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

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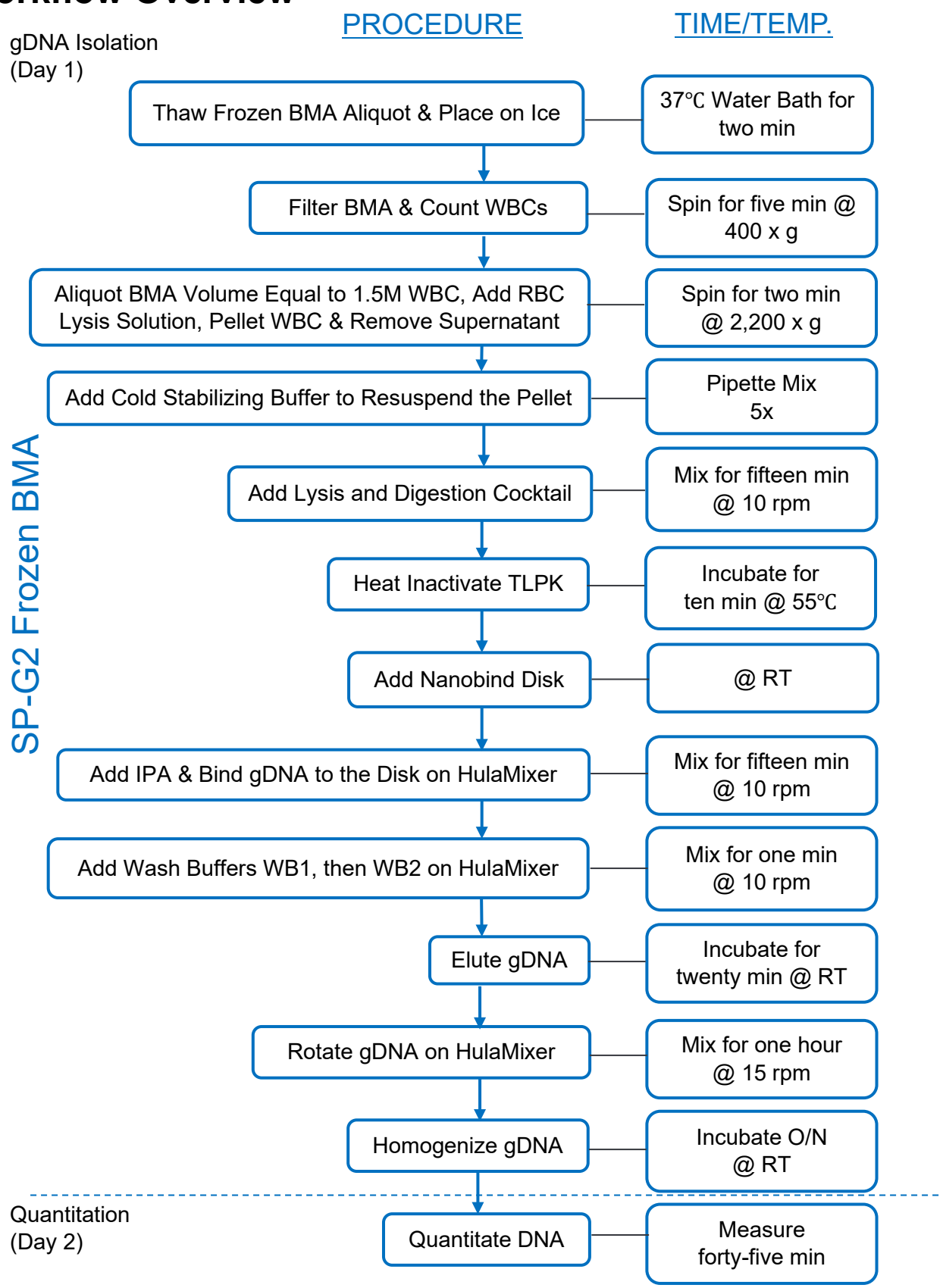
# Revision History

| REVISION | NOTES |
|---|---|
| **A** | Commercial release. |
| **B** | Formatting updates. |
| **C** | Formatting updates. |
| **D** | Updated to accommodate the change from 12 to 24 reaction kits. |

# Workflow Overview



# Bionano Prep SP-G2 Frozen Human BMA 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.

## Bionano Prep SP-G2 Bionano Prep SP BMA Add-On, 1 or 2 x (Part # 80062, 12 preps)

| Item | Amount | Part Number | Storage | Handling Considerations |
|---|---|---|---|---|
| BMA Filter | 24 ea. | 20464 | Room Temp (15-30°C) | |
| Microcentrifuge Tubes, 2 ml | 2 x 12 ea. | 20452 | Room Temp (15-30°C) | |
| DNA Stabilizer | 4 ml | 20398 | Room Temp (15-30°C) | |

