Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol

Document Number: 30268

Document Revision: D

Table of Contents

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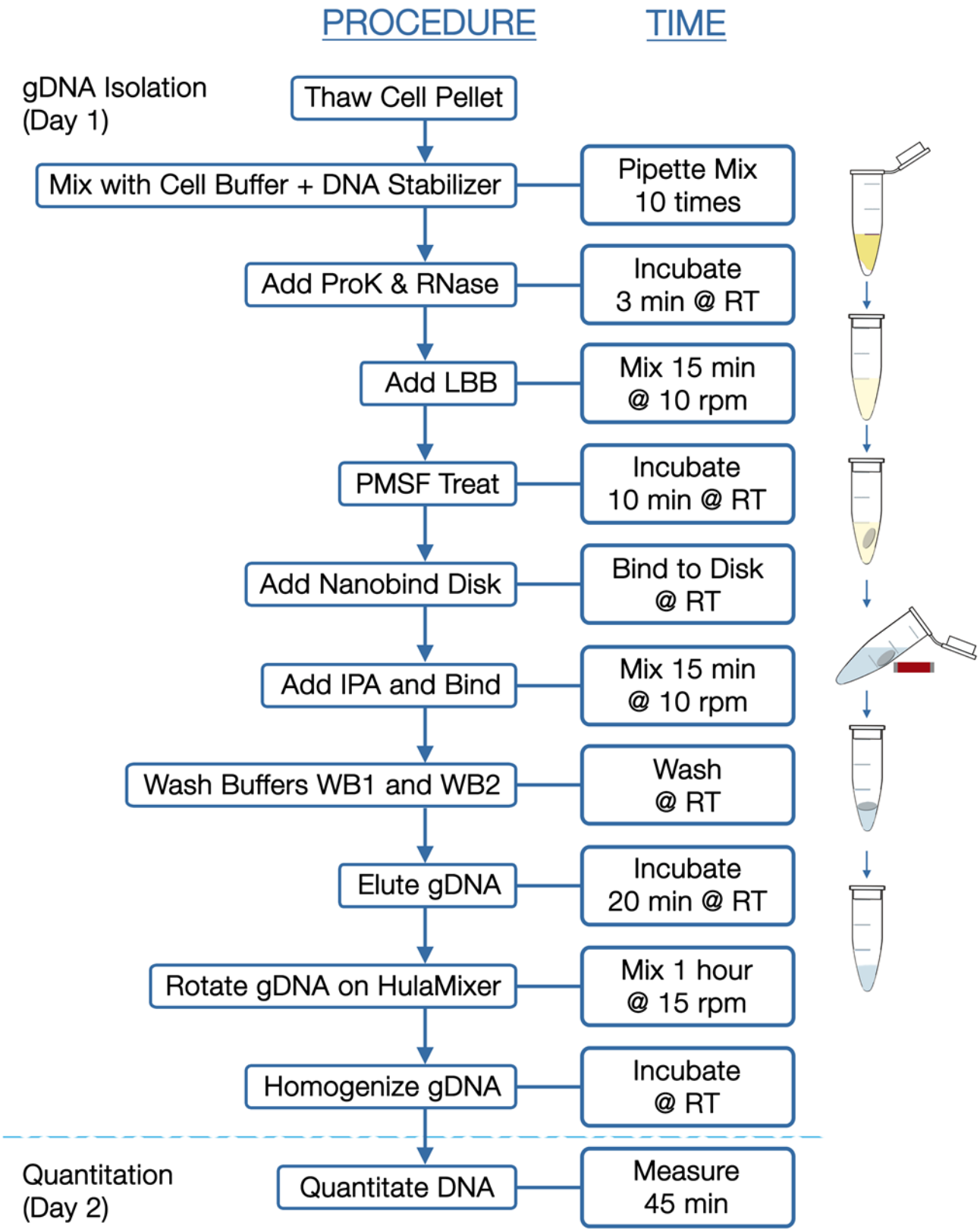
Revision History

