Bionano Prep SP-G2 Frozen Cell Pellet DNA Isolation Protocol

DOCUMENT NUMBER:
CG-00004

DOCUMENT REVISION:
B

Effective Date: 01/18/2023

bionano

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Legal Notice

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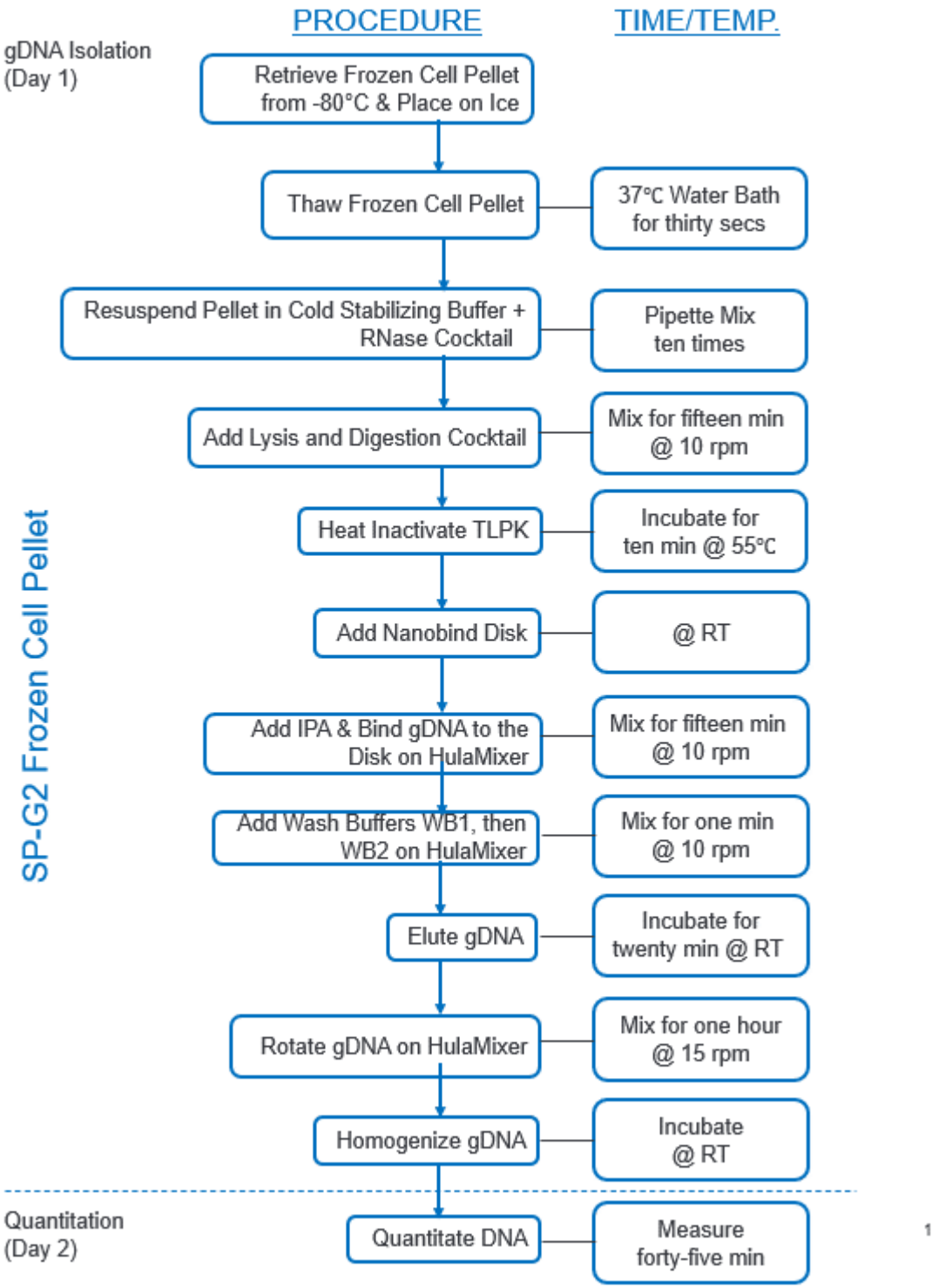
Revision History

REVISION	NOTES
A	Commercial release.
B	General formatting changes for release.

