Bionano Prep SP BMA DNA Isolation Protocol v2

Document Number: 30399

Document Revision: A

Table of Contents

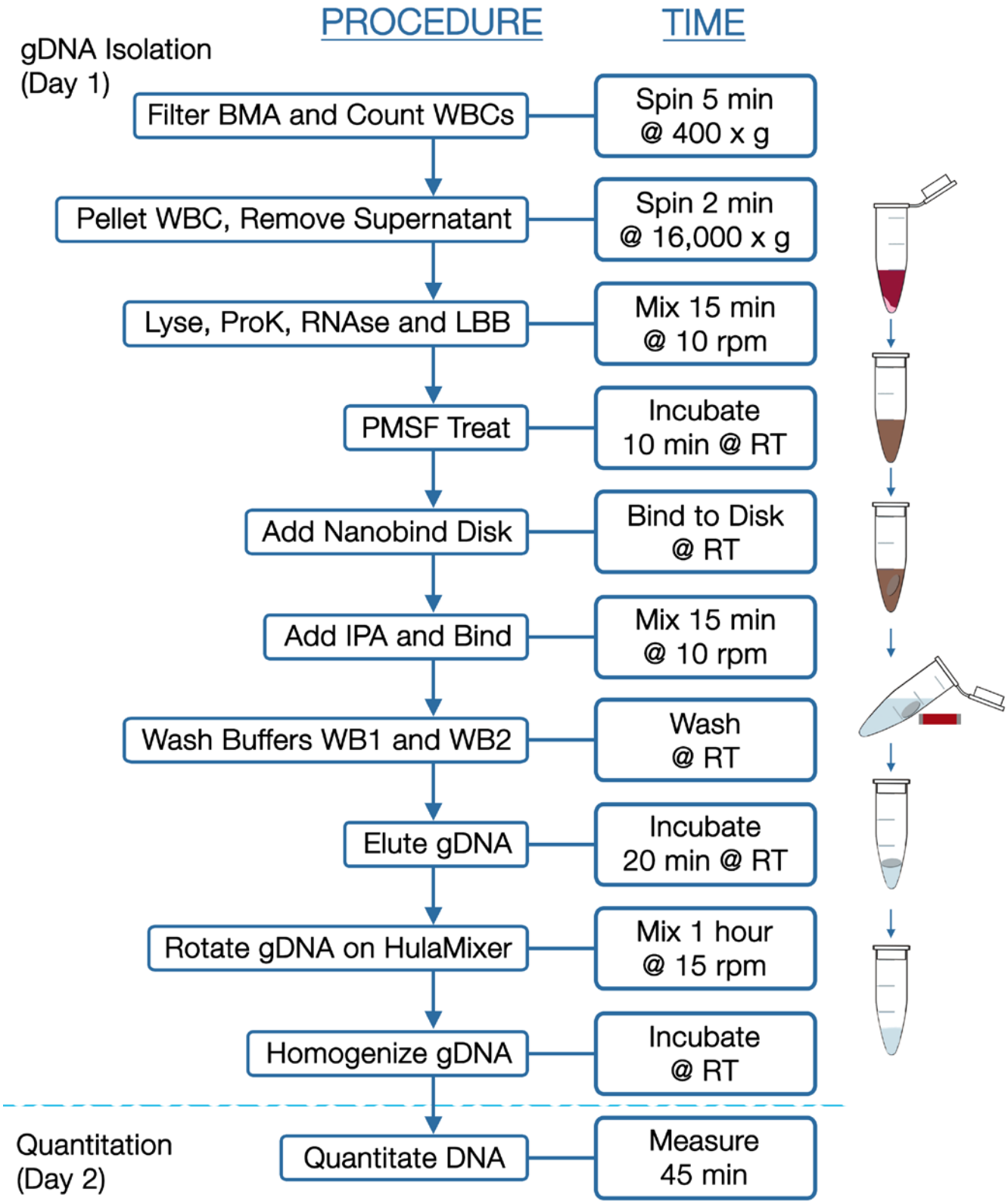
Legal Notice

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

Revision History

Revision	Release Date	Notes
1	03/02/2021	Early Access release of Bionano Prep SP Blood and Cell DNA Isolation Kit v2 (80042).
A	04/22/2021	Commercial release.

