Bionano Prep SP-G2 Fresh Human Bone Marrow Aspirate (BMA) DNA Isolation Protocol

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bionano

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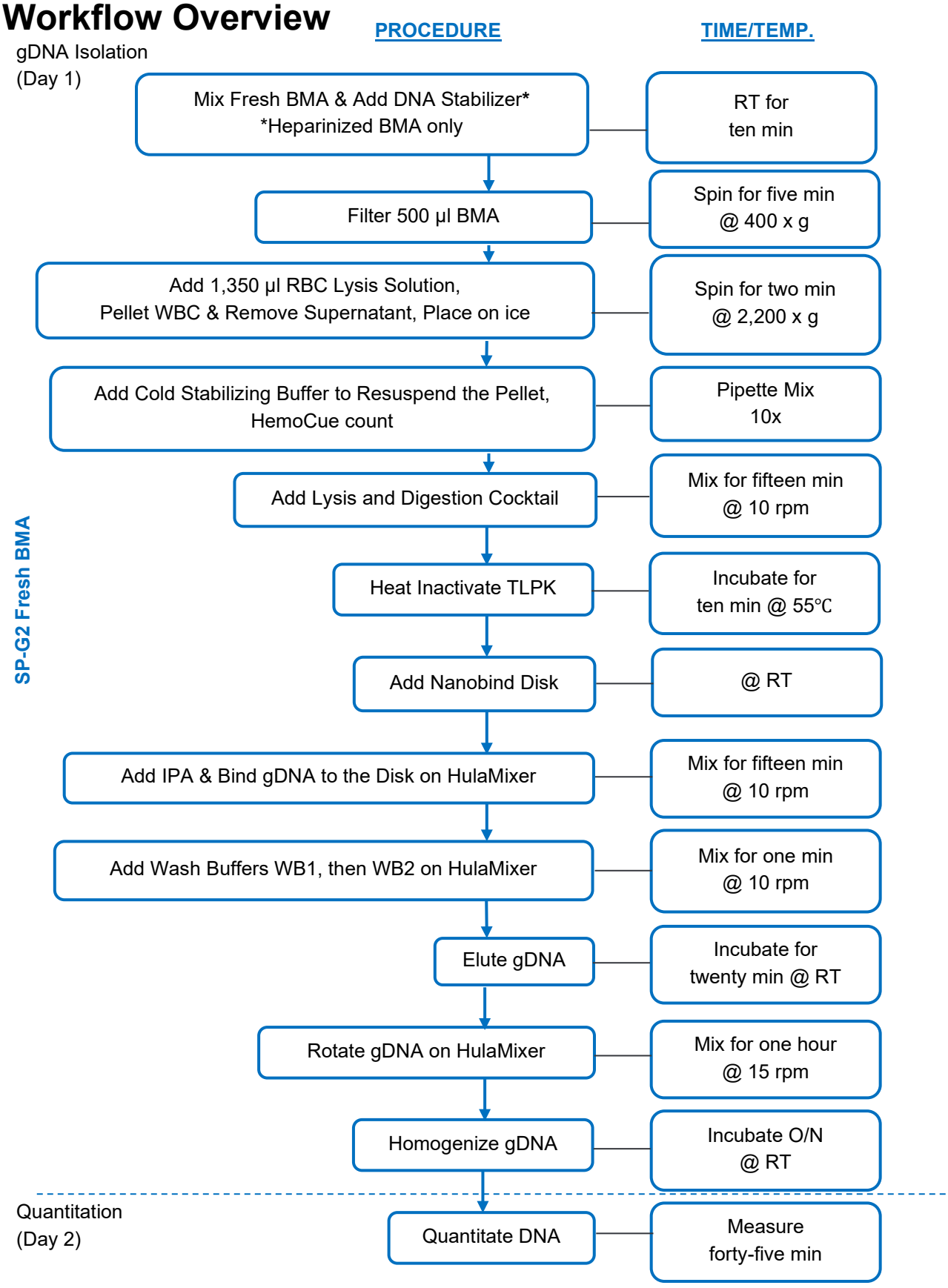
Revision History

REVISION	NOTES
A	Commercial release.

Workflow Overview



Bionano Prep SP-G2 Fresh Human BMA DNA Isolation Bundle Kit and User-Supplied Materials

The Bionano Prep SP-G2 Fresh Human BMA DNA Isolation Bundle Kit (P/N 90151) contains Bionano Prep SP-G2 Blood and Cell Culture DNA Isolation Kit contents (P/N 80060, 12 preps) and Bionano Prep SP-G2 Bionano Prep SP BMA Add-On contents (P/N 80062, 12 preps).

Table 1. Bionano Prep SP-G2 Blood and Cell Culture DNA Isolation Kit Contents (P/N 80060, 12 preps).

Component	Part #	Quantity	Storage	Handling Considerations
RBC Lysis Solution	20442	18 ml	15°C to 30°C	
Cell Buffer	20374	50 ml	15°C to 30°C	
Digestion Enhancer	20443	4 ml	15°C to 30°C	
Lysis and Binding Buffer (LBB)	20444	1.2 ml	15°C to 30°C	See Important Notes Section for hazardous waste information.
Wash Buffer 1 (WB1)	20445	4.5 ml	15°C to 30°C	See Important Notes Section for hazardous waste information.
Wash Buffer 2 (WB2)	20446	6 ml	15°C to 30°C	
Elution Buffer (EB)	20378	1.1 ml	15°C to 30°C	.
DE Detergent	20447	55 µl	15°C to 30°C	
4mm Nanobind Disks	20448	12 ea.	15°C to 30°C	
Protein LoBind Microcentrifuge Tubes, 1.5ml	20449	2 x 12 ea.	15°C to 30°C	
Protein LoBind Microcentrifuge Tubes, 0.5ml	20450	12 ea.	15°C to 30°C	
Magnetic Retriever Plastic Sheath	20451	12 ea.	15°C to 30°C	
Microcentrifuge Tubes, 2ml	20452	1 x 12 ea.	15°C to 30°C	
DNA Stabilizer	20423	350 µl	15°C to 30°C	
RNase A*	20455	150 µl	2°C to 8°C	

Component	Part #	Quantity	Storage	Handling Considerations
Ultrapure Water	20355	2 x 900 µl	2°C to 8°C	
Thermolabile Proteinase K (TLPK)	20441	150 µl	-15°C to -25°C	

*Not used in this protocol.