Workflow Overview



Bionano Prep SP-G2 Blood & Cell Culture DNA Isolation Kit and User-Supplied Materials

Bionano Prep SP-G2 Blood & Cell Culture DNA Isolation Kit Contents (Part # 80060, 12 preps)

Item	Amount	Part Number	Storage
RBC Lysis*	18 ml	20442	Room Temp (15-30°C)
Cell Buffer	50 ml	20374	Room Temp (15-30°C)
Digestion Enhancer	4.0 ml	20443	Room Temp (15-30°C)
Lysis and Binding Buffer (LBB)**	1.2 ml	20444	Room Temp (15-30°C)
Wash Buffer 1 (WB1)**	4.5 ml	20445	Room Temp (15-30°C)
Wash Buffer 2 (WB2)	6.0 ml	20446	Room Temp (15-30°C)
Elution Buffer (EB)	1.1 ml	20378	Room Temp (15-30°C)
DE Detergent	55 µl	20447	Room Temp (15-30°C)
4mm Nanobind Disks	12 ea.	20448	Room Temp (15-30°C)
Protein LoBind Microcentrifuge Tubes, 1.5 ml	2 x 12 ea.	20449	Room Temp (15-30°C)
Protein LoBind Microcentrifuge Tubes, 0.5 ml	12 ea.	20450	Room Temp (15-30°C)
Magnetic Retriever Plastic Sheath	12 ea.	20451	Room Temp (15-30°C)
Microcentrifuge Tubes, 2.0 ml	12 ea.	20452	Room Temp (15-30°C)
DNA Stabilizer	350 µl	20423	Room Temp (15-30°C)
RNase A	150 µl	20455	Refrigerate (2°C-8°C)
Ultrapure Water	2 x 900 µl	20355	Refrigerate (2°C-8°C)
Thermolabile Proteinase K (TLPK)	150 µl	20441	Freeze (-15°C to -25°C)

*Not used in this protocol.

**See Important Notes section for hazardous waste information.

User-Supplied Materials and Equipment

Item	Supplier	Catalog #
Day 1 – Pelleting, gDNA Isolation and Homogenization		
Bionano Prep SP Magnetic Retriever (2 pack)	Bionano Genomics (Training Kit)	80031
DynaMag-2 Magnetic Tube Rack	Thermo Fisher	12321D
HulaMixer Sample Mixer	Thermo Fisher	15920D
Microcentrifuge Tubes, 2.0 ml, Nuclease Free	Fisher Scientific or Equivalent	05-408-138
Microcentrifuge Tube, 5.0 ml, Nuclease Free	Thomas Scientific or Equivalent	1201T80
Ethanol, 200 Proof, Molecular Biology Grade	Sigma-Aldrich	E7023
Isopropanol (IPA), ≥ 99.5%, Molecular Biology Grade	Fisher Scientific	A461-212
Bleach for Blood Disposal	General Lab Supplier	
Conical Centrifuge Tubes, 50 ml, PP	Thermo Fisher or Equivalent	14-432-22
15 ml Conical Tube	Fisher Scientific	05-539-12
Centrifuge with 1.5 ml Tube Rotor (2,200 x g spin)	Cole-Parmer or Equivalent	EW-17701-11
Water Bath, 37°C	General Lab Supplier	
Ice Bucket and Ice	General Lab Supplier	
Thermomixer, 55°C	Eppendorf or Equivalent	5382000023
Parafilm	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
Filtered 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	
Aluminum Cooling Block for 1.5 ml and 2.0 ml (optional)	Sigma-Aldrich or Equivalent	Z743497
Cryopreservation Box (for 1.5 ml microcentrifuge tubes)	General Lab Supplier	
Day 2 - Quantitation		
Benchtop Vortexer	VWR or Equivalent	10153-838
Bath Sonicator	General Lab Supplier	
15 ml Conical Tube	Fisher Scientific	05-539-12
Fluorometer, Qubit	Thermo Fisher or Equivalent	Q33216
Qubit dsDNA BR 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® SP-G2 Frozen Cell Pellet DNA Isolation Protocol can provide ultra-high molecular weight (UHMW) genomic DNA (gDNA) in approximately three and a half hours from 1.5 million frozen cells. It utilizes an improved 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 evaluated using an Epstein Barr Virus (EBV) immortalized human lymphoblastoid cell line (GM12878) that grows in suspension culture. gDNA prepared using this protocol has been validated only with Direct Label and Stain (DLS) labeling. See Training Video for technically critical steps and troubleshooting; the steps mentioned in the video correspond to the SP-G2 Frozen Human Blood Protocol, but they are the same processes described here.

