# Bionano Prep® SP-G2 Fresh and Cryopreserved Amniocyte and CVS DNA Isolation Tech Note

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CG-00075

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A

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bionano

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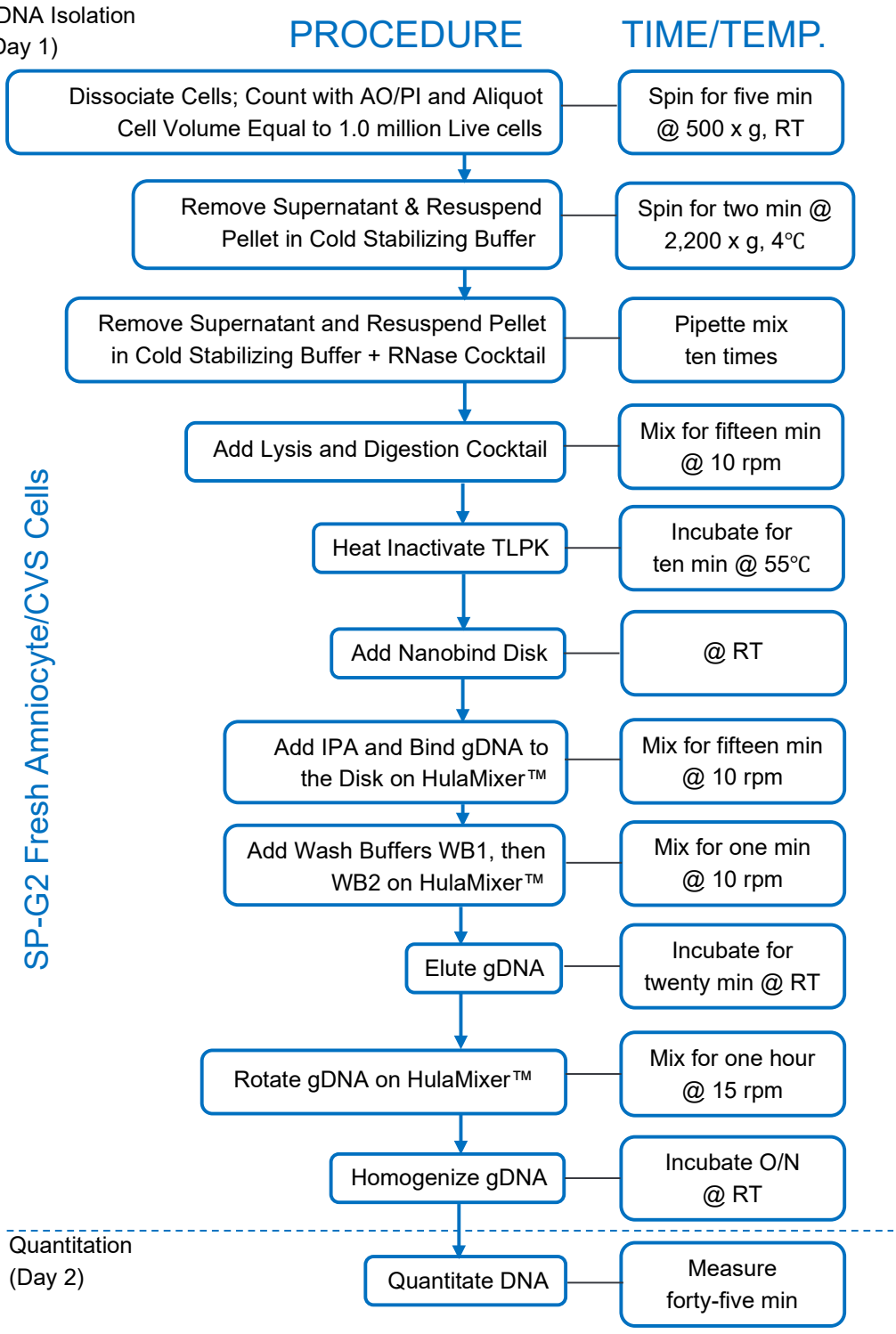
# Revision History