Workflow Overview



Bionano Prep SP BMA DNA Isolation Kit and User-Supplied Materials

Bionano Prep SP Bone Marrow Aspirate (BMA) DNA Isolation Kit v2 Contents (Part # 90057, 10 preps)

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

Item	Amount	Part Number	Storage
4 mm Nanobind Disks	10 disks	20402	Room Temp (18-25°C)
Protein LoBind Microcentrifuge Tubes, 1.5 ml	10 tubes	20422	Room Temp (18-25°C)
Protein LoBind Microcentrifuge Tubes, 0.5 ml	10 tubes	20421	Room Temp (18-25°C)
RNase A Enzyme	200 µl	20373	Refrigerate (4°C)
DNA Stabilizer	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

Bionano Prep SP BMA Add-On (Part # 80037, 10 reactions)

Item	Amount	Part Number	Storage
DNA Stabilizer	4 ml	20398	Room Temp (18-25°C)
Standard Microfuge Tubes, 2.0 ml	2 x 10 tubes	20396	Room Temp (18-25°C)
BMA Filters, 100 µm	20 filters	20401	Room Temp (18-25°C)

User-Supplied Materials

Item	Supplier	Catalog #
Day 1 – Pelleting, gDNA Isolation and Homogenization		
Bionano Prep SP Magnetic Retriever (2 pack)	Bionano Genomics	80031
HemoCue WBC Analyzer	Fisher Scientific (for US) Distributor (outside US)	22-601-017
HemoCue Microcuvettes	Fisher Scientific	22-601-018
Vari-Mix Test Tube Rocker	Thermo Fisher or Equivalent	M48725Q
DynaMag-2 Magnetic Tube Rack	Thermo Fisher	12321D
HulaMixer Sample Mixer	Thermo Fisher	15920D
Microcentrifuge Tubes, 1.5 ml, Nuclease Free	VWR	87003-294
Phenylmethylsulphonyl Fluoride Solution (PMSF),100 mM	Sigma-Aldrich	93482
Ethanol, 200 Proof, Molecular Biology Grade	Sigma-Aldrich	E7023
Isopropanol (IPA), ≥ 99.5%, Molecular Biology Grade	Fisher Scientific	A461-212
Disinfectant Concentrate, TexQ TX651	Texwipe	TX651
Bleach for Blood Disposal	General Lab Supplier	
Conical Centrifuge Tubes, 50 ml, PP	Thermo Fisher or Equivalent	14-432-22
Variable Speed MicroCentrifuge (400-16,000 x g spin)	General Lab Supplier	
Water Bath, 37°C	General Lab Supplier	
Ice Bucket and Ice	General Lab Supplier	
Sterile 5 and 10 ml Disposable Piettes (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	

Parafilm	General Lab Supplier	
Day 2 - Quantitation		
Benchtop Vortexer	General Lab Supplier	
Bath Sonicator (optional)	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 ul, C-10 for Pos. Displ. Pipette (optional)	Rainin or Equivalent	17008604

Introduction and Important Notes

Introduction:

This Bionano Prep® SP BMA DNA Isolation Protocol v2 can provide ultra-high molecular weight (UHMW) gDNA in less than four hours. 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 processing several bone marrow aspirates, with the donor bone marrow aspirates being processed up to two at a time. DNA Stabilizer was added to a 1 ml volume of fresh human bone marrow aspirate drawn into a heparin tube which was frozen and then processed without additional freeze/thaw cycles. gDNA prepared using this protocol has been tested only with DLS labeling. See Training Video for technically critical steps and troubleshooting. The current workflow is set up such that four BMAs can be comfortably processed in a typical workday, with two BMAs in the morning and two in the afternoon.

Overview

Cell lysis, Proteinase K and RNAse 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 pipet, 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.

- 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 or equal to 0.30. CV = standard deviation/mean.
- Typical gDNA concentration is 45-90 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 bore tip or positive displacement pipette.

Characteristics of High Quality gDNA for Bionano Mapping

- A clear gDNA solution is ideal, but a cloudy 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.

Batch Size

We recommend processing up to four samples at a time, with a maximum of eight samples per day with four processed in the morning and four processed in the afternoon.

Using the Bionano Prep SP Magnetic Retriever

a. Hold a plastic sheath on the sides near the top and insert the Bionano Magnetic SP Disk 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.

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.

