



Bionano Prep SP-G2 Frozen Human Blood DNA Isolation Protocol

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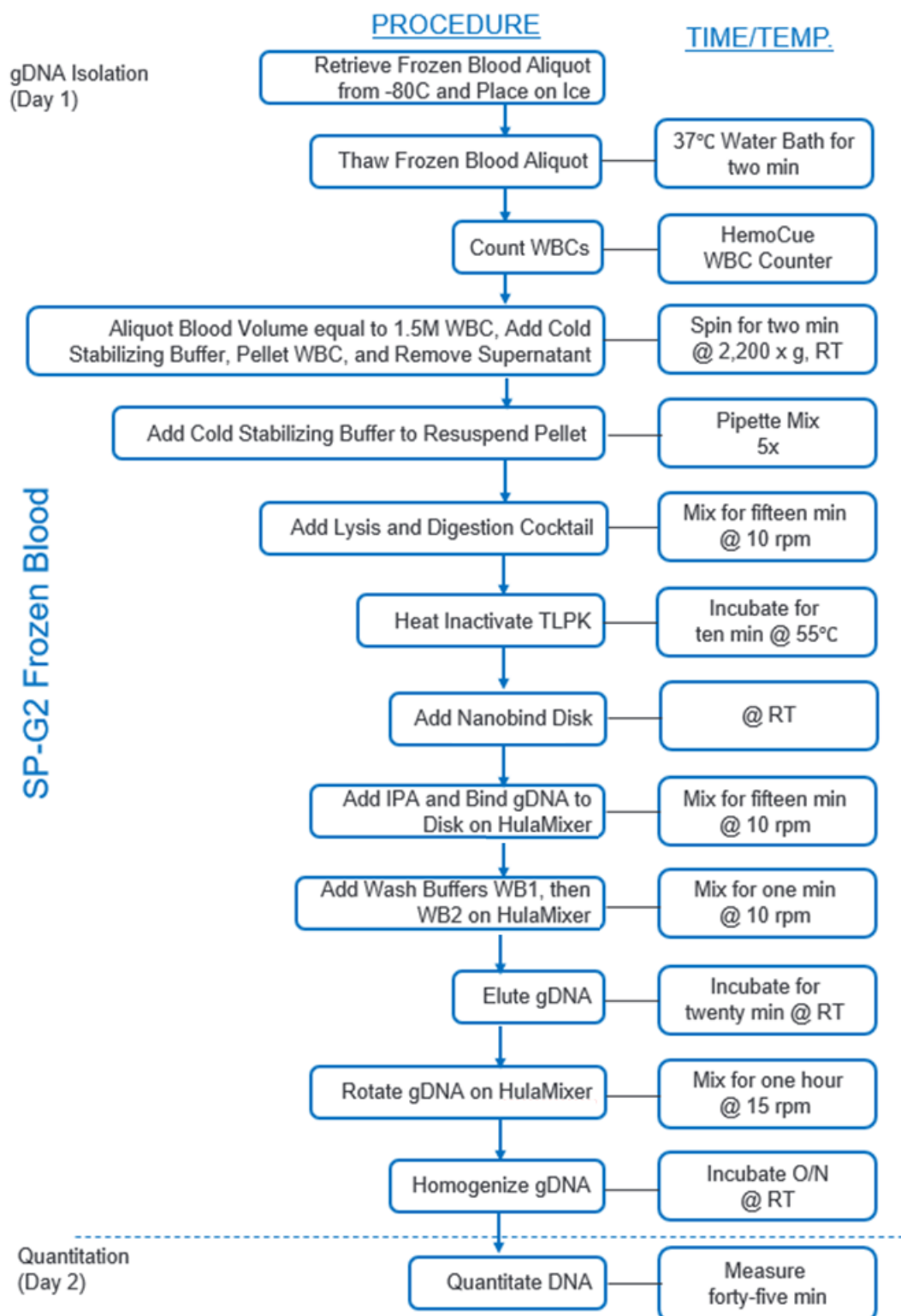
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Revision History

REVISION	NOTES
A	Commercial release.
B	General formatting changes for release.
C	Formatting updates.
D	Updated to accommodate the change from 12 to 24 reaction kits.