Overview

Cell lysis and Thermolabile 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 pipette 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 pipette, follow guidelines in the **Important Notes** for accurate pipetting. Standard assays for quantification of gDNA concentration will not provide accurate measurements of long gDNA due to its viscous nature:

- Sonication of sampled gDNA is necessary for accurate quantitation.
- Typical gDNA concentration is 45-120 ng/µl.

PIPETTING VISCOUS 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 gently 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 to 2 µl volume. 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 three to five 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, can produce a bubble at the top of the pipette tip indicating under-sampling (start over if this happens).

gDNA HANDLING

- Mixing of recovered gDNA (after homogenization steps) is always conducted with a wide bore pipette tip to prevent shearing.
- Recovered gDNA should never be frozen or vortexed.
- gDNA may become non-homogenous during extended storage.
- Pipetting of recovered gDNA for accurate sampling is always conducted with a standard bore 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.
- The 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 Coefficient of Variation (CV) of ≤ 0.30 (recommended).

USING THE BIONANO PREP SP MAGNETIC RETRIEVER

1. 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.
2. Insert the sheathed retriever into a 1.5 ml Protein LoBind microcentrifuge tube to attract the Nanobind Disk to the retriever in the sheath.
3. Carefully lift the sheathed retriever with the bound disk out of the tube and insert the sheathed retriever into a 0.5 ml Protein LoBind microcentrifuge tube until the disk is gently wedged at the bottom of the tube.
4. 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 stays in the 0.5 ml Protein LoBind tube.
5. Change sheath for each new sample.

BATCH SIZE

Processing no more than six samples at a time and up to two batches per working day is recommended.