| REVISION | NOTES |
|---|---|
| **A** | Commercial release. |

# Workflow Overview (Fresh Cell Pellets gDNA Isolation)



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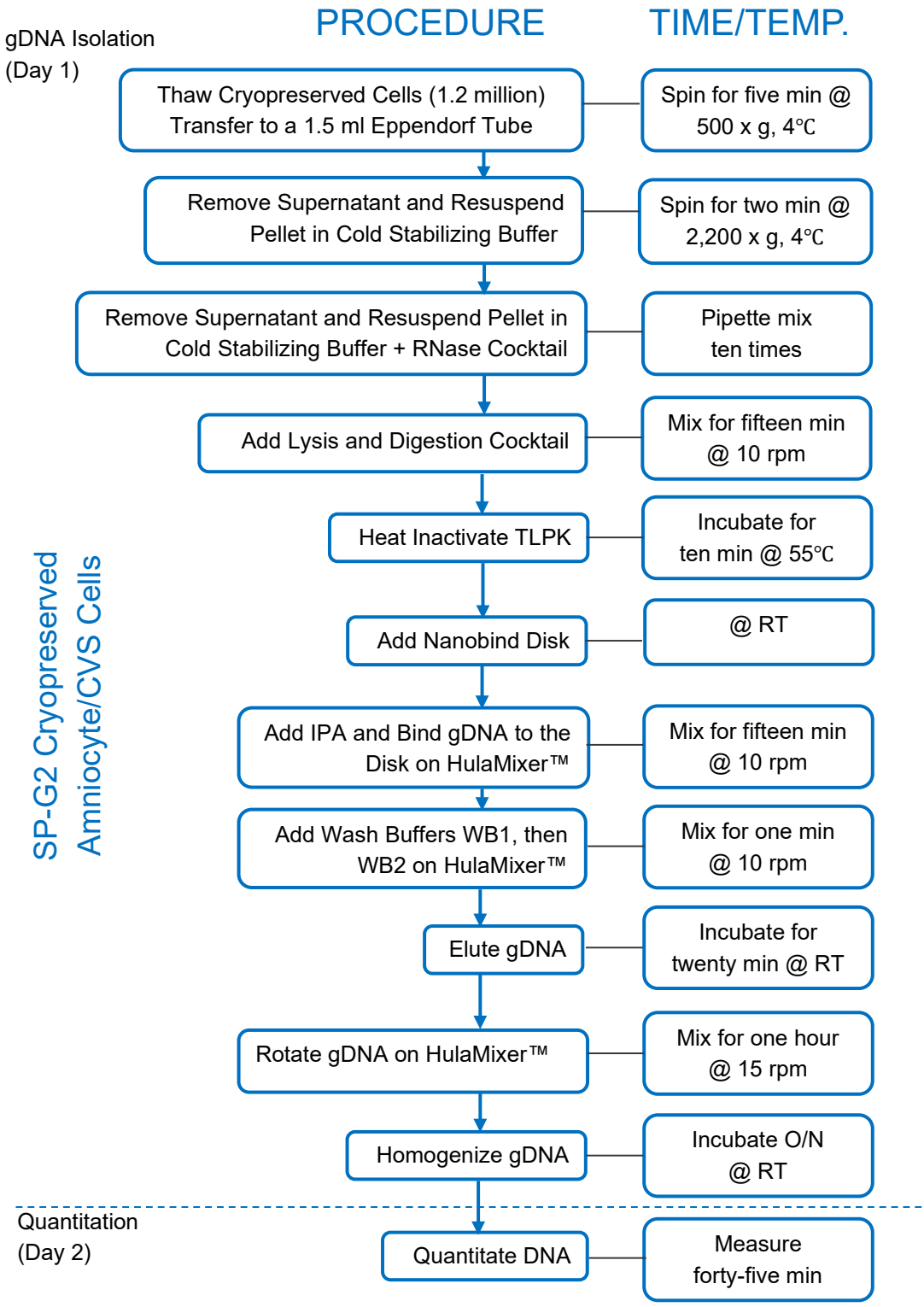