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 # 80118, 24 preps)

Item	Amount	Part Number	Storage	Handling Considerations
RBC Lysis*	2 x 18 ml	20442	Room Temp (15 - 30°C)	
Cell Buffer	50 ml	20374	Room Temp (15 - 30°C)	
Digestion Enhancer	6.5 ml	20457	Room Temp (15 - 30°C)	
Lysis and Binding Buffer (LBB)**	2.0 ml	20458	Room Temp (15 - 30°C)	See Important Notes Section for hazardous waste information.
Wash Buffer 1 (WB1)**	13.0 ml	20459	Room Temp (15 - 30°C)	See Important Notes Section for hazardous waste information.
Wash Buffer 2 (WB2)	17.5 ml	20460	Room Temp (15 - 30°C)	
Elution Buffer (EB)	3.6 ml	20463	Room Temp (15 - 30°C)	
DE Detergent	110 µl	20545	Room Temp (15 - 30°C)	
4mm Nanobind Disks	24 ea.	20539	Room Temp (15 - 30°C)	
Protein LoBind Microcentrifuge Tubes, 1.5 ml	2 x 24 ea.	20540	Room Temp (15 - 30°C)	
Protein LoBind Microcentrifuge Tubes, 0.5 ml	24 ea.	20541	Room Temp (15 - 30°C)	
Magnetic Retriever Plastic Sheath	24 ea.	20543	Room Temp (15 - 30°C)	
Microcentrifuge Tubes, 2.0 ml	24 ea.	20542	Room Temp (15 - 30°C)	
DNA Stabilizer	700 µl	20469	Room Temp (15 - 30°C)	
RNase A	300 µl	20462	Refrigerate (2 - 8°C)	
Ultrapure Water	2 x 1800 µl	20532	Refrigerate (2 - 8°C)	
Thermolabile Proteinase K (TLPK)	325 µl	20456	Freeze (-25 to -15°C)	

*Not used in this protocol.

**See Important Notes section for hazardous waste information.

Or

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

Item	Amount	Part Number	Storage	Handling Considerations
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)	See Important Notes Section for hazardous waste information.
Wash Buffer 1 (WB1)**	4.5 ml	20445	Room Temp (15 - 30°C)	See Important Notes Section for hazardous waste information.
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 - 8°C)	
Ultrapure Water	2 x 900 µl	20355	Refrigerate (2 - 8°C)	
Thermolabile Proteinase K (TLPK)	150 µl	20441	Freeze (-25 to -15°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
HemoCue WBC Analyzer	Fisher Scientific (for US) Distributor (<u>outside US</u>)	22-601-017
HemoCue Microcuvettes	Fisher Scientific	22-601-018
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
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 or heat block, 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
Day 2 - Quantitation		
Benchtop Vortexer	VWR or Equivalent	10153-838
Bath Sonicator	General Lab Supplier	
15 ml Conical Tube	Fisher Scientific	05-539-12

Item	Supplier	Catalog #
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 Human Blood DNA Isolation Protocol can provide ultra-high molecular weight (UHMW) genomic DNA (gDNA) in less than four hours from 1.5 million white blood cells (WBC) of frozen human blood. 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 human blood drawn into EDTA tubes, aliquoted and frozen at -80°C within four days of the draw date and processed without additional freeze/thaw cycles. For best results, it is recommended to store blood at 4°C for no longer than five days post collection before freezing. Alternatively, blood samples can be maintained at room temperature (22-25°C) for up to sixty-six hours, with up to six hours of that time being at elevated temperature (30-40°C) before freezing. Storage of blood samples at room temperature beyond sixty-six hours or prolonged exposure (> six hours) at elevated temperature can lead to reduced gDNA molecule length and compromised assay results. 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.