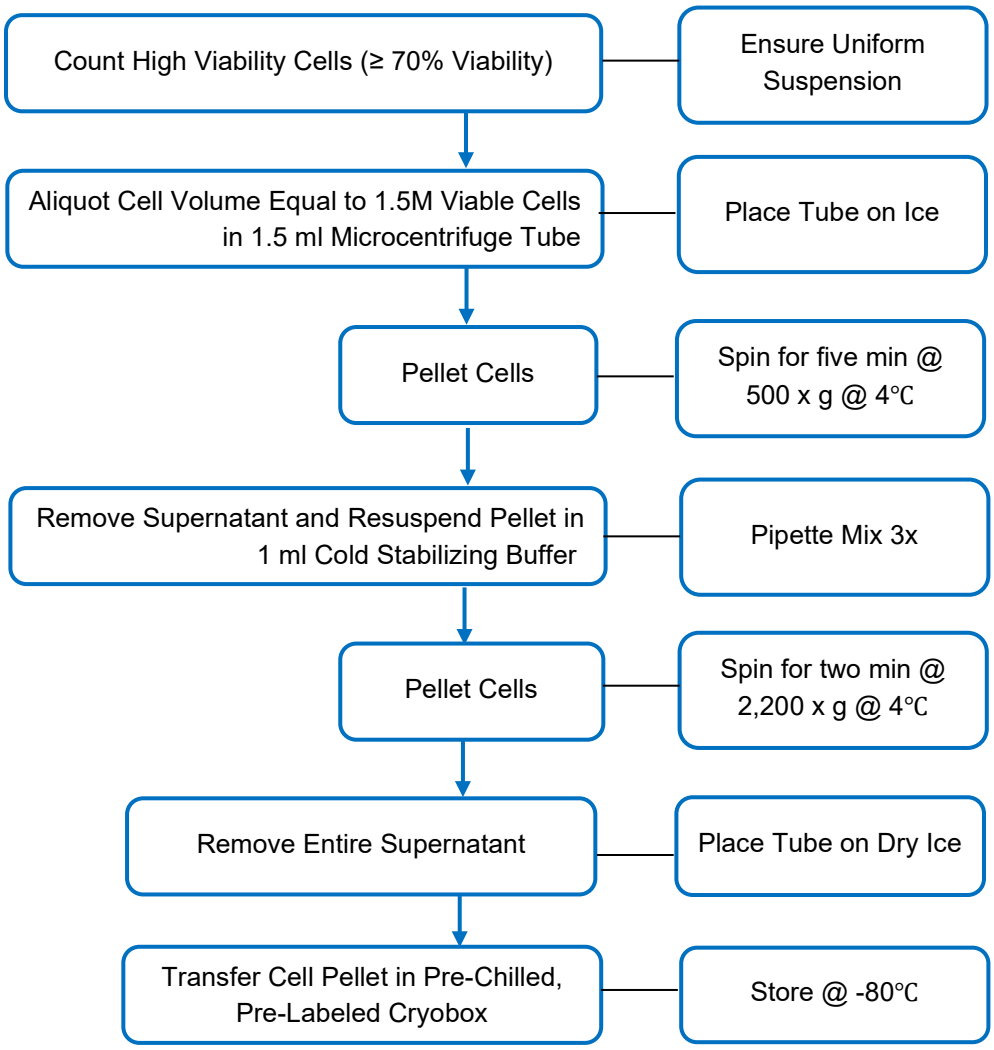
HAZARDOUS WASTE DISPOSAL

Buffers Digestion Enhancer, 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 following local environmental, health and safety regulations for decontamination and disposal of all solutions mixed with GuHCl.

Preparing Frozen Cell Pellets for Storage

Recommended Input: 1.5E+06 viable mammalian cells with ≥ 70% live cell viability

MAKING FROZEN CELL PELLETS PROCEDURE



SET UP

1. Gather materials and verify needed equipment.
   a. Set water bath to 37°C. Verify temperature with thermometer.
   b. Pre-warm cell culture media in 37°C water bath.
   c. Prepare hemacytometer and phase-contrast microscope or automated cell counter.
   d. Verify access to swinging bucket rotor centrifuge that can accommodate 15 ml conical tubes. Set spin settings to 500 x g, 5 minutes, room temperature.
   e. Cool microcentrifuge for 1.5 ml microcentrifuge tubes to 4°C. Set spin settings to 500 x g, 5 minutes.
   f. Retrieve pipettes and tips
   g. Add ice to an ice bucket.
   h. Add dry ice to a second ice bucket.
   i. For waste disposal, prepare two 50 ml conical tubes each with 5 ml bleach and 20 ml water. Invert several times to mix.
   j. Label desired number of 1.5 ml Protein Lo-Bind microcentrifuge tubes for cell pellets.
   k. For each sample, prepare 1,200 µl Stabilizing Buffer (SB) by mixing 1,176 µl Cell Buffer with 24 µl DNA Stabilizer. Multiply by the number of preps if batch size is more than one. Vortex to mix. Place on ice.
   l. Label a cryobox for storing cell pellets and pre-chill box at -80°C.