Freezing Fresh Heparinized Human Bone Marrow Aspirates for Storage with DNA Stabilizer

The gDNA content is obtained from the white blood cells (WBC). For each BMA sample, two heparinized BMA aliquots (~1 ml each) should be frozen (-80°C) in separate tubes and stored without thawing until gDNA isolation. Typically, only one aliquot will be required for this protocol, with the second serving as a backup. Samples should be frozen within 24 hours of aspiration, keep at 4°C until frozen at -80°C.

a. Mix fresh heparinized human BMA thoroughly at room temperature to ensure for good uniformity (15 minutes on a rocker at room temperature).

b. Processing one BMA sample at a time, transfer two 1 ml aliquots into DNAse/RNAse free 1.5 ml tubes.

c. Add 15 µl of DNA Stabilizer to each tube containing the 1 ml volume of fresh human BMA.

d. Cap tubes, invert 10 times to mix, then pulse-spin tubes **for one second** to collect any material from the microfuge tube lid, and immediately move aliquots to -80°C for long term storage.

e. Do not thaw aliquot from -80°C until proceeding with gDNA isolation.

Bionano Prep SP BMA DNA Isolation Protocol v2

Preparation for gDNA Isolation

Before First Use

- Verify access to a variable speed microcentrifuge (400 - 16,000 x g).
- PMSF decomposes rapidly in aqueous solutions. Create aliquots of 120 µl in 1.5 ml screw cap tubes and store the stock and aliquots at 4°C. Each aliquot will be sufficient for ten gDNA isolations.
- Add 100% Ethanol to Wash Buffers (WB1 and WB2), mix thoroughly and check “Ethanol Added” boxes:
    - 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).
- 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 (disposed as hazardous waste).
- For each sample, label one 0.5ml Protein LoBind Tube (Bionano), one 1.5ml Protein LoBind Tube (Bionano), and three 2.0 ml microfuge tubes (Bionano).
    - Seat a BMA filter (Bionano) into two of the labeled 2.0 ml microfuge tubes.
- Invert tubes of PMSF, Proteinase K (Bionano) and RNAse (Bionano) three times to mix, pulse spin briefly. Place PMSF and RNAse on ice.
- Prepare a strip of Parafilm (~ 2 cm) for HemoCue, ready microcuvettes and HemoCue system.
- Set up water bath to 37°C. Verify temperature with thermometer.

gDNA Isolation (~3 hours)

Thaw up to Four Aliquots of BMAs Containing DNA Stabilizer, Pellet WBCs, and Remove Supernatant

1. For each sample, remove a single 1 ml aliquot of frozen heparinized BMA containing DNA Stabilizer from the -80°C freezer and thaw in a 37°C water bath for 2 minutes using a floating tube rack. Remove aliquots from the water bath and keep at room temperature.

   Important Note: If you are unsure whether or DNA Stabilizer was added to the 1 ml aliquot of heparinized BMA before freezing, add 15 µl of DNA Stabilizer to tube upon thaw and proceed to Step 2. If the frozen heparinized BMA is not in a 1 ml aliquot with added DNA Stabilizer, refer to Appendix A.

2. Processing the aliquots from up to four samples simultaneously:

   a. Invert BMA aliquot tubes 10 times to mix, then pulse spin tubes **for one second** to collect any material from the microfuge tube lid.

b. For each thawed BMA sample, transfer 500 µl into two separate BMA filters seated in labeled 2.0 ml microfuge tubes.

c. Carefully place the tubes with seated filters in the benchtop microcentrifuge and centrifuge for 5 minutes at 400 x g at room temperature.

Note: Orient the microfuge tubes with filter inserts such that the caps face the center of the rotor.

d. Carefully remove the tubes from the centrifuge and discard the filters into a Biohazard waste container.

e. Gently pipet mix the entire volume of the filtered BMA 10 times with a 1,000 µl tip.

f. Following pipet mixing, pool the two filtered sample volumes into either of the 2 ml tubes.

g. Cap and invert tube containing pooled and filtered bone marrow aspirate 10 times to mix, then pulse spin tube **for one second** to collect material from the lid.

3. Determine WBC count <u>one</u> sample at a time:

a. Gently pipette mix the entire volume of the filtered BMA 10 times to mix, then pulse spin tube for one second to collect any material from the lid.

b. Immediately dispense 20 µl onto Parafilm and use HemoCue cuvette to measure WBCs.

c. Record HemoCue Reading in the table on the next page.

d. Perform the following calculations to fill out the table in this section for each sample:

- Transfer Volume (µl) = 1,500 ÷ HemoCue Reading
- Removal Volume (µl) = (Transfer Volume – 40 µl)

Note: The HemoCue gives readings in cells/L, but calculation is based on cells/µl to aliquot 1.5 x 10^6 WBC. **Calculation**: µl blood for 1.5 million cells = 1.5 x 10^6 (cells) / WBC count (cells/µl).