Table 2. Bionano Prep SP-G2 Bionano Prep SP BMA Add-On (P/N 80062, 12 preps).

Component	Part #	Quantity	Storage	Handling Considerations
BMA Filter	20464	24 ea.	15°C to 30°C	
Microcentrifuge Tubes, 2ml	20452	2 x 12 ea.	15°C to 30°C	
DNA Stabilizer	20398	4 ml	15°C to 30°C	

User-Supplied Materials and Equipment

Table 3. User-Supplied Materials.

Item	Description	Catalog #
Bionano Prep SP Magnetic Retriever (2 pack)	Bionano Genomics (Training Kit)	
Vari-Mix Tube Rocker	Thermo Fisher or equivalent	M48725
HemoCue WBC Analyzer	Fisher Scientific (for US) Distributor (outside US)	
HemoCue Microcuvettes	Fisher Scientific	22-601-018
Quaternary Ammonium Disinfectant	VAI or Equivalent	DQ100-01
DynaMag-2 Magnetic Tube Rack	Thermo Fisher	12321D
HulaMixer Sample Mixer	Thermo Fisher	15920D
Microcentrifuge Tubes, 2.0ml, Nuclease Free	Fisher Scientific or Equivalent	05-408-138
Microcentrifuge Tubes, 5.0ml, Nuclease Free	Thomas Scientific or Equivalent	1201T80
Ethanol, 200 Proof, Molecular Biology Grade	Sigma-Aldrich	E7023

Item	Description	Catalog #
Isopropanol (IPA), ≥ 99.5%, Molecular Biology Grade	Sigma-Aldrich	A461-212
Conical Centrifuge Tubes, 50ml, PP	Thermo Fisher or Equivalent	14-432-22
Centrifuge with 1.5ml Tube Rotor	Cole-Parmer or Equivalent	EW-17701-11
Ice Bucket and Ice	General Lab Supplier	
Microcentrifuge for 0.2 ml, 0.5 ml, and 1.5 ml Tubes, Nuclease Free	General lab supplier	
Eppendorf ThermoMixer® C & Eppendorf SmartBlock™ 1.5 mL, Thermoblock set to 55□C (Or 55□C heat block for 1.5ml tubes or water bath as alternatives)	Eppendorf or Equivalent	5382000023, 5360000038
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
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
Benchtop Vortexer	VWR or Equivalent	10153-838
Bath sonicator (optional)	Branson or equivalent	CPX 952-119R
15ml Conical Tube	Fisher Scientific	05-539-12
Qubit Fluorometer	Thermo Fisher or Equivalent	Q33238
Qubit Assay Tubes	Thermo Fisher or Equivalent	Q32856
Qubit BR (Broad Range) dsDNA Assay Kit	Thermo Fisher	Q32853
Positive-displacement pipet MR-10 (recommended)**	Rainin or equivalent	17008575
Pipet tips, 10 µl, C-10 for pos. displacement (recommended)**	Rainin or equivalent	17008604

*(Items in blue) Users are strongly encouraged not to substitute this equipment but to order the exact item recommended. Using this equipment will lend the most successful outcome from the workflow.

**A positive displacement pipet is a special pipet with a plunger that operates a built-in piston in special tips that go with the pipet. Such a pipet and tips are highly effective in accurately pipetting and dispensing small volumes of viscous liquids, and therefore are highly recommended to be used while aliquoting Ultra High Molecular Weight gDNA isolated using Bionano's protocols.

Introduction and Important Notes

Introduction

This Bionano Prep® SP-G2 Fresh Human Bone Marrow Aspirates (BMA) DNA Isolation Protocol can provide ultra-high molecular weight (UHMW) genomic DNA (gDNA) in approximately 5 hours from 1.5 million white blood cells (WBC) from fresh human BMA. 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 by processing fresh bone marrow aspirates from healthy donors, with aspirates collected in both heparin and EDTA tubes, which were shipped Room Temperature (RT) after collection and processed the day following collection. Fresh BMA should be stored ambient upon collection until processing and processed within 72 hours of collection. 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 current workflow is set up such that up to six BMAs can be comfortably processed in a typical workday.

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 to use for labeling.

gDNA QUANTITATION

gDNA quantitation is used to measure concentration and serves as a gauge of UHMW gDNA homogeneity. Qubit DNA 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.

- 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 at 4°C.
- 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 4E+9 cells/L HemoCue reading is strongly 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 RBC Lysis Solution and GuHCl should be safely decontaminated with a quaternary ammonium disinfectant before disposal in a hazardous waste stream. We recommend following local environmental, health and safety regulations for decontamination and disposal of all solutions mixed with RBC Lysis Solution and/or GuHCl. **NOTE:** VAI DECON-QUAT 100 Quaternary Ammonium Solution is used for protocol development at Bionano.

Bionano Prep SP-G2 Fresh Human BMA 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 equipment (see “User Supplied Material” section above).
   a. Pipettes and tips
   b. Prepare strips of Parafilm (~ 2 cm) for HemoCue; ready microcuvettes and HemoCue system.
   c. Ice bucket and ice and place aluminum cooling block on ice
   d. Verify microcentrifuge spin speed is 400 x g, 5 minutes room temperature for the BMA filtration spin.
   e. For waste disposal, prepare:
      One 50 ml conical designated for RBC Lysis Solution and GuHCl liquid waste (disposed as hazardous waste as per local environmental, health and safety regulations. For instance, for each gDNA isolation at Bionano, 100 µl of VAI DECON-QUAT 100 Quaternary Ammonium Solution is used to treat the RBC Lysis Solution and GuHCl liquid waste before disposal in a hazardous waste stream)
   f. HulaMixer Sample Mixer
   g. 100% IPA
   h. DynaMag-2 Magnetic Tube Rack

i. Bionano Prep SP Magnetic Retriever
j. Set a Thermomixer to 55°C, ten min, no shaking.
k. Pointed Forceps
l. 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 BMA DNA Isolation Bundle Kit: BMA Filters (100 µm), RBC Lysis Solution, 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 500 µl Stabilizing Buffer (SB) by mixing 490 µl Cell Buffer with 10 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 pre-chilled aluminum block on ice.
   c. For each sample, label a single 2.0 ml microcentrifuge tube (Bionano) for BMA filtration step. Insert a 100 µm BMA filter into the 2.0 ml tube. Place the tube, with seated BMA filter insert, in a rack at room temperature.
   d. For each sample, label one 2.0 ml microcentrifuge tube (Bionano) for 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 4. 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 in Step 19 in gDNA Isolation.

gDNA Isolation (Up to 3 hours 45 minutes)

MIX/FILTER/ADD RBC LYSIS SOLUTION, PELLET WBCs, REMOVE SUPERNATANT, ADD COLD STABILIZING BUFFER, RESUSPEND PELLET, COUNT/ALIQUOT WBCs

Recommended Input: 1.5E+06 WBCs

1. For each sample, gather a 0.5 ml aliquot of fresh heparinized BMA, containing DNA Stabilizer, and/or a 0.5 ml aliquot of fresh BMA in EDTA at RT.