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 for 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** section 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 45-90 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 a 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 also 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.

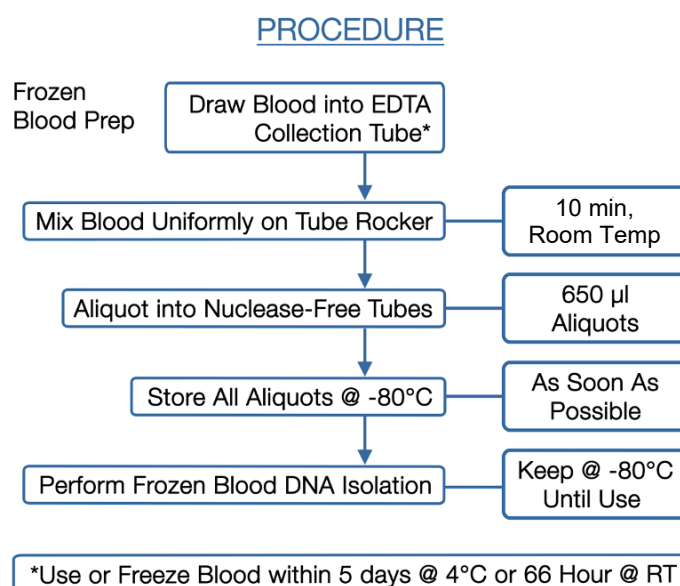
BATCH SIZE AND WBC COUNTS

Processing no more than six samples at a time and up to two batches per working day is recommended. A minimum of 2.5E+9 cells/L is required.

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.

FREEZING BLOOD FOR STORAGE



The gDNA content is obtained from the WBC. Typically, 200 - 600 µl of normal patient human whole blood drawn into an EDTA tube will be sufficient for this process. Two blood aliquots (650 µl/aliquot) should be frozen (-80°C) in separate tubes and stored without thawing until gDNA isolation. For best results, it is recommended to store blood at 4°C for no longer than five days post collection before freezing. Alternatively, blood samples can be maintained at room temperature (22-25°C) for up to sixty-six hours, with up to six hours of that time being at elevated temperature (30-40°C) before freezing. Storage of blood samples at room temperature beyond sixty-six hours or prolonged exposure (> six hours) at elevated temperature can lead to reduced gDNA molecule length and compromised assay results. Typically, only one aliquot will be required for this protocol, with the second serving as a backup for any failure.

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- Mix fresh human whole blood thoroughly at room temperature to ensure good uniformity (ten minutes on a test tube rocker at room temperature).
 - Transfer two 650 µl aliquots into nuclease-free 1.5 ml microcentrifuge tubes (user-supplied material).
 - Cap tubes and immediately move aliquots to -80°C for long term storage.
 - Do not thaw aliquot from -80°C until proceeding with gDNA isolation.

Bionano Prep SP-G2 Frozen Human Blood DNA Isolation Protocol

Preparation for gDNA Isolation (30 minutes)

BEFORE FIRST USE

1. Add 1.5X volume of 100% Ethanol to Wash Buffers (WB1 and WB2) according to the given volumes on the WB1 and WB2 bottles and mix thoroughly.

SET UP

1. Gather materials and verify equipment (see “User Supplied Material” section above).
 - a. Set water bath to 37°C. Verify temperature with thermometer.
 - b. Pipettes and tips
 - c. Prepare strips of Parafilm (~ 2 cm) for HemoCue, ready microcuvettes and [HemoCue system](#).
 - d. Ice bucket and ice
 - e. Verify microcentrifuge spin speed is 2,200 x g, two minutes, room temperature.
 - f. For waste disposal, prepare:
 - One 50 ml conical with 5 ml bleach + 20 ml water; invert several times to mix.
 - One 50 ml conical designated for GuHCl liquid waste (disposed as hazardous waste as per local environmental, health and safety regulations)
 - g. HulaMixer Sample Mixer
 - h. 100% IPA
 - i. DynaMag-2 Magnetic Tube Rack
 - j. Bionano Prep SP Magnetic Retriever
 - k. Set a Thermomixer to 55°C, ten min, no shaking.
 - l. Pointed Forceps
 - m. Label one 2.0 ml microcentrifuge tube for a batch size of three or fewer samples or one 5.0 ml microcentrifuge tube for a batch size of 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, Digestion Enhancer, DE Detergent, Ultrapure Water, Nanobind Disk, microcentrifuge tubes, sheath, LBB, WB1, WB2, and EB.
 - a. 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 and place on ice.