Revision	Notes
A	Initial Release
B	Updated name for Bionano Prep SP Magnetic Retriever
C	Included additional indications, pointing the user towards the Appendix for preparing frozen cell pellets.
D	Corrected Part Number of Standard Microfuge Tubes, 2.0 ml

Workflow Overview



Bionano Prep SP DNA Isolation Kit and User-Supplied Materials

Table 1: Bionano Prep SP Blood & Cell Culture DNA Isolation Kit Contents (Part # 800030, 10 preps)

Item	Amount	Part Number	Storage
Nanobind Disks	10 disks	20379	Room Temp (18-25°C)
Protein LoBind Microcentrifuge Tubes, 1.5 ml	20 tubes	20380	Room Temp (18-25°C)
RNase A Enzyme	200 µl	20373	Refrigerate (4°C)
DNA Stabilizer	20 µl	20397	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

Table 2: User-Supplied Materials

Item	Supplier	Catalog #
Day 1 – Counting, Pelleting, gDNA Isolation and Homogenization		
Bionano Prep SP Magnetic Retriever (2 pack)	Bionano Genomics	80031
Hemocytometer & Phase Contrast Microscope or Automated Cell Counter	General Lab Supplier	
DynaMag-2 Magnetic Tube Rack	Thermo Fisher	12321D
HulaMixer Sample Mixer	Thermo Fisher	15920D
Microcentrifuge Tubes, 1.5 ml, Nuclease Free	VWR	87003-294
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
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
Centrifuge with 1.5 ml Tube Rotor (2,200 x g spin)	General Lab Supplier	
Centrifuge with a Swinging Bucket Rotor for 15 ml Conical Tubes to Concentrate Cells From Media (2,200 x g spin)	General Lab Supplier	
Ice Bucket and Ice	General Lab Supplier	
Sterile 5 and 10 ml Disposable Pipettes (TD+)	General Lab Supplier	
Mini Benchtop Microcentrifuge (2,200 x g spin)	Labnet	C1301B
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	
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 Frozen Cells DNA Isolation Protocol 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 EBV immortalized human lymphoblastoid cell line (GM12878) that grows in suspension culture. gDNA prepared using this protocol has been validated 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 here.

Overview

Cell lysis and Proteinase K digestion occurs 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 to make the UHMW gDNA more homogeneous. 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

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. Be patient. 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 (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 tip of the pipette tip indicating under-sampling (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 Qubit gDNA quantitation assay with 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 tube and insert the sheathed retriever into a new Protein LoBind microfuge tube.
d. Holding the sheath on the side near the top, with one hand pull the retriever up until the Nanobind Disk disassociates from the sheath and drops into the new tube.
e. Change 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. This conforms to disposal requirements in the state of California, US, but may be different for your location. Please consult local requirement for decontamination and disposal.

Bionano Prep SP Frozen Cells DNA Isolation Protocol

Preparation for gDNA Isolation from Frozen Cell Pellets

Note: For best results, we encourage preparing frozen cell pellets as described in the Appendix.

Before First Use

- Verify access to tabletop centrifuge with swinging bucket rotor that can accommodate 15 ml polypropylene conicals to concentrate cells from media.
- Verify mini benchtop microcentrifuge 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.

Set Up

- Gather materials (see “User Supplied Material” section above).
- Prepare 37°C water bath or heat block to thaw frozen cell pellets. Verify temperature with thermometer.
- For each sample, prepare Stabilizing Buffer by mixing Cell Buffer (Bionano) with DNA Stabilizer (Bionano):
    - 50 µl Stabilizing Buffer = 49 µl Cell Buffer + 1 µl DNA Stabilizer, vortex to mix and pulse spin.
- 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 two Protein LoBind Tubes (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 frozen Cell Pellets, add Stabilizing Buffer, Resuspend Cells and Transfer to Protein LoBind Tubes

Note: For important instructions on preparing Frozen Cell Pellets, please refer to Appendix.