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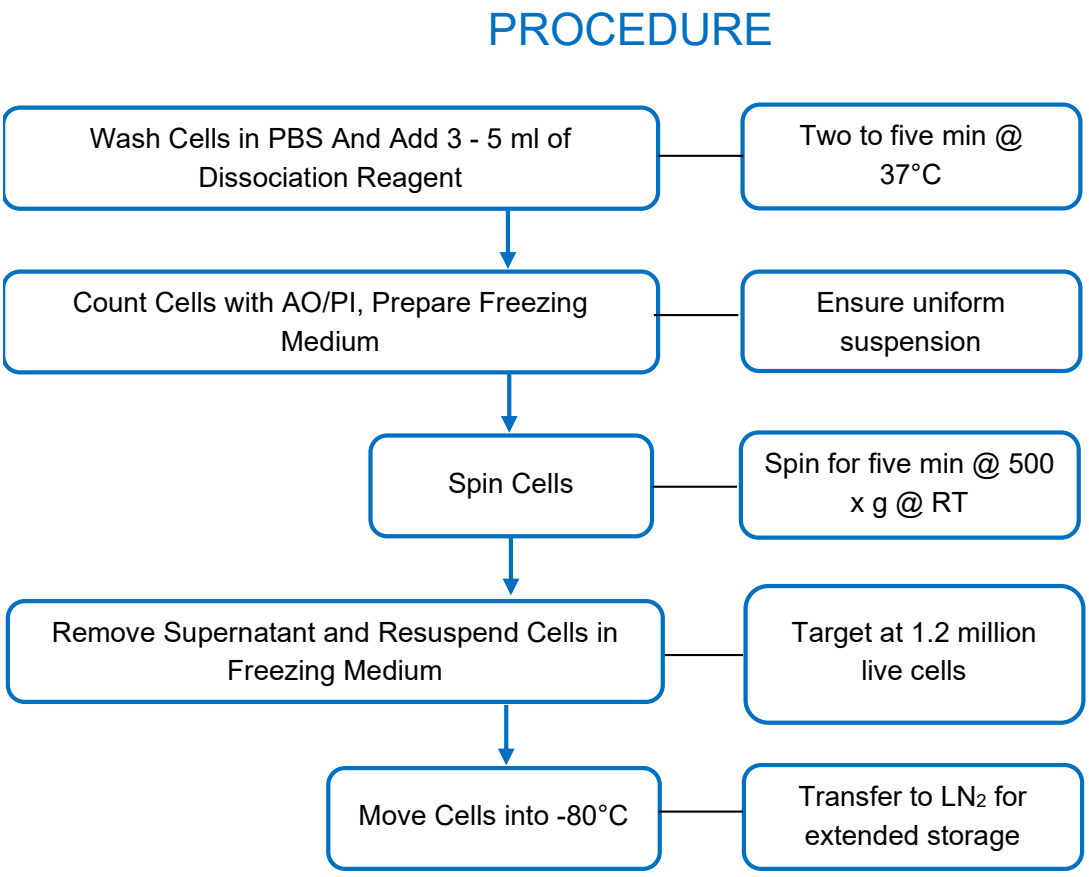


# Bionano Prep® SP-G2 Fresh and Cryopreserved Amniocyte and CVS DNA Isolation Kit and User-Supplied Materials

**Table 1**. *Bionano Prep® SP-G2 Fresh and Cryopreserved Amniocyte and CVS DNA Isolation Tech Note* (CG-00075) utilizes Bionano Prep® SP-G2 Blood & Cell Culture DNA Isolation Kit Contents (Part # 80060, 12 preps).

| Component | Part # | Quantity | Storage | Handling Considerations |
|---|---|---|---|---|
| RBC Lysis* | 20442 | 18 ml | 15-30°C | |
| Cell Buffer | 20374 | 50 ml | 15-30°C | |
| Digestion Enhancer | 20443 | 4.0 ml | 15-30°C | |
| Lysis and Binding Buffer (LBB) | 20444 | 1.2 ml | 15-30°C | See “Important Notes” section for hazardous waste information. |
| Wash Buffer 1 (WB1) | 20445 | 4.5 ml | 15-30°C | See “Important Notes” section for hazardous waste information. |
| Wash Buffer 2 (WB2) | 20446 | 6.0 ml | 15-30°C | |
| Elution Buffer (EB) | 20378 | 1.1 ml | 15-30°C | |
| DE Detergent | 20447 | 55 µl | 15-30°C | |
| 4mm Nanobind Disks | 20448 | 12 ea. | 15-30°C | |
| Protein LoBind Microcentrifuge Tubes, 1.5 ml | 20449 | 2 x 12 ea. | 15-30°C | |
| Protein LoBind Microcentrifuge Tubes, 0.5 ml | 20450 | 12 ea. | 15-30°C | |
| Magnetic Retriever Plastic Sheath | 20451 | 12 ea. | 15-30°C | |
| Microcentrifuge Tubes, 2.0 ml | 20452 | 12 ea. | 15-30°C | |
| DNA Stabilizer | 20423 | 350 µl | 15-30°C | |
| RNase A | 20455 | 150 µl | 2°C-8°C | |
| Ultrapure Water | 20355 | 2 x 900 µl | 2°C-8°C | |
| Thermolabile Proteinase K (TLPK) | 20441 | 150 µl | -15°C to -25°C | |

*Not used in this Tech Note.

