. For the Qubit DNA standards, add 10 µl of Standards 1 and 2 to the Assay Tubes containing BR Buffer from step 2b.
7. Once sonication is complete, retrieve assay tubes and pulse spin briefly. Vortex tubes for 5 seconds at maximum speed, then pulse spin again.
8. Add 180 µl of Working Solution to each sonicated DNA aliquot and Qubit DNA Standard aliquot. Vortex for 5 seconds, and pulse spin the tubes.
9. Incubate samples for at least 2 minutes, then read on the Qubit Fluorometer.
10. Coefficient of Variation (CV = standard deviation/mean) from three separate readings should be < 0.30.

Note: If CV > 0.30, gently pipette-mix the entire volume of gDNA with five strokes (1 stroke = 1 up stroke + 1 down stroke) using a wide bore tip. Let the gDNA rest at least overnight at room temperature before repeating quantitation.

Note: Typical DNA concentrations range from 50-120 ng/µl.

Sample ID	Left (ng/µl)	Middle (ng/µl)	Right (ng/µl)	CV (st dev/mean)

Labeling

Direct Label and Stain (DLS) labeling. See “Kits and Consumables” section at https://bionanogenomics.com/support/ for applicable kits and protocols.

Troubleshooting

See Training Video starting at 8:40 for video explanations of troubleshooting.

The gDNA comes unbound from the Nanobind Disk.

Evidence: gDNA is aspirated or becomes detached from disk during binding or during washes.

Steps to follow if sample is aspirated:

1. Leaving the sample tube racked on the magnet, dispense gDNA-containing liquid back into tube containing the disk.
2. Remove racked tube from the magnet and invert rack multiple times by hand to re-establish binding.

Alternatively:

1. Leaving the sample tube racked on the magnet, dispense gDNA-containing liquid back into the tube containing the disk.
2. Aspirate liquid from the tube such that a minimal volume (~50 µl) remains above unbound gDNA. Discard supernatant leaving the DNA in a minimal volume at bottom of the tube.
3. Carefully aspirate unbound gDNA containing the minimal liquid into the pipet tip and place directly onto the racked disk on the magnet to re-establish binding.

The gDNA is not homogeneous before labeling.

Evidence: The gDNA quantitation CV of three measurements (top, middle and bottom) is > 0.30.

Steps to follow:

1. Aspirate and dispense sample using a wide bore tip for a total of five times.
2. Incubate the gDNA at room temperature for 1 to 3 days.
3. After incubation, again aspirate and dispense the sample using a wide bore tip five times.
4. Quantitate with the Qubit BR Assay.

The gDNA is not viscous.

Evidence: Sample consistency is very thin and easily pipetted, but concentration is > 35 ng/µL.

The sample likely does not have high molecular weight gDNA.

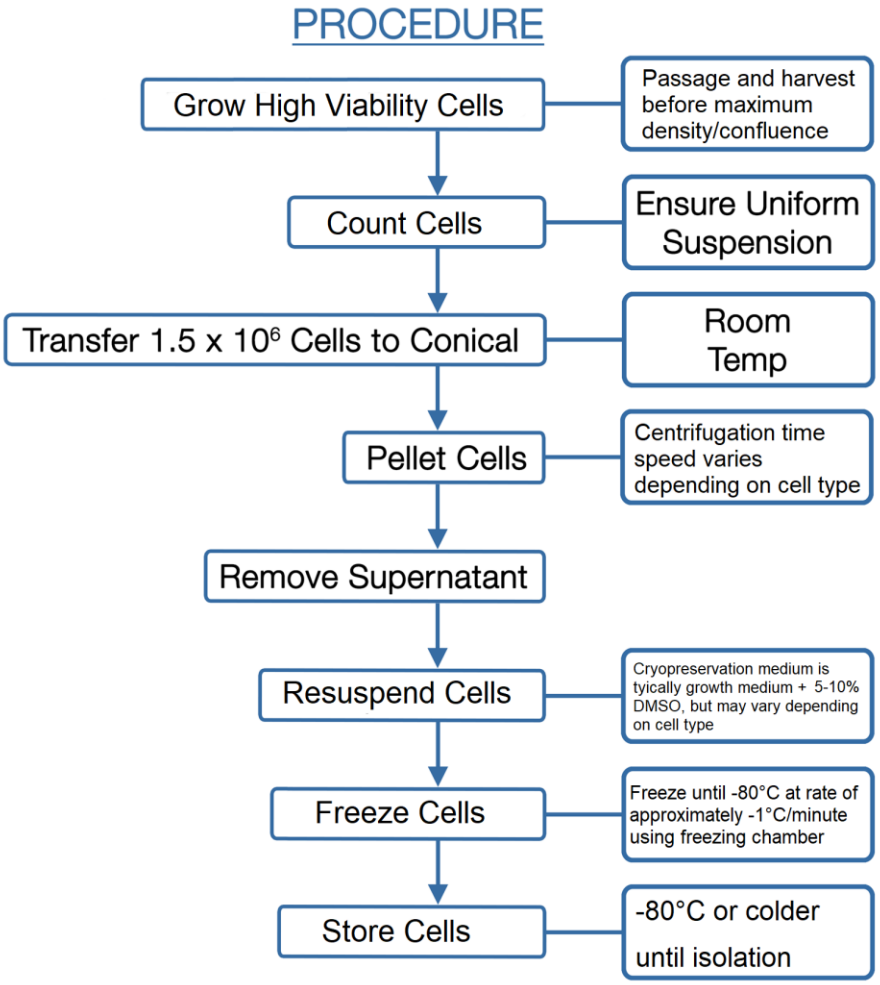
Check the sample using pulse field gel electrophoresis before labeling to confirm presence of high molecular weight gDNA.