- b. For each sample, label one 0.5 ml Protein LoBind Tube (Bionano) and one 1.5 ml Protein LoBind Tube (Bionano). Place the 1.5 ml Protein LoBind tube(s) on ice.
 - c. For each sample, label one 2.0 ml microcentrifuge tube (Bionano) for the gDNA homogenization step. Place in a rack at room temperature.
3. Prepare Lysis and Digestion Cocktail Master Mix in a 2.0 ml microcentrifuge tube for a batch size of three or fewer samples or in a 5.0 ml microcentrifuge tube for a batch size of four to six samples. Prepare the Master Mix following the component order of 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 right before use at Step 11 in gDNA Isolation.

gDNA Isolation (2 hours 35 minutes)

THAW/COUNT/ALICQUOT BLOOD, DILUTE BLOOD WITH COLD SB, PELLET WBCS, AND REMOVE SUPERNATANT

1. For each sample, remove one 650 μl aliquot of frozen blood from -80°C freezer and place on ice. Thaw up to six aliquots in a 37°C water bath for two minutes using a floating tube rack. After two minutes, remove the aliquot(s) from the water bath and place on ice.
2. Processing one blood sample at a time, for a batch size of no more than six samples:

- a. Remove one blood aliquot from ice, and invert ten times to mix, then pulse spin tube for one second to collect any material from the microcentrifuge tube lid. Immediately dispense 20 µl onto a piece of Parafilm and use HemoCue cuvette to measure WBCs. Place sample on ice.

- b. Record Sample ID and HemoCue Reading (in E+03 cells/µl) in **Table 2**.

NOTE: If the concentration of WBCs of the blood sample is too high ($> 30\text{E}+09$ cells/L) and falls outside the range of detection, the HemoCue instrument display will read “HHH.” Typically, blood samples which give a HemoCue concentration of “HHH” can be diluted in Cell Buffer and then re-counted to accurately determine WBC concentration (see below).

- i. Invert the blood sample ten times to mix. Pulse spin sample for one second to collect any material from the microcentrifuge tube lid. Place on ice.
 - ii. Immediately transfer 25 µl of the blood samples to a 1.5 ml tube containing 75 µl of Cell Buffer (to make a 1:4 dilution of the blood sample).
 - iii. Gently pipette mix the entire volume ten times with a 200 µl standard bore tip. Pulse spin for two seconds.
 - iv. Immediately dispense 20 µl onto Parafilm and use HemoCue cuvette to measure WBCs.
 - v. Perform the following calculations and record the values in **Table 2**.
 - $\text{HemoCue Reading} = \text{WBC count (after Cell Buffer dilution)} \times \text{DF} (=4)$
- c. For each sample, perform the following calculations and record the values in **Table 2**.
 - $\text{Transfer Volume } (\mu\text{l}) = 1.5\text{E}+06 \text{ cells} \div \text{HemoCue Reading in E}+03 \text{ cells}/\mu\text{l}$
 - $\text{Cold Stabilizing Buffer Dilution Volume } (\mu\text{l}) = 1,200 \mu\text{l} - \text{Transfer Volume } (\mu\text{l})$
 - $\text{Removal Volume } (\mu\text{l}) = 1,160 \mu\text{l}$

NOTE: The HemoCue gives readings in E+09 cells/L, but the calculation is based on E+03 cells/µl to aliquot $1.5\text{E}+06$ WBCs into a pre-chilled 1.5 ml Protein LoBind tube.