# User-Supplied Materials and Equipment

| Item | Supplier | Catalog # |
|---|---|---|
| Bionano Prep SP Magnetic Retriever (2-pack) | Bionano Genomics (Training Kit) | 80031 |
| Hemocytometer and Phase Contrast Microscope or Automated Cell Counter with Acridine Orange/Propidium Iodide (AO/PI) stain solution | General Lab Supplier | |
| Serological Pipettes, Sterile | General Lab Supplier | |
| Pipette-Aid | General Lab Supplier | |
| DynaMag™-2 Magnet | Thermo Fisher | 12321D |
| HulaMixer™ Sample Mixer | Thermo Fisher | 15920D |
| Cryovials, 1 ml | General Lab Supplier | |
| 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 Liquid Biohazardous Waste Disposal | General Lab Supplier | |
| Conical Centrifuge Tubes, 50 ml, Polypropylene | Thermo Fisher or Equivalent | 14-432-22 |
| Conical Centrifuge Tubes, 15 ml, Polypropylene | Thermo Fisher or Equivalent | 05-539-12 |
| Centrifuge with a Swinging Bucket Rotor for 15 ml Conical Tubes | General Lab Supplier | |
| Refrigerated Centrifuge with 1.5 ml Tube Rotor (2,200 x g spin) | Cole-Parmer or Equivalent | EW-17701-11 |
| Water Bath, 37°C | General Lab Supplier | |
| T25/T75 cell culture flasks | General Lab Supplier | |
| Growth Medium (ex. AmnioMAX II™) | General Lab Supplier | |
| Phosphate Buffered Saline (PBS), pH 7.2, without calcium, magnesium, and phenol red | Thermo Fisher or Equivalent | 20012027 |
| Dissociation Enzyme Reagent (TrypLE™ or equivalent) | Thermo Fisher or equivalent | 12604013 |
| Quaternary Ammonium Disinfectant | VAI or Equivalent | DQ100-01 |
| Sterile 5- and 10-ml Disposable Pipettes (TD+) | General Lab Supplier | |
| Dimethyl Sulfoxide (DMSO) | Sigma-Aldrich or Equivalent | D2650 |

| Item | Supplier | Catalog # |
|---|---|---|
| Ice Bucket and Ice | General Lab Supplier | |
| Eppendorf® ThermoMixer® C and Eppendorf SmartBlock™ 1.5 ml, Thermoblock set to 55°C<br>(Or alternatively a 55°C heat block for 1.5ml tubes or water bath as alternatives) | Eppendorf or Equivalent | 5382000023, 5360000038 |
| Pointed Forceps | Electron Microscopy Sciences or Equivalent | 78141-01 |
| Wide-bore Pipette Tips, Filtered, Aerosol, 200 µl | VWR or Equivalent | 46620-642 |
| Pipettes (10, 20, 200, and 1000 µl) and Nuclease Free, Filtered Pipette Tips | General Lab Supplier | |
| Foam or isopropanol freezing chamber | Corning or equivalent | P659-432008 |
| 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 | Branson or equivalent | CPX 952-119R |
| 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 pipette MR-10 (recommended)** | Rainin or equivalent | 17008575 |
| Pipette 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 lead to the most successful outcome from the workflow.

**A positive displacement pipette is a special pipette with a plunger that operates a built-in piston in special tips that go with the pipette. Such a pipette 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 and Cryopreserved Amniocyte and Chorionic Villus Samples CVS DNA Isolation Tech Note* can provide ultra-high molecular weight (UHMW) genomic DNA (gDNA) in less than four hours from 1.0 million live fresh Amniocyte/CVS cells or 1.2 million live cryopreserved cells. It utilizes an improved lyse, bind, wash, and elute procedure that is common for silica-based gDNA extraction technologies in combination with a novel paramagnetic disk. Unlike magnetic beads and silica spin columns, which shear large gDNA, the Nanobind Disk binds and releases gDNA with significantly less fragmentation, resulting in UHMW gDNA. High gDNA binding capacity is the result of a novel nano structured silica on the outside of the thermoplastic paramagnetic disk. This tech note was evaluated using patient-derived cultured amniocyte and CVS cell cultures. gDNA prepared using this protocol has been validated only with Direct Label and Stain Generation 2 (DLS-G2) labeling. See Training Video for technically critical steps and troubleshooting. The current workflow is set up such that up to 6 cell cultures can be comfortably processed in a batch.