PREPARING FROZEN CELL PELLETS PROCEDURE

1. Count Cells in Stock Cell Culture
   a. Resuspend stock cell culture to create a uniform cell suspension for counting.
   b. Count number of viable cells with a cell counting device.
      Note: Cells should be in log phase with high percent cell viability (≥ 70%) as this maximizes quality and size of isolated gDNA. Record number/percent of viable cells.
   c. Calculate the volume of original stock cell culture required for up to twelve cell pellets, each containing 1.5E+06 viable cells. If the viable cell density is < 1.25E+06 viable cells/ml, proceed to Step 2. If viable cell density is ≥ 1.25E+06 viable cells/ml, proceed to Step 3.
2. Concentrate Cells (if cell concentration is low)
   a. Transfer appropriate volume of stock cell culture into a 15 ml conical tube.
   b. Centrifuge 15 ml conical tube at room temperature at 500 x g for five minutes in a swinging bucket rotor to pellet cells.
   c. Remove supernatant and resuspend cells with a smaller volume of growth media to obtain a live cell concentration of at least 1.25E+06 live cells/ml.
   d. Count number of viable cells with a cell counting device.
   e. Calculate concentrated stock cell culture volume to yield 1.5E+06 viable cells per pellet.

3. Aliquot Cells
    a. Pipette mix stock cell culture suspension to ensure homogenous cell suspension.
    b. Aliquot the target cell volume of stock cell culture suspension into each pre-labeled, pre-chilled 1.5 ml Protein LoBind tube. Place on ice.
4. Pellet Cells
    a. Centrifuge cells at 4°C at 500 x g for five minutes in a fixed angle rotor microcentrifuge.
    b. Remove entire supernatant without disturbing the pellet. Discard supernatant in the 50 ml conical tube containing bleach. Place sample on ice.
5. Wash Cells with Cold Stabilizing Buffer
    a. Add 1 ml of cold Stabilizing Buffer to each pellet.
    b. Resuspend pellet by pipetting up and down three times with a P1000 pipette set to 1,000 µl.
    c. Centrifuge the cells at 4°C at 2,200 x g for two minutes in a fixed angle rotor microcentrifuge.
    d. After centrifugation, place samples on ice.
    e. Aspirate entire supernatant and discard into the 50 ml conical tube containing bleach. Use a P200 pipette to remove residual liquid from cell pellet.
    f. Keep samples on ice until all supernatants have been removed.
6. Freeze Cell Pellets on Ice
    a. Place cell pellets on dry ice and incubate for five minutes to snap-freeze.
    b. Transfer snap-frozen cell pellets to pre-labeled, pre-chilled (-80°C) cryobox.

    Note: Frozen cell pellets can be used for gDNA isolation the next day.

Bionano Prep SP-G2 Frozen Cell Pellet DNA Isolation Protocol

Preparation for gDNA Isolation (30 minutes)

BEFORE FIRST USE

1. Add 100% Ethanol to Wash Buffers (WB1 and WB2) and mix thoroughly:
   a. Add 6.75 ml 100% Ethanol to Wash Buffer 1 (WB1) for a final volume of 11.25 ml.
   b. Add 9.00 ml of 100% Ethanol to Wash Buffer 2 (WB2) for a final volume of 15.00 ml.

SET UP

1. Gather materials and verify needed equipment (see “User Supplied Material” section above).
   a. Set water bath to 37°C. Verify temperature with thermometer.
   b. Pipettes and tips
   c. Ice bucket and ice
   d. For waste disposal, prepare:
      - One 50 ml conical designated for GuHCl liquid waste (disposed as hazardous waste as per local environmental, health and safety regulations)
   e. HulaMixer Sample Mixer
   f. DynaMag-2 Magnetic Tube Rack
   g. 100% IPA
   h. Bionano Prep SP Magnetic Retriever
   i. Set a Thermomixer to 55°C, ten min, no shaking.
   j. Pointed Forceps
   k. Label one 2.0 ml tube (for batch size of three or fewer), or one 5.0 ml tube (for four to six samples) for the Lysis and Digestion Cocktail Master Mix.
2. Gather the following reagents and materials from the SP-G2 kit: Cell Buffer, DNA Stabilizer, RNase A Digestion Enhancer, DE Detergent, Ultrapure Water, Nanobind Disk, microcentrifuge tubes, sheath, LBB, WB1, WB2 and EB.
   a. For each sample, prepare 50 µl Stabilizing Buffer by mixing 49 µl Cell Buffer with 1 µl DNA Stabilizer. Multiply by the number of preps if batch size is more than one. Vortex to mix. Pulse spin for two seconds and place on ice.
   b. Flick RNase A 3x to mix. Pulse spin and place on ice.
   c. For each sample, prepare 48 µl Stabilizing Buffer/RNase A Cocktail Master Mix by mixing 36 µl Stabilizing Buffer with 12 µl RNase A. Multiply by the number of preps if batch size is more than one. Vortex briefly to mix. Pulse spin and place on ice.