Example Sample Calculations, i.e., HemoCue reading: $5.0\text{E}+09$ cells/L = $5.0\text{E}+03$ cells/µl

- $\text{Transfer Volume } (\mu\text{l}) = 1.5\text{E}+06 \text{ cells} \div 5.0\text{E}+03 \text{ cells}/\mu\text{l} = 300 \mu\text{l}$
- $\text{Cold Stabilizing Buffer Dilution Volume } (\mu\text{l}) = 1,200 \mu\text{l} - 300 \mu\text{l} = 900 \mu\text{l}$
- $\text{Removal Volume } (\mu\text{l}) = 1,160 \mu\text{l}$

Table 2. Frozen Blood Sample Worksheet

Sample ID	HemoCue Reading * (E+03 cells/ μ l)	Transfer Volume (1.5 E+06 cells \div HemoCue Reading)	Cold Stabilizing Buffer Dilution Volume (1,200 μ l - Transfer Volume)	Removal Volume
		μ l	μ l	1,160 μ l
		μ l	μ l	1,160 μ l
		μ l	μ l	1,160 μ l
		μ l	μ l	1,160 μ l
		μ l	μ l	1,160 μ l
		μ l	μ l	1,160 μ l

*Multiply by DF (=4) if Cell Buffer is used to dilute blood sample due to high WBC count (HemoCue displays HHH, gDNA Isolation Step 2.b.).

3. Processing one sample at a time:

- Invert ten times to mix and pulse spin.
- Transfer the calculated [Transfer Volume] into the previously labeled, pre-chilled 1.5 ml Protein LoBind tube. Cap the tube and place on ice. Change tips between samples.

NOTE: Typical transfer volume for a normal healthy individual will range between 200 and 600 μ l.

4. To each sample add the [Cold Stabilizing Buffer Dilution Volume], calculated in **Table 2**. Cap the tube and place on ice.

NOTE: Adding Cold Stabilizing Buffer dilutes the blood to help visualize the WBCs after the centrifugation step and wash away cellular contaminants/debris.

5. Invert sample ten times to mix then place sample on ice. Spin balanced sample for two minutes at 2,200 x g at room temperature.

NOTE: It is helpful to align the tube hinge to the outer edge of the centrifuge, so that the pellet will always be localized on the same side.

6. During the centrifugation for WBC pelleting, retrieve TLPK from -20°C storage and place on ice. Discard any remaining unused blood into the bleach waste container and place the empty frozen blood aliquot tube into the biohazardous waste bag.

7. Remove sample from the centrifuge after the spin. Inspect the bottom of the tube to visualize the WBC pellet and note its location. Place sample on ice.

8. After centrifugation, remove 1,160 μ l of supernatant from each sample tube in two passes:

- Using an extra-long, filtered 1000 μ l tip remove and discard 1000 μ l of supernatant into the bleach conical. Change tips between samples.

- b. Using a P200 remove 160 µl supernatant and discard into the bleach conical. Aspirate from the meniscus but do not disturb the pellet. Cap the tube and place on ice. After supernatant removal, there should be approximately 40 µl of supernatant with the WBC pellet. Change tips between samples.

RESUSPEND, LYSE/DIGEST WBCS, AND INACTIVATE THERMOLABILE PROTEINASE K

9. To each sample, add 20 µl of cold Stabilizing Buffer on the top of the ~40 µl containing supernatant and WBC pellet.
10. Processing one sample at a time, using a 200 µl standard bore pipette tip gently scratch 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 five 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 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.
11. 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, placing it back in the rack at room temperature. Place the TLPK on ice.
NOTE: Do not vortex. From this step forward, samples will be handled at room temperature.
12. Add 430 µl of Complete Lysis and Digestion Cocktail Master Mix to each sample. Cap the tube. Change tips between samples.
13. Invert mix each sample fifteen times.
14. Rotate sample on HulaMixer for fifteen minutes at room temperature at 10 rpm, no shaking or vibration.
15. 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. Fill the bleach conical to 50 ml with water, cap conical, invert mix, and dispose the contents down the sink.
16. Remove sample from HulaMixer, and pulse spin sample for two seconds.
17. Incubate sample in a Thermomixer pre-set to 55°C for ten mins, with no shaking.
18. Remove sample from the Thermomixer and turn the Thermomixer off.