   NOTE: If DNA Stabilizer has not been added to fresh heparinized BMA, refer to the 'Appendix' section below.

2. Processing one sample at a time, for a batch size of no more than six samples:
   a. Invert one BMA aliquot 10X to mix. Pulse spin sample for one second to collect any material from the microcentrifuge tube lid.
   b. Transfer entire volume onto BMA filter, seated in a labeled 2.0 ml microcentrifuge tube.
3. Carefully place the tubes with seated filters in the benchtop microcentrifuge and centrifuge balanced for 5 minutes at 400 x g at room temperature.

   NOTE: Orient the microcentrifuge tubes with filter inserts such that the tube caps face the center of the rotor.
4. Carefully remove the tubes from the microcentrifuge and place on a rack at room temperature.
5. Remove and discard the filters into a biohazardous waste container.
6. Complete RBC Lysis on each filtered sample as follows:
   Using a filtered 1,000 µl pipette, set at 450 µl, gently pipet mix sample 10X to mix, using a new pipette tip for each sample.
   a. Add 1,350 µl of RBC Lysis Solution to each tube.
   b. Cap tubes and invert 10X to mix.
   c. Incubate tubes for 5 minutes at room temperature.
   d. After incubation, invert tubes 10X to mix.
7. Carefully place the tubes in the benchtop microcentrifuge and centrifuge balanced for 2 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.
8. Carefully remove the tubes from the microcentrifuge and place back to the rack at room temperature. Inspect the bottom of the tube to visualize the WBC pellet and note its location.

   NOTE: See Figure 1 in the 'Appendix' section for a visual example.
9. After centrifugation, remove supernatant from each sample as follows:
   a. Using a filtered 1,000 µl pipette tip, remove 1,000 µl of bulk supernatant from each tube (aspirate from top of liquid and away from the pellet), using a new pipette tip for each sample. Discard supernatants into conical tube containing quaternary ammonium disinfectant and discard pipette tips.

NOTE: For the supernatant removal described above, there may be remnant solution that does not get removed during aspiration that is adhered to the side walls of the tube. **DO NOT** attempt to go back into the tube and remove with a pipet tip. **DO NOT** spin down tube before proceeding to Step 9b below.

b. Using a new filtered 1,000 µl pipette tip, remove an additional 650 µl of bulk supernatant from each tube (aspirate from top of liquid and away from pellet), using a new pipette tip for each sample. Discard supernatants into conical tube containing quaternary ammonium disinfectant.

NOTE: For the supernatant removal described above, there may be remnant solution that does not get removed during aspiration that is adhered to the side walls of the tube. **DO NOT** attempt to go back into the tube and remove with a pipet tip. **DO NOT SPIN DOWN** tube before proceeding to Step 10 below. See **Figure 2** in the Appendix for a visual example.

10. Slowly add 300 ul of cold Stabilizing Buffer to each tube containing ~150ul residual with pellet, while avoiding any remnant solution on the side walls of the tube. **DO NOT** MIX and **DO NOT SPIN DOWN** tubes. Place tubes on pre-chilled aluminum block on ice. Use a new tip per sample.

NOTE: DO NOT SPIN DOWN tubes before proceeding to Step 11 below.

11. Pipet mix and HemoCue count each sample as follows, processing <u>one</u> sample at a time:

a. Gently pipet mix the sample ten times, using a 1000 uL pipet tip. Set the pipet to 400 uL and use the tip to scratch the pellet in a circular fashion while aspirating to dislodge and fully resuspend the pellet.

b. Immediately dispense 20 µl onto Parafilm. Place sample on aluminum block. Allow sample on Parafilm to equilibrate to room temperature for 1 minute.

c. Use a HemoCue microcuvette to measure WBCs.

NOTE: The rate of capillary action for WBCs in Stabilizing Buffer is faster than for 'crude' filtered fresh BMA (i.e. pre-RBC lysis fresh BMA). It's important to visually inspect the HemoCue microcuvette after filling to ensure that there are no large bubbles and fill the microcuvette quickly (<u>≤1 second</u>) once making contact with the solution to ensure that liquid is retained in the microcuvette for proper reading. See **Figure 3, Figure 4, Figure 5, Figure 6,** and **Figure 7** in the Appendix for visual examples.

d. Discard used HemoCue microcuvette in biohazardous waste.

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

NOTE: If upon reading the HemoCue microcuvette the HemoCue display reads 'Err01', Repeat Step 11 above, loading a new HemoCue microcuvette.

f. For each sample, perform the following calculations and record the values in **Table 2**.

- Transfer Volume (µl) = 1.5E+06 cells ÷ HemoCue Reading in E+03 cells/µl
- Removal Volume (µl) = Transfer Volume – 40 µl

NOTE: If the concentration of WBCs in the BMA 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).

For a concentrated Bone Marrow Aspirate displaying 'HHH' by initial HemoCue reading only:

Determine the WBC count one sample at a time:

g. Invert bone marrow aliquot tube 10 times to mix, then pulse spin tube for one second to collect any material from the lid.
h. 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). For Research Use Only.
i. Pipet mix the entire volume 10 times gently with a standard 200 µl tip.
j. Immediately dispense 20 µl onto Parafilm and use HemoCue microcuvette to measure WBCs.
k. Perform the following calculation to determine sample WBC concentration and record HemoCue Reading in the table:
   - HemoCue Reading in E+03 cells/µl x 4 = undiluted HemoCue Reading
l. 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 13b.
   - Cell Buffer Addition Volume (µl) = (40 µl – Transfer Volume)

12. Repeat Step 11 above for remaining samples.

Table 5. Fresh BMA Sample Worksheet

Sample ID	HemoCue Reading (E+03 cells/µl)	Transfer Volume (µl) (1.5 E+06 cells ÷ HemoCue Reading)	Removal volume (µl) (Transfer Volume (µl) – 40 µl)
		µl	µl
		µl	µl
		µl	µl
		µl	µl
		µl	µl
		µl	µl

13. Processing one sample at a time:
    a. Gently pipet mix sample 5X for homogeneity with a filtered 1,000 µl pipette tip, set at 380 µl.

b. Transfer the calculated [Transfer Volume] into the previously labeled, pre-chilled 1.5 ml Protein LoBind tube. Cap the tube and place on pre-chilled aluminum block on ice. Change tips between samples.