## 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)<br>Distributor (outside US) | 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 | 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 |

| Item | Supplier | Catalog # |
|---|---|---|
| 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 |
| 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 BMA DNA Isolation Protocol can provide ultra-high molecular weight (UHMW) genomic DNA (gDNA) in approximately five hours from 1.5 million white blood cells (WBC) of frozen human bone marrow aspirates (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 several bone marrow aspirates. The donor bone marrow aspirate was drawn into a heparin tube, frozen and then processed without additional freeze/thaw cycles. 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 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 45-120 ng/µl.

### PIPETTING VISCOUS gDNA

To draw viscous gDNA, hold the stock tube for close-up visualization, depress the pipette plunger until the first stop, submerge the pipette tip and gently and slowly release the plunger to start drawing the viscous gDNA into the tip while carefully monitoring uptake. Keep the tip submerged even after the viscous solution stops moving upward and levels off. Be patient. Viscous gDNA can take a few seconds to fill 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

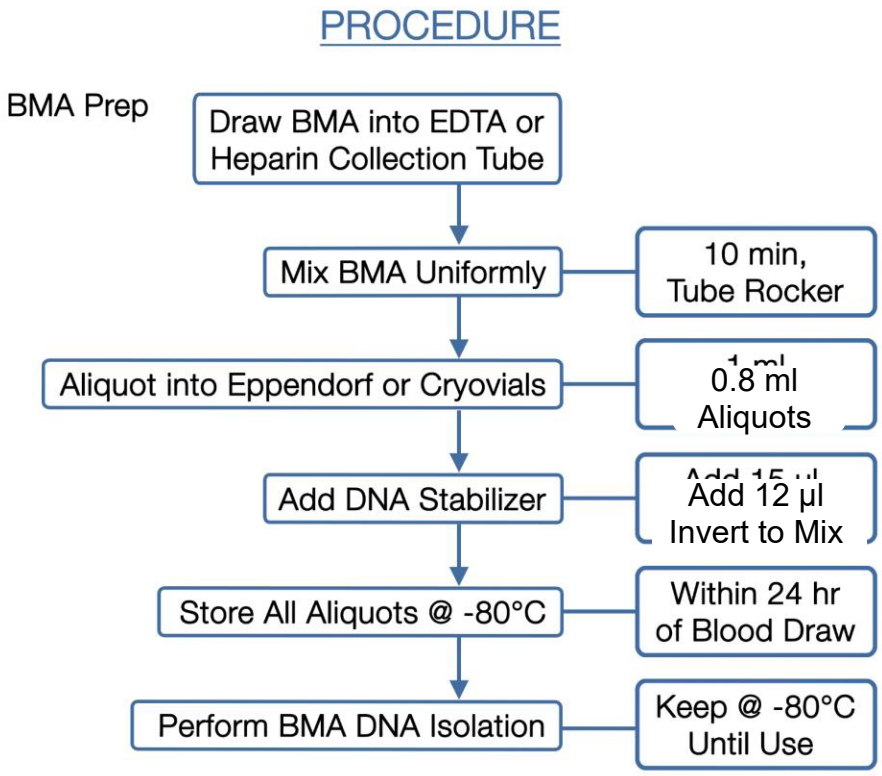
Processing no more than six samples at a time and up to two batches per working day is recommended. A minimum of 2.5E+9 cells/L HemoCue reading is required.

**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 FRESH HEPARINIZED HUMAN BONE MARROW ASPIRATES (BMA) FOR STORAGE WITH DNA STABILIZER**

The gDNA content is obtained from the WBC. Recommended input is 1.5E+06 WBCs. For each BMA sample, two heparinized BMA aliquots (~0.8 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 one serving as a backup. Samples should be frozen within 24 hours of aspiration, keep at 4°C until frozen at -80°C.




1. Mix fresh heparinized human BMA thoroughly at room temperature to ensure good uniformity (ten minutes on tube rocker at room temperature).
2. Processing one BMA at a time, transfer two 0.8 ml aliquots into nuclease-free 1.5 ml tubes.
3. Add 12 µl of DNA Stabilizer to each tube containing the 0.8 ml volume of fresh human BMA.
4. Cap tubes, invert ten times to mix. Pulse-spin tubes for one second to collect any material from the microcentrifuge tube lid, and immediately move aliquots to -80C for long term storage.
5. Do not thaw aliquot from -80°C until proceeding with gDNA isolation.