Note: If the concentration of WBCs in the BMA containing DNA Stabilizer is high and falls outside the range of detection, the HemoCue instrument display will read ‘HHH’. Typically, BMAs which give a HemoCue reading of ‘HHH’ can be diluted in Cell Buffer and then re-counted to accurately determine WBC concentration in the bone marrow aspirate (See below).

<u>For a concentrated Bone Marrow Aspirate displaying ‘HHH’ by initial HemoCue reading only:</u>

Determine the WBC count <u>one</u> sample at a time:

e. Invert bone marrow aliquot tube 10 times to mix, then pulse spin tube for one second to collect any material from the lid.

f. Immediately transfer 25 µl of bone marrow aspirate to a 1.5 ml tube containing 75 µl of Cell Buffer (to make a 1:4 dilution of the bone marrow).

g. Pipet mix the entire volume 10 times gently with a standard 200 µl tip.

h. Immediately dispense 20 µl onto Parafilm and use HemoCue cuvette to measure WBCs.

i. Perform the following calculation to determine sample WBC concentration and record HemoCue Reading in the table on the next page:

- HemoCue Reading x 4 = **undiluted** HemoCue Reading

j. Perform the following calculations to fill out the table in this section for each sample:

- Transfer Volume (µl) = 1,500 ÷ **undiluted** HemoCue Reading
- Removal Volume (µl) = (Transfer Volume – 40 µl)

Note: If the Transfer Volume is 40 µl, no removal volume is required for the sample. If the Transfer Volume is < 40 µl, determine the amount of Cell Buffer to be added to the sample at Step 4.

- Cell Buffer Addition Volume (µl) = (40 µl – Transfer Volume)

Note: The HemoCue gives readings in cells/L, but calculation is based on cells/µl to aliquot 1.5 x 10^6 WBC. **Calculation**: µl blood for 1.5 million cells = 1.5 x 10^6 (cells) / WBC count (cells/µl).

4. After counting WBCs, invert each filtered BMA 10 times to mix, pulse spin for one second to collect material from the microfuge tube lid. Using a pipette set to [Transfer Volume], transfer BMA volume into the previously labeled 1.5 ml Protein LoBind tube.

a. **If the [Transfer Volume] determined in Step 3 = 40 µl**, do not complete Steps 5 and 6, and proceed to Step 7.

b. **If the [Transfer Volume] determined in Step 3 is < 40 µl**, add Cell Buffer to the transferred bone marrow aspirate to 40 µl, do not complete Steps 5 and 6, and proceed directly to Step 7.

c. **If the [Transfer Volume] determined in Step 3 is > 40 µl**, proceed to Step 5.

5. Centrifuge balanced BMA aliquots at 16,000 x g for 2 minutes at room temperature.

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

6. Set an appropriate pipette to [Removal Volume] to remove supernatant with a standard tip. Dispense supernatant into bleach conical. After removal, there should be approximately 40 µl of solution with the WBC pellet.

Note: Angle tube and draw from top of the liquid meniscus, on the side opposite the pellet very slowly. If initial aliquot volume is ≤ 240 µl, perform supernatant removal in one pass. If initial aliquot volume is ≥ 240 µl, perform supernatant removal in multiple passes with 200 µl tip, changing the tip with each pass. Once supernatant from all samples have been removed, you may fill the bleach-containing conical tube to 50 ml with water, cap conical, invert conical to mix, and dispose of the contents down the sink.

Sample ID	Undiluted HemoCue Reading	Transfer Volume	Removal Volume	Cell Buffer Volume
	cells/L	µl	µl	µl
	cells/L	µl	µl	µl
	cells/L	µl	µl	µl
	cells/L	µl	µl	µl
	cells/L	µl	µl	µl
	cells/L	µl	µl	µl
	cells/L	µl	µl	µl
	cells/L	µl	µl	µl

Lyse and Digest WBCs

7. Add 50 µl of Proteinase K (Bionano) directly onto each WBC pellet. Do not pipet mix.
8. Add 20 µl of RNAse A (Bionano) to each tube. Do not pipet mix.
9. Pipette mix sample 5 times with 200 µl standard tip set to 110 µl to resuspend pellet.