NOTE: If the [Transfer Volume] determined in Step 11 = 40 µl, do not compete Steps 14 and 15, and proceed to Step 16. If the [Transfer Volume] determined in Step 11 is < 40 µl, add cold Stabilizing Buffer to the transferred BMA to 40 µl, do not complete Steps 14 and 15, and proceed to Step 16. If the [Transfer Volume] determined in Step 11 is > 40 µl, proceed to Step 14.

NOTE: Retrieve TLPK from -20C and place on pre-chilled aluminum block on ice.

14. Carefully place the tubes in the benchtop microcentrifuge and centrifuge balanced for 2 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. Carefully remove the tubes from the microcentrifuge and place on pre-chilled aluminum block on ice. Inspect the bottom of the tube to visualize the WBC pellet and note its location.

NOTE: See **Figure 8** in the Appendix for a visual example.

15. After centrifugation, remove supernatant from each sample tube as follows:

a. Using a 200 µl tip, remove the supernatant volume equal to [Removal Volume] from each tube, aspirating from the top of the solution and away from the pellet and place tube on pre-chilled aluminum block on ice, using a new pipette tip for each sample. Discard supernatants into conical tube containing quaternary ammonium disinfectant and discard pipette tips. After supernatant removal, there should be approximately 40 µl of supernatant with the WBC pellet.

NOTE: If the Removal Volume is > 200 µl, divide the Removal Volume by two, and use a P200 to remove the supernatant, changing tips between passes.

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

16. To each sample, add 20 µl of cold Stabilizing Buffer on top of the ~40 µl containing supernatant and WBC pellet and place tube on pre-chilled aluminum block on ice.
17. Processing one sample at a time, use a 200 µl standard bore pipette tip, set at 55 µl to 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.

18. Flick the TLPK tube three times and pulse spin for 2 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, sample will be handled at room temperature.

19. Add 430 µl of Complete Lysis and Digestion Cocktail Master Mix to each sample and place tube at room temperature. Cap the tube. Change tips between samples.
20. Invert mix each sample 15 times.
21. Rotate sample on HulaMixer for 15 minutes at room temperature at 10 rpm, no shaking or vibration.
22. 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.
23. Remove sample from HulaMixer, and pulse spin sample for 2 seconds.
24. Incubate sample in a Thermomixer pre-set to 55°C for 10 minutes, with no shaking.
25. Remove sample and turn the Thermomixer off.

gDNA BIND, WASH AND ELUTE

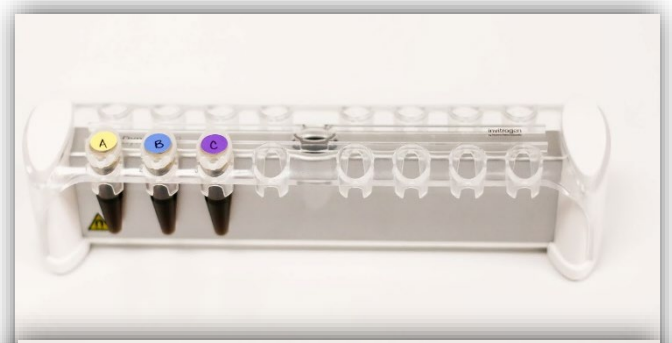
26. Using pointed forceps, carefully add a single 4 mm Nanobind Disk to the lysate.
    NOTE: Disks can sometimes stick together.
27. Add 480 µl of 100% IPA to each sample.
28. Invert mix each sample 5 times.
29. Rotate sample on HulaMixer for 15 minutes at room temperature at 10 rpm, no shaking or vibration.
    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.
30. Remove sample from the HulaMixer.
31. Combine the clear Dynamag rack with the magnetic base as outlined below, making sure the Nanobind Disk is secured by the magnet near the top of the liquid level. If 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 user.

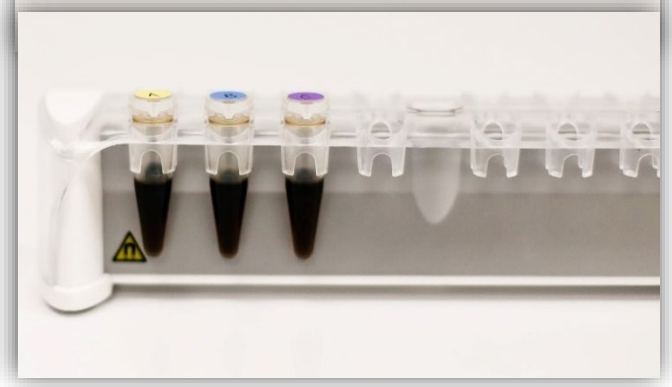
b. Invert Dynamag magnetic base and lower onto clear rack.

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 front.



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



32. Set one P1000 pipette to 1,000 µl and a second to 700 µl.
33. 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 2 seconds for the 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.

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

34. 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 31a through 31e.
    g. Remove supernatant as described in Step 33.
    NOTE: 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 *Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide* (CG-30608).

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

36. 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 31a through 31e.
g. Remove supernatant as described in Step 33.
NOTE: 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 *Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide* (CG-30608).

37. Repeat Wash WB2, Step 36.
38. 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.
39. 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.
40. 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.
41. 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 sheath between samples.

ELUTING THE gDNA

42. Add 65 µl of EB to the 0.5 ml Protein LoBind tube containing the Nanobind Disk and cap the tube.
43. Spin the tube on benchtop microcentrifuge for 5 seconds.
44. Using a 10 µl standard tip, gently nudge the Nanobind Disk towards the bottom of the tube, making sure that it is fully submerged in liquid. The disk should remain parallel to the bench surface (See Training Video).
45. Incubate submerged Nanobind Disk in EB at room temperature for 20 minutes.
46. Collect extracted gDNA by transferring eluate to the labeled 2.0 ml microcentrifuge tube with a 200 µl standard tip.
47. Spin the tube with the Nanobind Disk in benchtop microcentrifuge for 5 seconds to separate the residual eluate from the Nanobind Disk.
48. 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.
49. Pulse spin samples for 2 seconds.