d. Retrieve TLPK from -20°C storage and place on ice.
e. For each sample, label one 0.5 ml Protein LoBind Tube.
f. For each sample, label, and store on ice one 1.5 ml Protein LoBind tube (if frozen cell pellet sample was not previously made and stored at -80°C in a 1.5 ml Protein LoBind tube).
g. For each sample, label one 2.0 ml microcentrifuge tube for gDNA homogenization step. Place in a rack at room temperature.

3. Prepare Lysis and Digestion Cocktail Master Mix in a 2.0 ml tube for a batch size of three or fewer samples or in a 5.0 ml tube for a batch size of four to six samples. Prepare the Master Mix following the order of component addition listed in **Table 1**. Cap the tube, invert mix fifteen times, and place the tube on a tube rack at room temperature.

   Note: Do not vortex. Do not add TLPK to the Cocktail Master Mix yet.

Table 1. Lysis and Digestion Cocktail Master Mix Preparation Worksheet

Master Mix Component	Master Mix Component Volume (µl)	No. of Samples	Master Mix Excess	Master Mix Component Total Volume = Master Mix Component Volume x No. of Samples x Master Mix Excess	Order of Addition
Digestion Enhancer	270		1.2		1
Nuclease-free Water	66.25		1.2		2
LBB*	80		1.2		3
DE Detergent*	3.75		1.2		4
TLPK**	10		1.2		5
Total	**430**				

*Pipette LBB and DE Detergent slowly due to high viscosity and risk of bubble formation.
**Add immediately before use at Step 4 in gDNA Isolation.

gDNA Isolation (2 hours)

THAW FROZEN CELL PELLETS, ADD SB/RNASE, AND RESUSPEND CELLS

Recommended Input: 1.5E+06 Viable Cells

1. For each sample, remove one frozen cell pellet from -80°C freezer and place on ice. Thaw up to six cell pellets each containing 1.5E+06 cells in a 37°C water bath for thirty seconds using a floating tube rack. After thirty seconds, remove cell pellet(s) from the water bath and place on ice.
2. Add 40 µl cold Stabilizing Buffer/RNase on top of each pellet. Place on ice.
3. Processing one sample at a time, use a 200 µl standard bore pipette tip to gently scratch the pellet in a circular fashion three to five times to dislodge the pellet into solution. Then using the same tip, slowly pipette mix the sample ten times to resuspend the pellet. Place sample on ice. Change tips between samples.

Note: Aspirate the entire sample volume into the tip and visually inspect the tube while mixing to ensure that the pellet is being resuspended fully during mixing, such that by the end of mixing there is no visible pellet remaining on the side of the tube. Avoid generating bubbles.

LYSE, DIGEST, AND INACTIVATE THERMOLABILE PROTEINASE K

4. Flick the TLPK tube three times and pulse spin for two seconds. Add the TLPK volume calculated for the batch size in **Table 1** to the Lysis and Digestion Cocktail Master Mix to make the Complete Lysis and Digestion Cocktail Master Mix. Cap and invert the Master Mix fifteen times to mix, place it back in the rack at room temperature. Place the TLPK on ice.
   Note: Do not vortex. From this step forward, sample will be handled at room temperature.
5. Add 430 µl of Complete Lysis and Digestion Cocktail Master Mix to each sample. Cap the tube. Change tips between samples.
6. Invert mix each sample fifteen times.
7. Rotate sample on HulaMixer for fifteen minutes at room temperature at 10 rpm, no shaking or vibration.
8. During the rotation, return the TLPK back to -20°C storage. Discard any remaining unused Lysis and Digestion Cocktail Master Mix (with TLPK) into the 50 ml conical designated for GuHCl liquid waste.
9. Remove sample from HulaMixer, and pulse spin sample for two seconds.
10. Incubate sample in a Thermomixer pre-set to 55°C for ten mins, with no shaking.
11. Remove sample from the Thermomixer and turn the Thermomixer off.