# Bionano Prep SP-G2 Frozen Human BMA DNA Isolation Protocol

## Preparation for gDNA Isolation (30 minutes)

**BEFORE FIRST USE**

1. Add 1.5.X 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 400 x g, five minutes room temperature for the BMA filtration spin.
   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: 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 50 µl Stabilizing Buffer (SB) by mixing 49 µl Cell Buffer with 1 µl DNA Stabilizer. Multiply by the number of preps if batch size is more than one. Vortex to mix 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 two 2.0 ml microcentrifuge tubes (Bionano) for BMA filtration step. Insert a 100 µm BMA filter into each 2.0 ml tube. Place the two tubes, with seated BMA filter inserts, 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 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 in Step 14 in gDNA Isolation.

## gDNA Isolation (Up to 3 hours 45 minutes)

**THAW/FILTER/COUNT/ALIQUOT BMAS, ADD RBC LYSIS SOLUTION, PELLET WBCS, AND REMOVE SUPERNATANT**

**Recommended Input: 1.5E+06 WBCs**

1. For each sample, remove one 0.8 ml aliquot of frozen heparinized BMA containing DNA Stabilizer from the -80°C freezer and place on ice. Thaw up to six frozen BMA 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.

   **NOTE**: If unsure whether DNA Stabilizer was added to the 0.8 ml aliquot of heparinized BMA before freezing, add 12 µl of DNA Stabilizer to tube upon thaw and proceed to Step 2. If the frozen heparinized BMA is not in a 0.8 ml aliquot with added DNA Stabilizer, refer to the Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide (P/N 30608).

2. Processing one sample at a time, for a batch size of no more than six samples:

   a. Remove one BMA aliquot from ice and invert ten times to mix. Pulse spin sample for one second to collect any material from the microcentrifuge tube lid. Place sample on ice.
   b. For each thawed BMA sample, transfer 400 µl into two separate BMA filters (split entire BMA volume equally), each seated in a labeled 2.0 ml microcentrifuge tube.
   c. Carefully place the tubes with seated filters in the benchtop microcentrifuge and centrifuge for five 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.
   d. Carefully remove the tubes from the microcentrifuge.
   e. Remove and discard the filters into a biohazardous waste container. Cap the tubes and place on ice.
   f. Pool the two filtered sample volumes into either of the 2.0 ml microcentrifuge tubes after gently pipette mixing (to avoid bubbles) the entire volume of filtered sample in each tube 10x with a P1000. Cap the filtered and pooled BMA sample tube and place on ice. Discard the remaining 2.0 ml tube.

3. Processing one filtered and pooled BMA sample at a time, for a batch size of no more than six samples:
   a. Invert the BMA sample ten times to mix. Pulse spin sample for one second to collect any material from the microcentrifuge tube lid. Immediately dispense 20 µl onto 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 filtered and pooled BMA is too high (> 30E+09 cells/L) and falls outside the range of detection, the HemoCue instrument display will read “HHH.” Typically, BMAs 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 BMA 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 BMA sample to a 1.5 ml tube containing 75 µl of Cell Buffer (to make a 1:4 dilution of the BMA 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 values in **Table 2**.
   - HemoCue Reading = WBC count (after Cell Buffer dilution) x DF (=4)

c. 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
   - RBC Lysis Solution Volume (µl) = Transfer Volume x 3 (µl)
   - Removal Volume 1 (µl) = RBC Lysis Volume (µl)
   - Removal Volume 2 (µl) = Transfer Volume – 40 µl; or
   - If splitting filtered BMA into two 1.5 ml tubes for RBC Lysis steps due to low HemoCue reading (≤ 2.5E+09 cells/L): Removal Volume 2 (µl) = Transfer Volume – 10 µl

**NOTE:** The HemoCue gives readings in E+09 cells/L, but calculation is based on E+03 cells/µl to transfer 1.5E+06 WBCs into a pre-chilled 1.5 ml Protein LoBind tube.

**Example Sample Calculations:**

i. **Use a single 1.5 ml Protein LoBind tube when HemoCue reading ≥ 5.0E+09 cells/L (5.0E+03 cells/µl).**
**For instance, HemoCue reading = 7.5E+09 cells/L (7.5E+03 cells/µl)**
   - Transfer Volume (µl) = 1.5E+06 cells ÷ 7.5E+03 cells/µl = 200 µl
   - RBC Lysis Solution Volume (µl) = 200 µl x 3 = 600 µl
   - Removal Volume 1 (µl) = RBC Lysis Solution Volume = 600 µl
   - Removal Volume 2 (µl) = Transfer Volume - 40 µl = 160 µl