   Note: Pull up 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 left in the bottom or side of the tube.
10. Incubate at room temperature for 3 minutes.
11. Add 225 µl Buffer LBB to samples using 1,000 µl tip. Cap and invert tube 15 times to mix.

    Note: 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.
12. Rotate samples on HulaMixer for 15 minutes at room temperature at 10 rpm. No shaking/vibration.
13. Pulse spin tube for 2 seconds to collect liquid at the bottom of the tube.
14. 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.
15. Incubate at room temperature for 10 minutes.

gDNA Bind, Wash and Elute

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

    Note: Disks can sometimes stick together, ensure only one disk is transferred to the tube.
17. Add 340 µl 100% isopropanol to all tubes. Cap and invert tubes 5 times to mix.

18. 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.

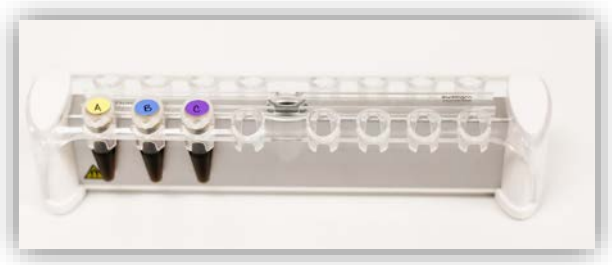
19. Examine gDNA association with Nanobind Disk and invert to increase binding (See Training Video, 0:25):
    a. Place sample tubes into clear Dynamag tube rack and visually inspect all tubes in rack to ensure that gDNA is tethered to the Nanobind Disk.
    b. If gDNA strands are visibly hanging near the bottom of the tube, 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.

20. 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 you.

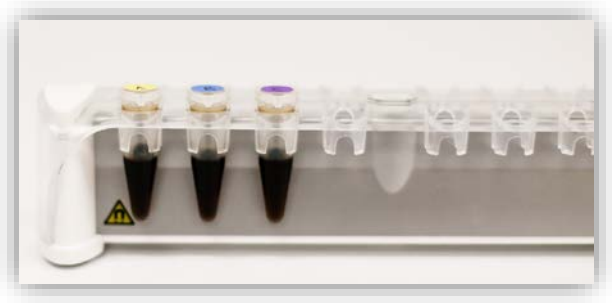
    b. Invert Dynamag magnetic base and lower onto clear rack.

    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.



21. Set one 1,000 µl pipette to 1,000 µl and a second to 700 µl.
22. Remove supernatant as outlined below, being careful not to aspirate the gDNA (See Training Video, 1:15):
    a. Angle entire rack at a 45° angle by holding in one hand (grasping the entire apparatus from below with tubes visible 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.
23. Perform Wash WB1 (See Training Video, 2:21): Dispense 700 µl of Buffer WB1 directly onto the disks in the tubes and cap tubes.
    a. Lift clear tube rack to separate from magnetic base.
    b. Invert clear rack with tubes 180° 10 times to wash.
    c. Re-rack clear tube rack and tubes with magnetic base as described in Step 20.
    d. Remove supernatant as described in Step 22.

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

25. Perform Wash WB2:
    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° 20 times to wash.
    d. Re-rack clear tube rack and tubes with magnetic base as described in Step 20.
    e. Remove supernatant as described in Step 22.

    ⚠ 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.

26. Repeat Wash WB2, Step 25.

    ⚠ If the gDNA associated with the Nanobind still has color after 20 inversions, see Troubleshooting

27. Open tube lid fully (parallel to lab bench) and lift each tube apart from base.
28. In close proximity to a 0.5ml Protein LoBind tube, transfer Nanobind Disk to the 0.5ml 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).

    ⚠ Ensure than the gDNA remains adhered to the disk during transfer.

29. Spin the Protein LoBind tube in benchtop microcentrifuge for 5 seconds.
30. 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 in small circular motion to remove all residual liquid from bottom of tube.

31. Add 65 µl of Buffer EB to Protein LoBind tube.
32. Spin the tube on benchtop microcentrifuge for 5 seconds.
33. 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).
34. Incubate submerged Nanobind Disk in Buffer EB at room temperature for 20 minutes.
35. Collect gDNA by transferring eluate to the labeled 2.0 ml microfuge tube with a standard 200 µl tip.
36. 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 labeled 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)

gDNA Homogenization

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

    - Repeat this process 5 times for a total of 6 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.