gDNA BIND, WASH AND ELUTE

12. Using pointed forceps, carefully add a single 4 mm Nanobind Disk to the lysate.
    Note: Disks can sometimes stick together.
13. Add 480 µl of 100% IPA to each sample. Cap the tube.
14. Invert mix each sample five times.
15. Rotate sample on HulaMixer for fifteen minutes at room temperature at 10 rpm, no shaking or 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 microcentrifuge tube until the Nanobind Disk goes back into the solution. Replace the tube on the HulaMixer and resume mixing.
16. Remove sample from HulaMixer.
17. Combine Dynamag 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).

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 front.

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

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

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

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

18. Set one P1000 pipette to 1,000 µl and a second to 700 µl.

19. Remove supernatant as outlined below, being careful not to aspirate the gDNA and changing tips between samples (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 lids towards user's other hand).
    b. Wait two seconds for gDNA to lay on the Nanobind Disk.
    c. Gently 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 the 50ml conical tube designated for GuHCl liquid waste.

    ⚠ Ensure that the gDNA has not been 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 Guide (30608).

20. Perform Wash WB1:
    a. Dispense 700 µl of Buffer WB1 into the tube and cap the tube.
    b. Separate the clear rack from the Dynamag rack, and transfer samples to the HulaMixer.
    c. Rotate samples on the HulaMixer for one minute at room temperature at 10 rpm, no shaking or vibration.

    Note: The Nanobind Disk may get stuck on the side of the tube, tube lid, or at the bottom of the tube. Do not stop the HulaMixer rotation or intervene if the Nanobind Disk gets stuck anywhere in the tube as this is normal.
    d. Remove samples from HulaMixer.
    e. Place samples into the clear Dynamag rack. Invert and gently shake the clear Dynamag rack until the Nanobind Disk in each sample is not attached to any part of the tube.
    f. Combine clear tube rack containing samples with magnetic base, as described in Step 17a through 17e.
    g. Remove supernatant as described in Step 19.

    ⚠ Ensure that the gDNA has not been 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 Guide (30608).

21. Set the second pipette to 500 µl (previously at 700 µl).

22. Perform Wash WB2:
    a. Dispense 500 µl of Buffer WB2 into the tube and cap the tube.
    b. Separate the clear rack from the Dynamag rack, and transfer samples to the HulaMixer.
    c. Rotate samples on the HulaMixer for one minute at room temperature at 10 rpm, no shaking or vibration.

Note: The Nanobind Disk may get stuck on the side of the tube, tube lid, or at the bottom of the tube. Do not stop the HulaMixer rotation or intervene if the Nanobind Disk gets stuck anywhere on the tube as this is normal.

d. Remove samples from HulaMixer.

e. Place samples into the clear Dynamag rack. Invert and gently shake the clear Dynamag rack until the Nanobind Disk in each sample is not attached to any part of the tube.

f. Combine clear tube rack containing samples with magnetic base, as described in Step 17a through 17e.

g. Remove supernatant as described in Step 19.

⚠ Ensure that the gDNA has not been 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 Guide (30608).

23. Repeat Wash WB2, Step 22.
24. After removing the second WB2 supernatant, transfer the samples with caps open to the tube rack that holds the previously labeled 0.5 ml Protein LoBind tubes.
25. Fully insert the Bionano Prep SP Magnetic Retriever into a clean Magnetic Retriever Plastic Sheath until the Retriever comes into full contact with the bottom of the sheath. Change sheaths between samples.
26. Insert the sheathed Bionano Prep SP Magnetic Retriever into the 1.5 ml Protein LoBind tube and place the sheathed retriever against the Nanobind Disk until it picks up the disk. Hold the sheathed Bionano Prep SP Magnetic Retriever so that it remains in full contact with the bottom of the sheath and the Nanobind remains magnetically captured.
27. Carefully lift the sheathed retriever with the bound disk out of the tube and insert the sheathed Bionano Prep SP Magnetic Retriever into a 0.5 ml Protein LoBind microcentrifuge tube until the disk is gently wedged at the bottom of the tube.
    Note: Change the sheath between samples.