Homogenization of gDNA Solution (70 minutes)

gDNA HOMOGENIZATION

50. Slowly pipette the entire gDNA volume into a standard 200 µl tip, then gently 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.
51. Place standard 2.0 ml microcentrifuge tube containing gDNA in rack of HulaMixer 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 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.
52. Remove microcentrifuge tube from rack of HulaMixer and spin tube on benchtop microcentrifuge for 2 seconds to pull the gDNA down to the bottom of the tube.
53. Allow the gDNA to equilibrate overnight at room temperature (25°C) to homogenize.
    NOTE: Most samples can be labeled the next day or within 48 hours post gDNA isolation using the *Bionano Prep Direct Label and Stain-G2 Protocol* (CG-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, pulse spin and 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 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 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 in **Table 6** below.
10. Calculate the CV = standard deviation/mean value for each sample and record it in **Table 6** 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 60-140 ng/µl.

Table 6. gDNA Quantitation (BR dsDNA) Worksheet

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

LABELING

gDNA samples are ready for Bionano Prep DLS-G2 Protocol labeling within 48 hours post isolation. See the “Kits and Consumables” section at https://bionano.com/support/ for applicable kits and protocols.

Appendix A: Guidelines for Fresh BMA Collection Vessel and Shipping

Fresh BMA should be collected in vessel(s) which have minimal dead space to ensure that contents are subjected to a minimal amount of mixing following collection during transport. Fresh BMA should be shipped ambient, without cold packs, but with bubble wrap and/or packing materials which provide cushion and insulation to also ensure a minimal amount of mixing and temperature fluctuation following collection, during transport, until receipt.

Appendix B: Preparing Fresh Heparinized Human BMA with DNA Stabilizer for Processing on Day of Receipt

The gDNA content is obtained from the WBC. Recommended input is 1.5E+06 WBCs. For each BMA sample collected in heparin, upon receipt, DNA Stabilizer should be added to aliquots (0.5 ml each) in separate tubes after thorough mixing. Typically, only one aliquot will be required for this protocol, with the second aliquot being stored at -80°C upon aliquoting and adding DNA Stabilizer, serving as a backup. Samples should be processed as early as the day following collection, and processed no longer than 72 hours from collection, and kept at ambient temperature from collection to processing.

1. Mix fresh heparinized human BMA thoroughly at room temperature to ensure good uniformity (10 minutes on rocker at room temperature).
2. Processing one BMA at a time, transfer two 0.5 ml aliquots into each of two nuclease-free 1.5 ml tubes.
3. Add 7.5 µl of DNA Stabilizer to tube containing the 0.5 ml volume of fresh human BMA.
4. Cap tubes and invert 10 times to mix. Pulse-spin tubes for one second to collect any material from the microcentrifuge tube lids. Store one tube at -80°C for backup and proceed with processing the other tube, storing the sample at ambient temperature, and processing sample immediately upon receipt.

NOTE: If the amount of the fresh heparinized human BMA aliquot is > 0.5 ml, using the following equation, add the appropriate amount of DNA Stabilizer to the well mixed fresh heparinized human BMA and mix as described in Step 4 above:

- µl of DNA Stabilizer to add = 15 x [µl of BMA]/1000

Appendix C: Preparing Fresh Human BMA in EDTA for Processing on Day of Receipt

The gDNA content is obtained from the WBC. Recommended input is 1.5E+06 WBCs. For each BMA sample collected in EDTA, upon receipt, fresh BMA should be aliquoted (0.5 ml each) in separate tubes after thorough mixing. Typically, only one aliquot will be required for this protocol, with the second aliquot being stored at -80°C

upon aliquoting, serving as a backup. Samples should be processed as early as the day following collection, and processed no longer than 72 hours from collection, and kept at ambient temperature from collection to processing.

1. Mix fresh human BMA in EDTA thoroughly at room temperature to ensure good uniformity (10 minutes on rocker at room temperature).
2. Processing one BMA at a time, transfer two 0.5 ml aliquots into each of two nuclease-free 1.5 ml tubes.
3. Cap tubes and store one tube at -80°C for backup and proceed with processing the other tube, storing the sample at ambient temperature, and processing the sample immediately upon receipt.
   NOTE: DO NOT add DNA Stabilizer to fresh Human BMA in EDTA for processing.