1. Thaw the cell pellets containing 1.5 million cells in a 37°C water bath or heat block for 30 seconds.
2. Add 40 µl of Stabilizing Buffer on the top of each pellet.
3. 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 previously labeled Protein LoBind tube with a standard 200 µl tip.

Lyse and Digest Cells

4. 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.**
5. Incubate at room temperature for 3 minutes.
6. 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 pipette tip. Dispense slowly and change tips between dispensing to ensure accuracy of dispense volume.
7. Rotate sample on HulaMixer for 15 minutes at room temperature at 10 rpm. No shaking/vibration.
8. Pulse spin tube for 2 seconds to collect liquid at the bottom of the tube.
9. Add 10 µl of 100 mM PMSF into the liquid portion of tube. Cap and invert tube 5 times to mix, pulse spin tube for 2 seconds to collect liquid at the bottom of the tube.
10. Incubate at room temperature for 10 minutes.

gDNA Bind, Wash and Elute

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

    Note: Disks can sometimes stick together.
12. Add 340 µl 100% isopropanol to all tubes. Cap and invert tubes 5 times to mix.
13. Rotate sample on HulaMixer for 15 minutes at room temperature at 10 rpm. No shaking/vibration.

    Note: Ensure that the Nanobind Disk does not remain in the lid of the tube during initial rotations. If it does, turn off rotator and invert microfuge tube until the Nanobind Disk goes back into the solution. Replace the tube on the HulaMixer and resume mixing.
14. Examine gDNA association with Nanobind Disk and invert to increase binding (See Training Video, 0:25):
    a. Place sample tubes into clear Dynamag tube rack and visually inspect all tubes in 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.

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

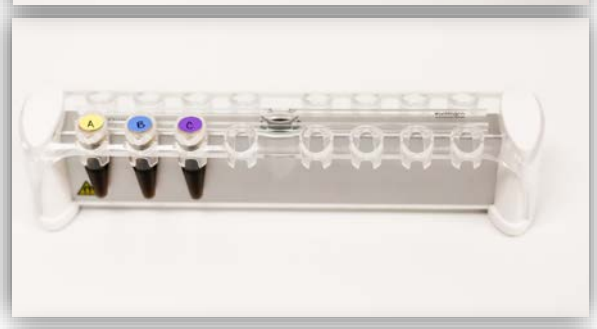
    Note: The color of liquid in the pictures below was modified for illustrative purposes.

    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 you.

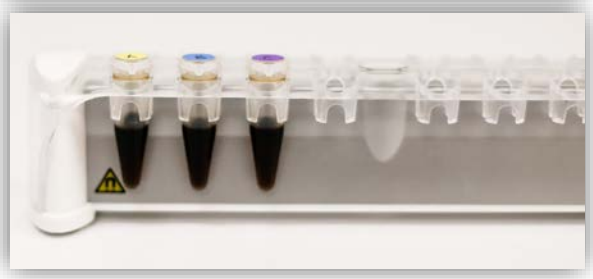
    b. Invert Dynamag magnetic base and lower onto clear rack.

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

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



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



16. Set one 1,000 µl pipette to 1,000 µl and a second to 700 µl.
17. Remove supernatant as outlined below, 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 to you and lids towards your other hand).
    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 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 Troubleshooting section below.

18. 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 Troubleshooting section below.

19. Set the second pipette to 500 µl (previously at 700 µl).
20. 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 Troubleshooting section below.

21. 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.