## Overview

Cell lysis and Thermolabile Proteinase K digestion occurs in a chaotropic buffer, and the released gDNA binds to the Nanobind Disk upon the addition of isopropanol. After three wash steps, the disk is transferred to a fresh tube and the gDNA is eluted from the disk. The recovered UHMW gDNA is subjected to limited shearing to make the UHMW gDNA more homogeneous. The gDNA is then mixed and equilibrated overnight at room temperature to facilitate DNA homogeneity and the concentration is determined. Typical gDNA size range is from 50 Kbp to ≥ 1 Mbp.

## Important Notes

### DNA HOMOGENEITY

Recovered gDNA is subjected to pipette mixing with a 200 µl standard pipette tip to increase homogeneity, ensuring consistent DNA sampling for labeling.

### gDNA QUANTITATION

gDNA quantitation is used to measure concentration and serves as a gauge of UHMW gDNA homogeneity. Qubit™ quantitation is preferred over other quantitation methods since it can also be used for measuring the 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* sections 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

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

## HAZARDOUS WASTE DISPOSAL

Buffers Digestion Enhancer, LBB and WB1 contain guanidine hydrochloride (GuHCl). GuHCl is harmful if swallowed or inhaled and causes skin and eye irritation. DO NOT mix with bleach or acidic reagents. Liquid waste containing GuHCl should be safely decontaminated with a quaternary ammonium disinfectant before disposal in a hazardous waste stream. We recommend following local environmental, health and safety regulations for decontamination and disposal of all solutions mixed with GuHCl.
**NOTE:** VAI DECON-QUAT 100® Quaternary Ammonium Solution is used for product development at Bionano.

# Bionano Prep® SP-G2 Fresh and Cryopreserved Amniocyte and CVS DNA Isolation Tech Note

### 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.

### Preparing Fresh Cell Pellets or Cryopreserved Cells for gDNA Isolation

#### SET UP

2. Gather materials and verify needed equipment (see “User Supplied Material” section above).
   a. Set water bath to 37°C. Verify temperature with thermometer.
   b. Pre-warm growth medium (ex. AmnioMAX II), PBS (pH 7.2), and dissociation enzyme (ex. TrypLE) in 37°C water bath.
   c. Prepare hemacytometer and phase-contrast microscope or automated cell counter with AO/PI stain solution.
   d. Prepare DMSO for cryopreservation. If DMSO is stored at 4°C, thaw in 37°C water bath. Place at room temperature.
   e. Verify access to a swinging bucket rotor centrifuge that can accommodate 15 ml conical tubes. Set spin settings to 500 x g, room temperature.
   f. Cool a microcentrifuge for 1.5 ml microcentrifuge tubes to 4°C.
   g. Retrieve pipettes and tips.
   h. Retrieve ice bucket and ice.
   i. 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)
   j. Label desired number of cryovials for cryopreservation.
   k. Prepare freezer chamber designed for cell cryopreservation. Follow manufacturing product protocol for use. Label desired number of 1.5 ml protein LoBind microcentrifuge tubes for cell pellets. Place on ice.
   l. HulaMixer Sample Mixer
   m. 100% IPA
   n. DynaMag-2 Magnetic Tube Rack
   o. Bionano Prep SP Magnetic Retriever
   p. Set a Thermomixer to 55°C, 10 minutes, no shaking.

q. Pointed Forceps
r. Label one 2.0 ml tube (for batch size of three or fewer), or one 5.0 ml tube (for four to six samples) for the Lysis and Digestion Cocktail Master Mix.