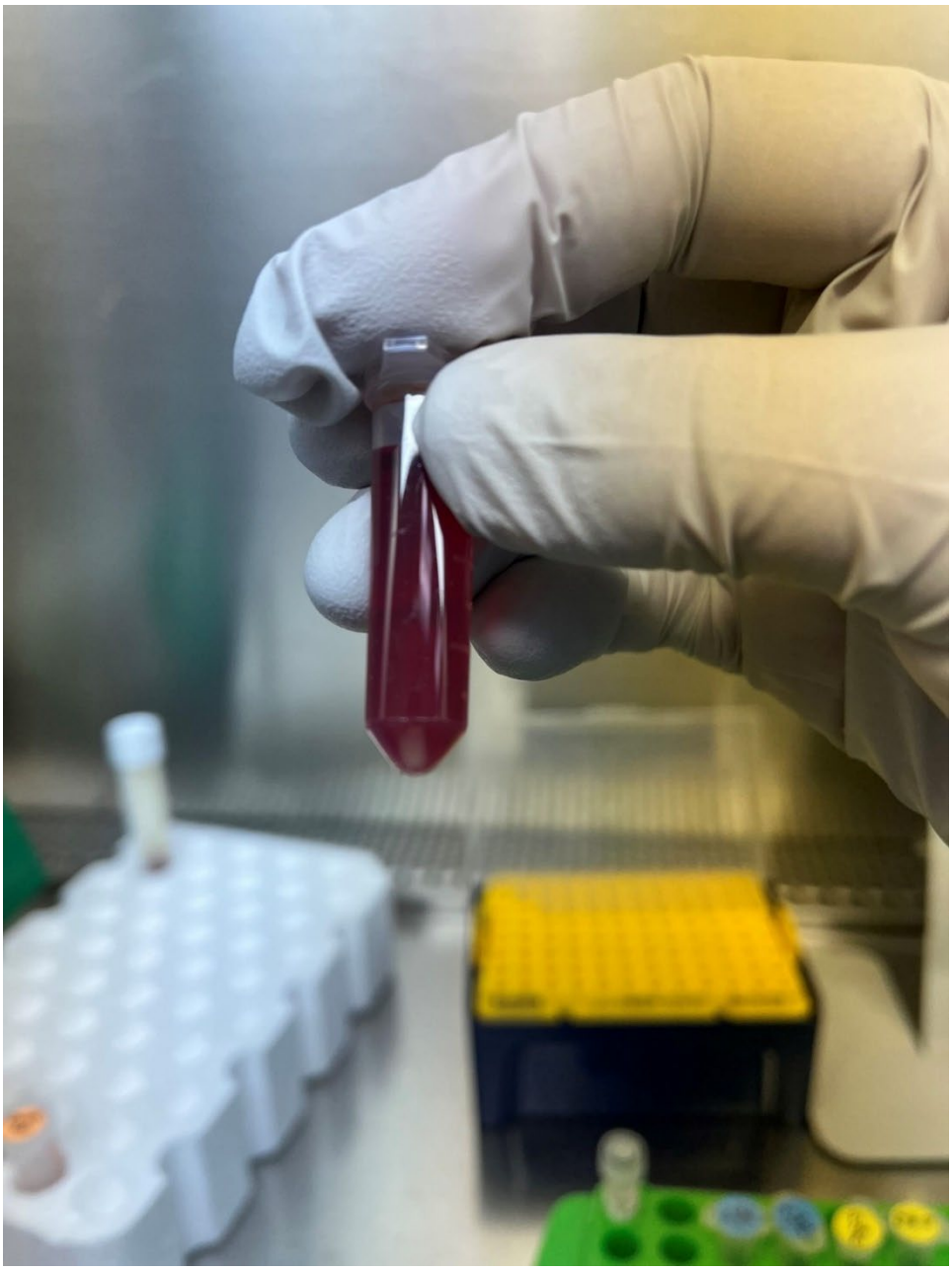


Figure 1. BMA (Heparin + DNA Stabilizer) following RBC lysis centrifugation

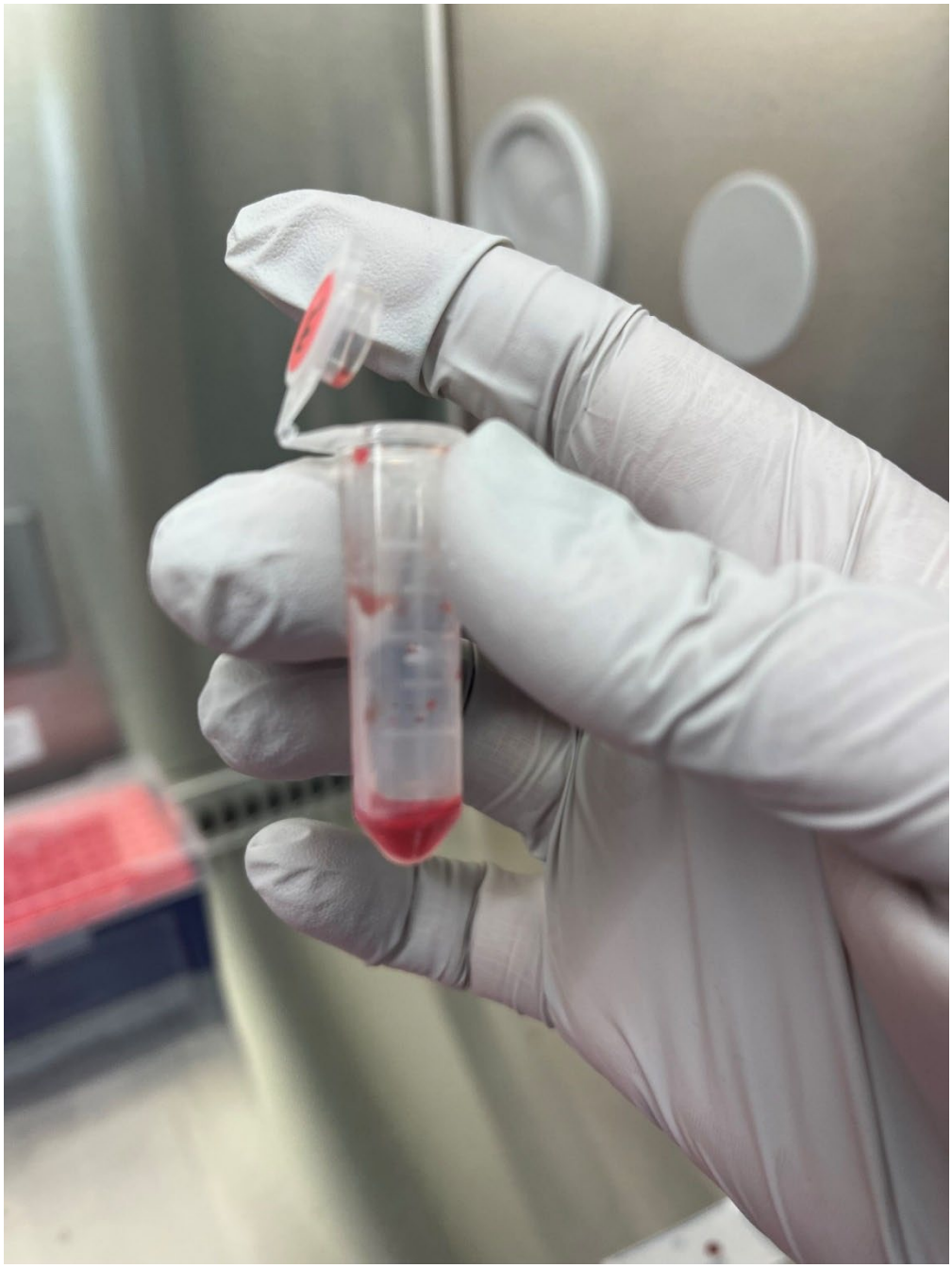


Figure 2. BMA (Heparin + DNA Stabilizer) following 1,650 µl supernatant removal

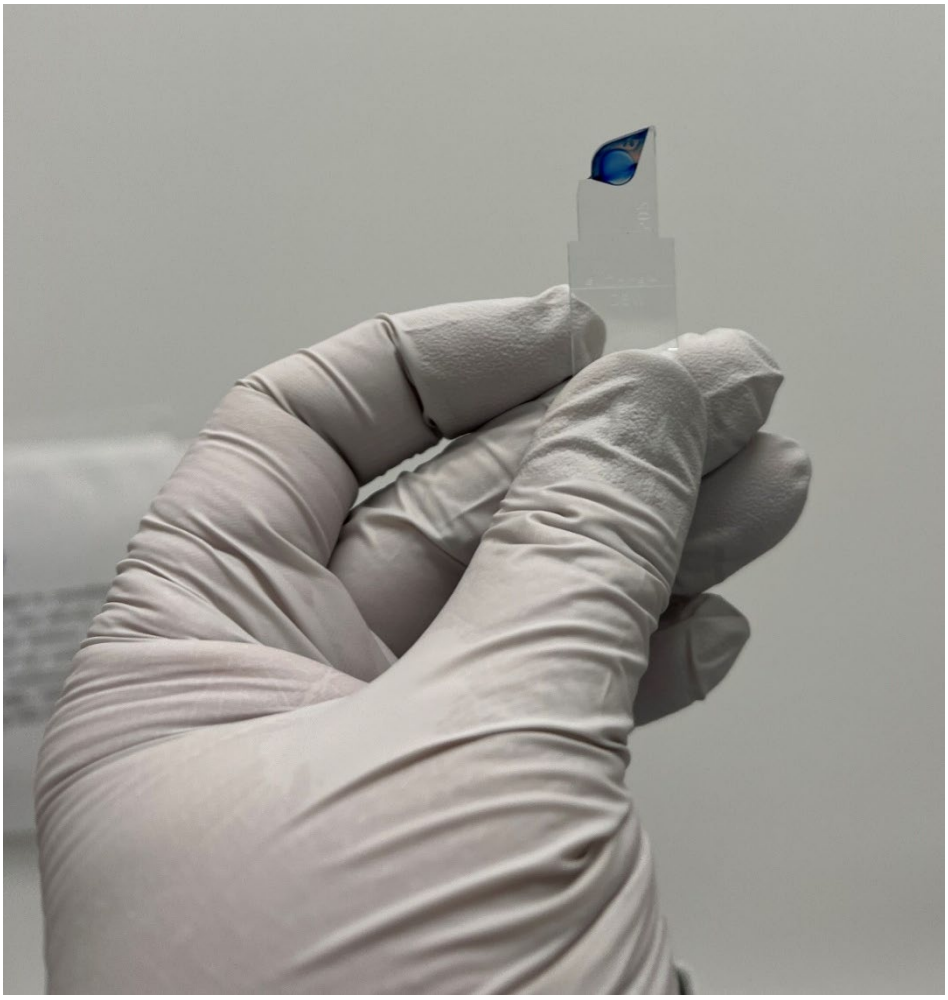