ELUTING THE gDNA

28. Add 65 µl of EB to 0.5 ml Protein LoBind tube containing the Nanobind Disk and cap the tube.
29. Spin the tube on benchtop microcentrifuge for five seconds.
30. Using a 10 µl standard tip, gently nudge the Nanobind Disk towards the bottom of the tube, ensuring that it is fully submerged in liquid. The disk should remain parallel to the bench surface (See Training Video, 8:20).
31. Incubate submerged Nanobind Disk in EB at room temperature for twenty minutes.
32. Collect extracted gDNA by transferring eluate to the labeled 2.0 ml microcentrifuge tube with a 200 µl standard tip.

33. Spin the tube with the Nanobind Disk on benchtop microcentrifuge for five seconds to separate the residual eluate from the Nanobind Disk.
34. Transfer the remaining eluate containing viscous gDNA to the same labeled 2.0 ml microcentrifuge tube with a standard 200 µl tip.

    Note: Almost all the viscous gDNA comes off the Nanobind Disk during the spin. Perform one to two more rounds of pulse spin if viscous gDNA is stuck between the disk and the bottom of the 0.5 ml Protein LoBind tube.
35. Pulse spin samples for two seconds.

Homogenization of gDNA Solution (70 minutes)

gDNA HOMOGENIZATION

36. Slowly pipette the entire gDNA volume into a standard 200 µl tip, then slowly dispense the gDNA. Avoid creating bubbles.

    Repeat this process three 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.

37. Place standard 2.0 ml microcentrifuge tube containing gDNA in rack of HulaMixer Sample Mixer and rotate at room temperature for one hour at 15 rpm.

    Note: During initial rotations, ensure that the gDNA gets drawn from the bottom of the microcentrifuge 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 HulaMixer and position the rack so that the microcentrifuge tube is oriented upside down. Gently flick the bottom of the microcentrifuge tube until the gDNA is drawn into the lid and resume mixing.
38. Remove microcentrifuge tube from rack of HulaMixer and spin tube on benchtop microcentrifuge for two seconds to pull the gDNA down to the bottom of the tube.
39. Allow the gDNA to equilibrate overnight at room temperature (25°C) to homogenize.

    Note: Most samples can be labeled the next day or within forty-eight hours post gDNA isolation using the DLS-G2 protocol (PN 30553).

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 **Important Notes** "Pipetting Viscous Genomic DNA (gDNA)" 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 and pulse spin 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 five times, being careful not to generate bubbles.

4. Using a new standard 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 ten minutes. Perform Steps 5 and 6 during sonication.

   Note: If a bath sonicator is not available, vortex for at least thirty seconds at maximum speed, then spin down briefly for two 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 five 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 five seconds, and pulse spin tubes.

9. Incubate samples for at least two minutes, then read on the Qubit Fluorometer. Record values in **Table 2** below.

10. Calculate the CV = standard deviation/mean value for each sample and record it in **Table 2** below.

    Note: If CV > 0.30, gently pipette-mix the entire volume of gDNA with 5 strokes (1 stroke = 1 up stroke + 1 down stroke) **using a wide bore tip**. Let the gDNA rest overnight at room temperature before repeating quantitation and performing DLS labeling the next day. Typical DNA concentrations range from 45-90 ng/µl.

Table 2. gDNA Quantitation (BR dsDNA) Worksheet

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

LABELING

gDNA samples are ready for Direct Label and Stain (DLS) labeling within forty-eight hours post isolation. See the "Kits and Consumables" section at https://bionanogenomics.com/support/ for applicable kits and protocols.

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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