38. 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.

    Note: To minimize the procedure time, the microfuge tubes can be left on the Hula Mixer overnight if the mixer is configured to stop after 1 hour. The following day, spin the tubes on benchtop microcentrifuge for 2 seconds to bring the gDNA to the bottom of the tube before quantitation.

39. 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 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 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 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. Record values below.
10. Coefficient of Variation (CV = standard deviation/mean) from three readings should be ≤ 0.30. Record 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 at least overnight at room temperature before repeating quantitation.

    Note: Typical DNA concentrations range from 45-90 ng/ul.

Sample ID	Left (ng/µl)	Middle (ng/µl)	Right (ng/µl)	Mean (ng/µl)	CV (stdev/mean)

Labeling

DNA is ready for Direct Label and Stain (DLS) (30206) labeling. See “Kits and Consumables” section at https://bionanogenomics.com/support/ for applicable kits and protocols.

Appendix A: Addition of DNA Stabilizer to Thawed Human Bone Marrow Aspirate in Heparin

Set Up

- Verify access to a variable speed microcentrifuge (400-16,000 x g).
- In order to account for significant variation in WBC concentration in heparinized BMAs from human samples, two 500 µl aliquots from each thawed heparinized BMA should be filtered and pooled. For each 500 µl volume of thawed BMA sample, label a 2.0 ml microfuge tube and sit a BMA filter on the tube.
- Set up water bath to 37°C. Verify temperature with thermometer.

Thaw Heparin Bone Marrow Aspirate, Filter, and Add DNA Stabilizer

1. Processing one sample at a time:

    a. Remove frozen heparinized BMA from -80°C freezer and thaw in 37°C water bath for 2 minutes (assuming ~ 2 ml in the tube) using a floating tube rack. Remove from the water bath and keep at room temperature.

    Note: The amount of time needed for thaw varies depending on how much volume of frozen bone marrow aspirate there is to thaw. For volumes ≤ 1 ml, 2 minutes of thaw at 37°C should be sufficient. For larger volumes, such as ≥ 4.8 ml, up to 8 minutes of thaw may be required.

    b. Invert thawed BMA tube 10 times to mix, pulse spin **for one second** to collect material from the microfuge tube lid.

    c. Transfer 500 µl of thawed BMA sample onto each of two BMA filters seated on labeled 2.0 ml microfuge tubes.

    Note: If starting material is less than 1 ml, then add equal volume of sample to each filter.

    d. Carefully place the tubes with seated filters in the benchtop microcentrifuge and centrifuge for 5 minutes at 400 x g at room temperature.

    Note: Orient the microfuge tubes with filter insert such that the caps face the center of the rotor.

    e. Carefully remove the tubes with seated filters from the centrifuge and discard the filters in a Biohazard waste container.

    f. Gently pipet mix the entire volume of the bone marrow aspirate 10 times to mix, then pulse spin tube **for one second** to collect material from the lid of the microfuge tube.

    g. Following pipet mixing, pool the two filtered sample volumes into either one of the 2 ml tubes.

    h. Cap and invert tube containing filtered bone marrow aspirate pool 10 times, then pulse spin tube **for one second** to collect material from the lid of the microfuge tube.

    i. Transfer the maximum **measured** volume to a labeled 1.5 ml microfuge tube.

j. Add the appropriate amount of DNA Stabilizer to the filtered pooled BMA using the following equation: µl of DNA Stabilizer to add = 15 x [µl of the filtered BMA]/1000.

k. Cap and invert tube 10 times to mix, then pulse spin tube **for one second** to collect any material from the microfuge tube lid. Proceed to Step 3b of the 'Bionano Prep SP BMA DNA Isolation Protocol'.

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 associated with the Nanobind still has color in the second WB2 wash

Evidence: After 20 inversions in the second WB2 wash the gDNA associated with the Nanobind still has color

Steps to Follow:

1. Lift clear rack to separate from magnetic base.
2. Invert clear rack upside down such that the NanoBind disks rest in the tube caps and **vigorously shake the tube rack continuously for 10 seconds**, making sure the Nanobind disks traverse the entire span of the microtubes from top to bottom while shaking.
3. Re-rack clear tube rack and tubes with magnetic base as described in Step 20 on page 15.
4. Remove supernatant as described in Step 22 on page 16.

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.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

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

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

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