Figure 3. BMA (EDTA) RBC lysed WBCs in Stabilizing Buffer in filled HemoCue cuvette.

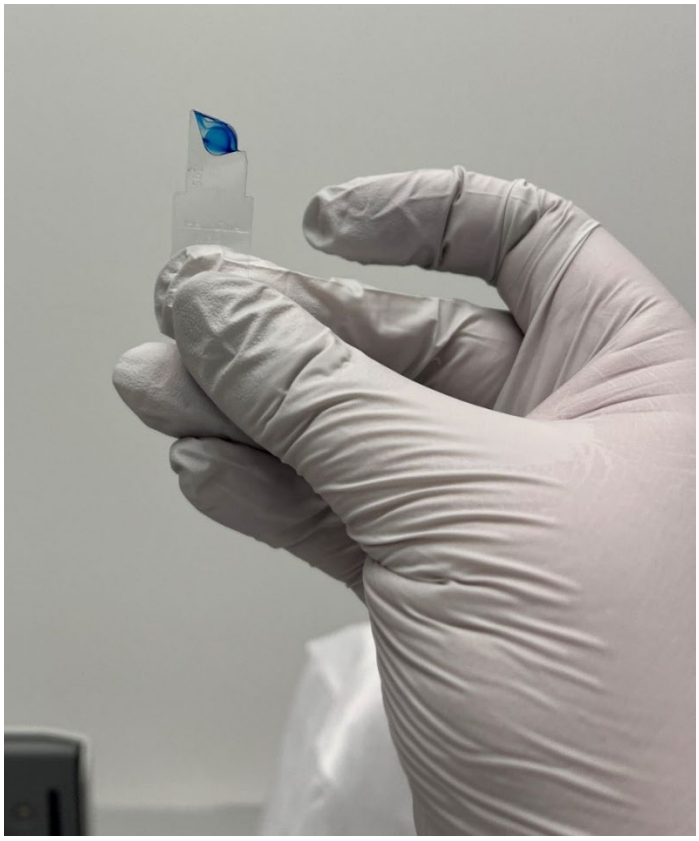


Figure 4. Non-filled (non-used) HemoCue cuvette (primarily for comparison to **Figure 3**)

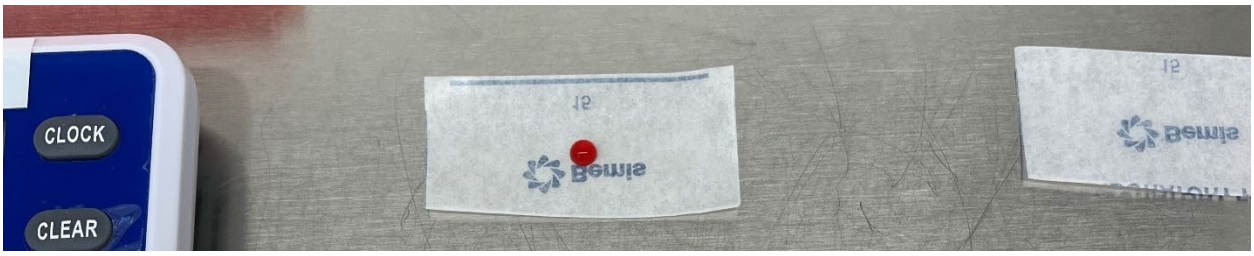


Figure 5. A 20 µl spot of RBC lysed WBCs from BMA (EDTA) in Stabilizing Buffer added to Parafilm