ii. **Use two 1.5 ml Protein LoBind tubes when 2.5E+09 cells/L ≤ HemoCue reading < 5.0E+09 cells/L.**
**For instance, HemoCue reading = 2.5E+09 cells/L (2.5E+03 cells/µl)**
   - Transfer Volume (µl) = 1.5E+06 cells ÷ 2.5E+03 cells/µl = 600 µl
   - Transfer 300 µl to each of the 1.5 ml Protein LoBind tube
   - RBC Lysis Solution Volume (µl) for each tube = 300 µl x 3 = 900 µl
   - Removal Volume 1 (µl) = RBC Lysis Solution Volume = 900 µl
   - Removal Volume 2 (µl) = Transfer Volume - 10 µl = 290 µl

**Table 2.** Frozen BMA Sample Worksheet

| Sample ID | HemoCue Reading * | Transfer Volume | RBC Lysis Solution Volume | If HemoCue Reading ≥ 5.0E+09 cells/L (One 1.5 ml tube for RBC Lysis) | | If 2.5E+09 cells/L ≤ HemoCue Reading < 5.0E+09 cells/L (Two 1.5 ml tubes for RBC Lysis) | |
|---|---|---|---|---|---|---|---|
| | (E+03 cells/µl) | (1.5 E+06 cells ÷ HemoCue Reading) | | Removal Volume 1 = RBC Lysis Solution Volume | Removal Volume 2 = Transfer Volume – 40 µl | Removal Volume 1 = RBC Lysis Solution Volume | Removal Volume 2 = Transfer Volume – 10 µl |
| | | µl | µl | µl | µl | µl | µl |
| | | µl | µl | µl | µl | µl | µl |
| | | µl | µl | µl | µl | µl | µl |
| | | µl | µl | µl | µl | µl | µl |
| | | µl | µl | µl | µl | µl | µl |
| | | µl | µl | µl | µl | µl | µl |

*Multiply by DF (=4) if Cell Buffer is used to dilute BMA sample due to high WBC count (HemoCue displays HHH, gDNA Isolation Step 3.b.).

4. Processing one sample at a time:
   a. Invert BMA sample ten times to mix. Pulse spin for one second to collect any material from the microcentrifuge tube lid. Place sample on ice.
   b. 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:** If the transfer volume is between 300 - 600µl split the transfer volume into two 1.5 ml Protein LoBind tubes.
5. To each sample add [RBC Lysis Solution Volume], calculated in **Table 2**. Cap the tube and place at room temperature.
6. Invert sample ten times to mix. Incubate sample at room temperature for five minutes.
7. After incubation, invert sample ten times to mix.
8. Spin the 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.
9. 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.

10. 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.
11. After centrifugation, remove supernatant from each sample tube as follows:
    a. Using an extra-long, filtered 1,000 µl pipette tip, remove the supernatant volume equal to Removal Volume 1 [RBC Lysis Solution Volume] from each tube (aspirate from top of liquid). Discard supernatant into bleach conical. Cap the tube and place sample on ice. The remaining supernatant volume should be the [Transfer Volume].
    **NOTE:** If the Removal Volume 1 is > 1,000 µl, change tips between passes.
    b. Using a P200 remove the supernatant volume equal to [Removal Volume 2] from each tube. Aspirate from the meniscus and do not disturb the pellet. Discard supernatant into bleach conical. Cap the tube and place sample on ice. After supernatant removal, there should be approximately 40 µl (or 10 µl for two 1.5 ml tubes) of supernatant including the WBC pellet.
    **NOTE:** If the Removal Volume 2 is > 200 µl, change tips between passes.

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

12. To each sample, add 20 µl of cold Stabilizing Buffer on top of the ~40 µl (or ~10 µl for two 1.5 ml tubes) containing supernatant and WBC pellet.
13. Processing one sample at a time, use a 200 µl standard bore pipette tip 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. Combine the two suspensions if two 1.5 ml Protein LoBind tubes were used for RBC Lysis. Place combined suspension 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.

14. 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, sample will be handled at room temperature.
15. Add 430 µl of Complete Lysis and Digestion Cocktail Master Mix to each sample. Cap the tube. Change tips between samples.
16. Invert mix each sample fifteen times.
17. Rotate sample on HulaMixer for fifteen minutes at room temperature at 10 rpm, no shaking or vibration.