gDNA BIND, WASH AND ELUTE

19. Using pointed forceps, carefully add a single 4 mm Nanobind Disk to the lysate.
NOTE: Disks can sometimes stick together.
20. Add 480 µl of 100% IPA to each sample. Cap the tube.
21. Invert mix each sample five times.
22. 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.

23. Remove sample from the HulaMixer.

24. 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).

- 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.



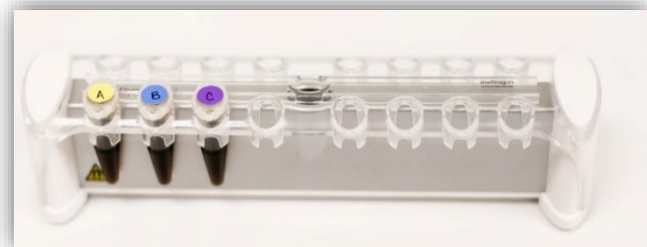
- b. Invert Dynamag magnetic base and lower onto clear rack.



- 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.



25. Set one P1000 pipette to 1,000 µl and a second to 700 µl.
26. 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 the 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 designated for GuHCl liquid waste.

 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 *Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide* (CG-30608).

27. 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 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 the 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 24a through 24e.
 - g. Remove supernatant as described in Step 26.



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 *Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide* (CG-30608).

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

29. 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: 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 tube 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 24a through 24e.
- g. Remove supernatant as described in Step 26.



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 *Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide* (CG-30608).

30. Repeat Wash WB2, Step 29.

31. 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.

32. 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.

33. 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 Disk remains magnetically captured.

34. Carefully lift the sheathed retriever with the bound disk out of the tube and insert it into a 0.5 ml Protein LoBind microcentrifuge tube until the disk is gently wedged at the bottom of the tube.

NOTE: Change the sheaths between samples.

ELUTING THE gDNA

35. Add 65 µl of EB to the 0.5 ml Protein LoBind tube containing the Nanobind Disk and cap the tube.

36. Spin the tube on benchtop microcentrifuge for five seconds.
37. 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).
38. Incubate submerged Nanobind Disk in EB at room temperature for twenty minutes.
39. Collect extracted gDNA by transferring eluate to the labeled 2.0 ml microcentrifuge tube with a 200 µl standard tip.
40. Spin the tube with the Nanobind Disk in benchtop microcentrifuge for five seconds to separate the residual eluate from the Nanobind Disk.
41. 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.

42. Pulse spin samples for two seconds.

Homogenization of gDNA Solution (70 minutes)

gDNA HOMOGENIZATION

43. 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.

44. 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 HulaMixer and position 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.

45. Remove microcentrifuge tube from rack of HulaMixer and spin tube on benchtop microcentrifuge for two seconds to bring the gDNA to the bottom of the tube.
46. Allow the gDNA to equilibrate overnight at room temperature to homogenize.

NOTE: Most samples can be labeled the next day or within forty-eight hours post gDNA isolation using the *Bionano Prep DLS-G2 Protocol* (CG-30553-1).

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 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 4°C, 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 fresh 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 3** below.

10. Calculate the CV = standard deviation/mean value for each sample and record it in **Table 3** below.

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 overnight at room temperature before repeating quantitation and performing DLS labeling the next day. Typical DNA concentrations range from 45-90 ng/ul.

Table 3. 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://bionano.com/support/> for applicable kits and protocols.

Technical Assistance

For technical assistance, contact Bionano 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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