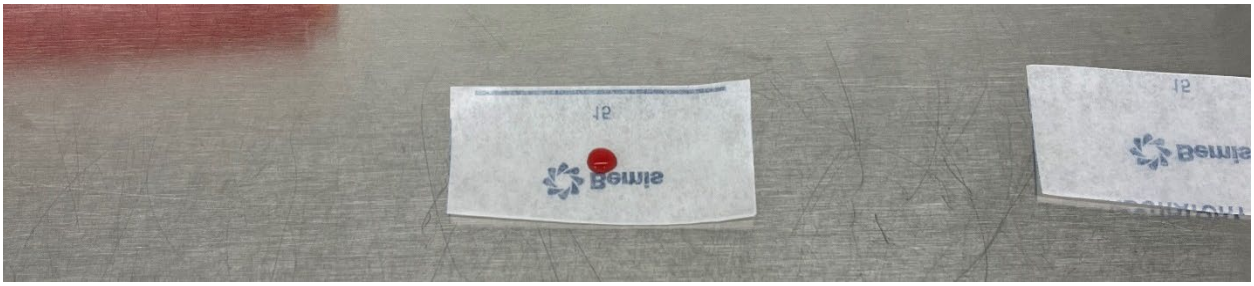


Figure 6. A 20 µl spot of RBC lysed WBCs from BMA (EDTA) in Stabilizing Buffer following proper filling of HemoCue microcuvette (i.e. RBC lysed WBCs in Stabilizing Buffer are significantly less viscous, requiring ≤ 1 second for liquid to fill the HemoCue cuvette when wicking from 20 µl spot on Parafilm, relative to 'crude' (i.e. non-RBC lysed WBCs from filtered BMA)

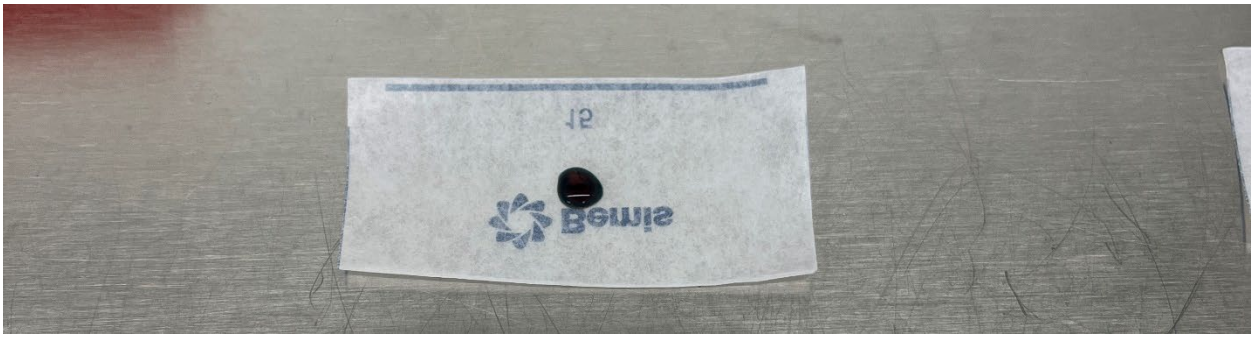


Figure 7. A 20 µl spot of RBC lysed WBCs from BMA (EDTA) in Stabilizing Buffer after **improper** filling of HemoCue microcuvette (i.e. RBC lysed WBCs in Stabilizing Buffer are significantly less viscous, requiring ≤ 1 second for liquid to fill the HemoCue cuvette when wicking from 20 µl spot on Parafilm. Here, the HemoCue cuvette was left on the surface of the 20 µl spot of WBCs for a total of 5 seconds. The leftover material on the Parafilm began changing from red to blue (indicating that the dye in the HemoCue microcuvette is leaking into spot of WBCs in as little as 3 seconds).

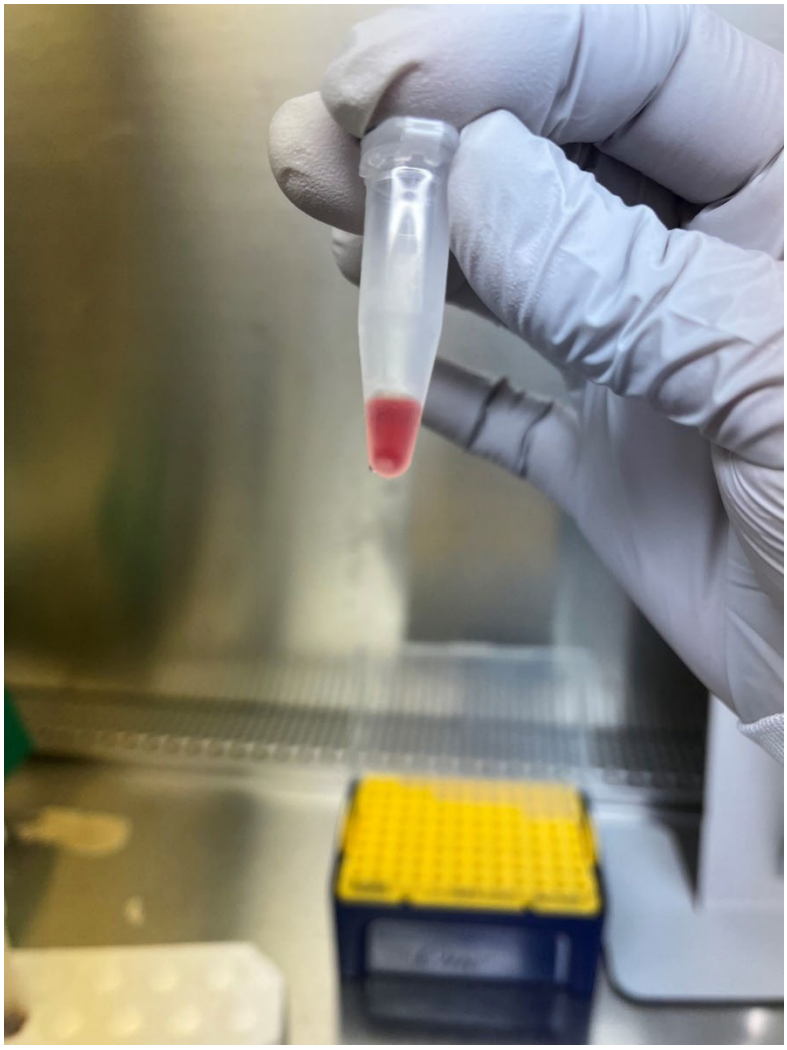


Figure 8. 1.5M BMA (heparin) WBCs following spin after transfer based on post RBC lysis HemoCue count

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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