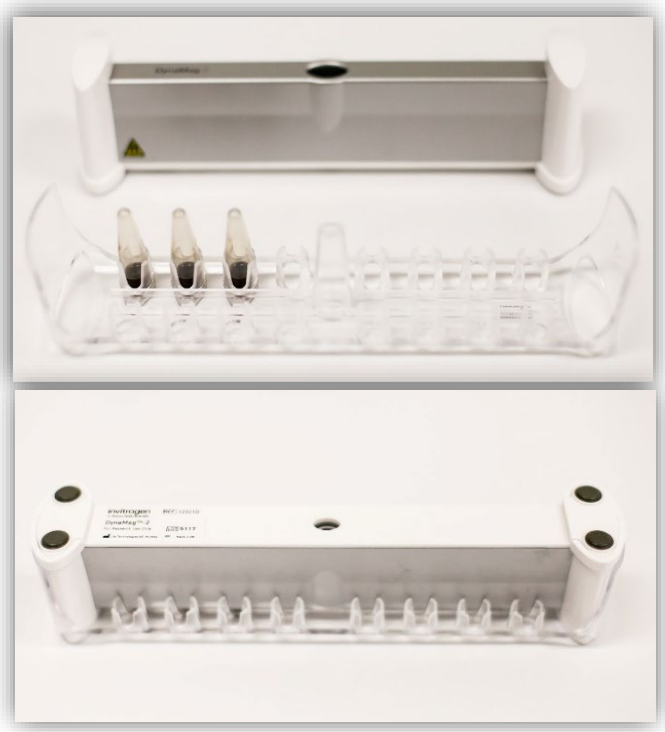
18. During the rotation return the TLPK back to -20°C storage. Discard any remaining unused Lysis and Digestion Cocktail Master Mix (with TLPK) into the 50 ml conical designated for GuHCl liquid waste. Fill the bleach conical to 50 ml with water, cap conical, invert mix, and dispose the contents down the sink.
19. Remove sample from HulaMixer, and pulse spin sample for two seconds.
20. Incubate sample in a Thermomixer pre-set to 55°C for ten mins, with no shaking.
21. Remove sample from the Thermomixer and turn the Thermomixer off.

**gDNA BIND, WASH AND ELUTE**

22. Using pointed forceps, carefully add a single 4 mm Nanobind Disk to the lysate.
    **NOTE:** Disks can sometimes stick together.
23. Add 480 µl of 100% IPA to each sample.
24. Invert mix each sample five times.
25. 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.
26. Remove sample from the HulaMixer.
27. 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.

28. Set one P1000 pipette to 1,000 µl and a second to 700 µl.
29. 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 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.

⚠ 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).

30. 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 27a through 27e.
    g. Remove supernatant as described in Step 29.

    ⚠ 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).

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

32. 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 27a through 27e.
    g. Remove supernatant as described in Step 29.

    ⚠ 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).

33. Repeat Wash WB2, Step 32.
34. 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.
35. 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.
36. 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.
37. 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

38. Add 65 µl of EB to the 0.5 ml Protein LoBind tube containing the Nanobind Disk and cap the tube.
39. Spin the tube on benchtop microcentrifuge for five seconds.
40. 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).
41. Incubate submerged Nanobind Disk in EB at room temperature for twenty minutes.
42. Collect extracted gDNA by transferring eluate to the labeled 2.0 ml microcentrifuge tube with a 200 µl standard tip.
43. Spin the tube with the Nanobind Disk in benchtop microcentrifuge for five seconds to separate the residual eluate from the Nanobind Disk.
44. 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.
45. Pulse spin samples for two seconds.

## Homogenization of gDNA Solution (70 minutes)

#### gDNA HOMOGENIZATION

46. 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.

47. 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.
48. Remove microcentrifuge tube from rack of HulaMixer and spin tube on benchtop microcentrifuge for two seconds to pull the gDNA down to the bottom of the tube.
49. Allow the gDNA to equilibrate overnight at room temperature (25°C) to homogenize.

    **NOTE:** Most samples can be labeled the next day or within forty-eight hours post gDNA isolation using the *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 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 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 5 strokes (1 stroke = 1 up stroke + 1 down stroke) **using a wide bore tip**. Let the gDNA rest overnight at room temperature before repeating quantitation and performing DLS labeling the next day. Typical DNA concentrations range from 45-90 ng/µl.

**Table 3.** 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 Direct Label and Stain (DLS) labeling within forty-eight hours post isolation. See the "Kits and Consumables" section at https://bionanogenomics.com/support/ for applicable kits and protocols.

# Technical Assistance

For technical assistance, contact Bionano 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@bionano.com |
| **Phone** | Hours of Operation:<br>Monday through Friday, 9:00 a.m. to 5:00 p.m., PST<br>US: +1 (858) 888-7663 |
| **Website** | www.bionano.com/support |
| **Address** | Bionano, Inc.<br>9540 Towne Centre Drive, Suite 100<br>San Diego, CA 92121 |

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