Evaluate the sample prep method and the quality/age of the input material. Repeat DNA isolation from the biological sample.

Appendix: Preparing Cryopreserved Cells for Storage

User-Supplied Materials

Item	Supplier	Catalog #
Day 0 – Cryopreserved cells		
Centrifuge with a Swinging Bucket Rotor for 50 ml Conical Tubes to Concentrate Cells from Media (2,200 x g spin)	General Lab Supplier	
Conical Centrifuge Tubes, 50 ml, PP	Thermo Fisher or Equivalent	14-432-22
Bleach for Cell Media Disposal	General Lab Supplier	
Growth medium for cells	General Lab Supplier	
Dimethylsulfoxide (DMSO) for cell culture	Sigma-Aldrich or Equivalent	S-002-D
Fetal Bovine Serum (if applicable for cell type)	Sigma-Aldrich or Equivalent	12103C
Freezing container	Thermo Scientific or Equivalent	5100-0001
Sterile 5- and 10-ml Disposable Pipettes (TD+)	General Lab Supplier	
1000 µl Tips, Sterile	General Lab Supplier	
Cryovials, Sterile	General Lab Supplier	



Count Cells, Pellet, Remove Supernatant, Resuspend Cells, and Transfer to Labeled Microfuge Tubes

Recommended input: 1.5 million viable mammalian cells at a density of ≥ 2 million cells per ml cryopreservation medium. Cells less than this amount may not produce sufficient gDNA, and insufficient density may reduce cell viability. It is recommended that cells have ≥ 80% viability.

Note: Optimal cryopreservation conditions vary by cell type. The following protocol has been tested with the human lymphoblastoid cell line NA12878 and is provided as an example. If an existing protocol has been optimized to maintain high viability in a user's cell lines, it is recommended to continue to use that protocol. A typical cryopreservation medium consists of growth medium with 5-10% DMSO.

Example Cryopreservation Medium for Lymphoblastoid Cell Line

- RPMI 1640 (Sigma R8758 or Equivalent).
- 20% FBS (Gibco, p/n 10438-026 or Equivalent).
- 6% DMSO (Sigma-Aldrich S-002-D or Equivalent)

Recommended Culture Conditions

1. Seed/split cells so there is no less than 200,000 viable cells/mL
2. To avoid slow growth and/or low viability, split or harvest cells before they reach maximum density or confluence.
3. For best data quality, it is important to maximize cell viability.

Freezing Cells

1. Grow enough cells to seed/maintain a backup culture after removing ≥1.5 million viable cells for shipment. Flasks may be pooled if necessary.
2. Calculate total number of viable cells; pipette first to gently break up cell aggregates.
3. Centrifuge for 10 min at 120-300 x g at 4°C.
4. Resuspend pellet in freezing medium at a density of ≥ 2 x 10^6 cells/mL:
5. Aliquot ≥ 1.5 x 10^6 cells in freezing medium into cryovials.
6. Freeze overnight at -1°C/min in -80°C freezer using freezing chamber containing fresh isopropanol (see manufacturer's instructions).
7. After the cells have completely frozen, it is recommended to transfer cryovials to a liquid nitrogen tank for long-term storage.

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.

Type	Contact
Email	support@bionanogenomics.com
Phone	Hours of Operation: Monday through Friday, 9:00 a.m. to 5:00 p.m., PST US: +1 (858) 888-7663
Website	www.bionanogenomics.com/support

Bionano Genomics, Inc.
9540 Towne Centre Drive, Suite 100
San Diego, CA 92121