22. Open tube lid fully (parallel to lab bench) and lift each tube apart from base.

23. In close proximity to a new Protein LoBind tube, transfer Nanobind Disk to a new Protein LoBind tube using Bionano Prep SP Magnetic Retriever (see Important Notes section for proper usage). Cap tube to prevent disk drying (See Training Video, 7:30).
24. Spin the Protein LoBind tube in benchtop microcentrifuge for 5 seconds.
25. 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 tip around with small circular motion to remove all residual liquid.
26. Add 110 µl of Buffer EB to Protein LoBind tube.
27. Spin the tube on benchtop microcentrifuge for 5 seconds.
28. Using a 10 µl standard tip, gently nudge 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).
29. Incubate submerged Nanobind Disk in Buffer EB at room temperature for 20 minutes.
30. Collect extracted gDNA by transferring eluate to previously labeled 2.0 ml microfuge tube with a 200 µl standard tip.
31. Spin the tube with the Nanobind Disk on benchtop microcentrifuge for 5 seconds and transfer all of the remaining eluate containing viscous gDNA to the same standard 2.0 ml microfuge tube as in previous step with a standard 200 µl tip. You may remove the disk before aspirating remaining elution buffer.

    Note: Almost all of the viscous gDNA comes off the Nanobind Disk during the spin.

Homogenization of gDNA Solution (70 minutes)

Homogenization of gDNA Solution

32. 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.
33. Place standard 2.0 ml microfuge tube containing gDNA in rack of 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 Hula Mixer and position rack so the microfuge tube is oriented upside down. Gently flick the bottom of the microfuge tube until the gDNA is drawn to into the lid and resume mixing.

34. Remove microfuge tube from rack of Hula Mixer and spin tube on 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 (from the start 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 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 BR Buffer of corresponding Qubit Assay Tube, rinsing tip when dispensing. Place 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 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 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 (stdev/mean)

Labeling

DNA is ready for 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 disk.
2. Remove racked tube from 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 tube containing disk.
2. Aspirate liquid from tube such that a minimal volume (~50 µl) remains above unbound gDNA and discard supernatant leaving the DNA in a minimal volume at bottom of the tube.
3. Carefully aspirate unbound gDNA containing the minimal liquid into pipet tip and pipet directly onto racked disk on 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 5 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 5 times.
4. Quantitate with 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 is likely not to have high molecular weight gDNA.

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

Evaluate sample prep method and input material quality/age and repeat DNA isolation from biological sample.

Appendix: Preparing Frozen Cell Pellets for Storage

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

Recommended input: 1.5 million viable mammalian cells.

Note: Cells less than this amount may not produce sufficient gDNA, and excessive amounts of cells may produce gDNA that is less pure and more difficult to become homogeneous.

1. Prepare Stabilizer Buffer by combining 49 µl of Cell Buffer + 1 µl DNA Stabilizer for each of the pellets you plan to prepare.
2. For each sample, pipet isolated cells in growth media repeatedly to ensure a uniform suspension.

   Note: If possible, make sure the cells are actively growing with high viability as this maximizes quality and size of isolated gDNA.
3. Quickly remove an aliquot, and with or without dilution, count cells with cell counting device.
4. Calculate the volume of original cell stock required for 1.5 million cells.
5. After pipet mixing to ensure a uniform suspension, transfer volume for 1.5 x 10^6 cells to a labeled 15 ml polypropylene conical.

   Note: You can make multiple pellets of the sample cell line or a few pellets of multiple cell lines.
6. After all the samples are in labeled conicals, pellet the cells by centrifugation using a swinging bucket rotor at 2,200 x g for 2 minutes at room temperature.
7. Remove the supernatants by decanting into the waste conical with bleach and use a Kimwipe® to absorb residual liquid from inverted cell pellet conical.
8. Add 40 µl of Stabilizer Buffer on top of each pellet.
9. Disrupt the pellet with a 200 µl wide bore tip, then continue to resuspend the pellet by pipetting up and down 10 times.
10. Transfer the entire volume of suspension (>40 µl) into a labeled 1.5 ml microcentrifuge tube with a standard 200 µl tip.
11. Pellet the cells in a microcentrifuge by spinning at 2,200 x g for 2 minutes at room temperature.
12. Using a standard 200 µl tip, carefully remove as much of the supernatant as possible without disturbing the pellet.
13. Freeze and store the cell pellets at -80°C.

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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