3. Gather the following reagents and materials from the Bionano Prep SP-G2 kit: Cell Buffer, DNA Stabilizer, RNase A, Digestion Enhancer, DE Detergent, Ultrapure Water, Nanobind Disk, microcentrifuge tubes, sheath, LBB, WB1, WB2 and EB.
   a. For each sample, prepare 1,200 µl Stabilizing Buffer (SB) by mixing 1,176 µl Cell Buffer with 24 µl DNA Stabilizer. Multiply by the number of preps if batch size is more than one. Vortex to mix and place on ice.
   b. Flick RNase A 3x to mix. Pulse spin and place on ice.
   c. For each sample, prepare 48 µl Stabilizing Buffer/RNase A Cocktail Master Mix by mixing 36 µl SB with 12 µl RNase A. Multiply by the number of preps if batch size is more than one. Vortex briefly to mix. Pulse spin and place on ice.
   d. 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.
   e. For each sample, label one 2.0 ml microcentrifuge tube (Bionano) for gDNA homogenization step. Place in a rack at room temperature.

**HARVESTING AND COUNTING CELLS (0.5 – 1.5 HOURS)**

**NOTE:** Bionano recommends growing Amniocyte or CVS cultures in T25 flasks targeting 90% confluence.

4. Remove all growth medium from flask using a sterile serological pipette. Discard into the bleach waste.
5. Rinse flask with 5 ml of warm PBS (pH 7.2). Swirl around flask briefly and remove PBS from the flask using a sterile serological pipette, discard into bleach waste.
6. Add 1.5 - 3 ml (depending on flask size) of 1x TrypLE or equivalent dissociation enzyme. Gently swirl solution around the flask to ensure the enzyme coats full surface area of flask. Then place flask in 37°C $CO_2$ incubator for 2 - 5 minutes, until cells visibly dissociate under microscope.
7. Add 5 - 10 ml of growth medium (ex. AmnioMAX II) to the flask and pipette medium against the growth surface of the flask several times to fully remove cells from the plastic substratum. Remove entire volume using a sterile serological pipette to a new 15 ml conical and count live cells with a hemocytometer or automated cell counter using AO/PI stain solution as per manufacturer's instructions.
   **NOTE:** To prepare fresh cell pellets for gDNA isolation, go to **Step 8**; To prepare cryopreserved cells for future gDNA isolation, go to **Step 16**.

**PREPARING FRESH CELL PELLETS FOR gDNA ISOLATION**

**NOTE:** Recommended Input is 1.0 million live cells with ≥ 70% live cell viability.

8. Calculate the volume of original stock cell culture containing 1.0 million live cells. If the live cell density is < 0.7 million live cells/ml, proceed to **Step 9**. If live cell density is ≥ 0.7 million live cells/ml, proceed to **Step 10**.
9. Concentrate cells (if cell density is < 0.7 million live cells/ml).

a. Transfer appropriate volume equivalent to the 1.0 million live cells of stock cell culture into a 15 ml conical tube.
b. Centrifuge 15 ml conical tube at room temperature at 500 x g for 5 minutes in a swinging bucket rotor to pellet cells.
c. Using a sterile serological pipette, remove supernatant, discard into the 50 ml conical containing bleach, and resuspend cells with a smaller volume of growth medium to obtain a live cell concentration of at least 0.7 million live cells/ml.
d. Count number of live cells with a cell counting device using AO/PI staining solution.
e. Calculate concentrated stock cell culture volume to yield 1.0 million live cells per pellet.

10. Aliquot cells
    a. Pipette mix stock cell culture suspension to ensure homogenous cell suspension.
    b. Aliquot the calculated volume of concentrated stock cell culture containing 1.0 million live cells (calculated in **Step 8**) into each pre-labeled, pre-chilled 1.5 ml Protein LoBind tube. Place on ice.
11. Pellet cells
    a. Centrifuge cells at 4°C at 500 x g for five minutes in a refrigerated centrifuge with 1.5 ml tube fixed angle rotor microcentrifuge.
    b. Using a 1000 µl pipette, remove entire supernatant without disturbing the pellet. Discard supernatant in the 50 ml conical tube containing bleach. Place sample on ice.
12. Wash cells with Cold Stabilizing Buffer
    a. Add 1 ml of cold Stabilizing Buffer to each pellet.
    b. Resuspend pellet by pipetting up and down three times with a 1000 µl pipette set to 1,000 µl. Place sample on ice.
    c. Centrifuge the cells at 4°C at 2,200 x g for two minutes in a refrigerated centrifuge with 1.5 ml tube fixed angle rotor microcentrifuge.
    d. During the centrifugation, retrieve TLPK from -20°C storage and place it on ice.
    e. After centrifugation, place samples on ice.
    f. Using a 1000 µl pipette, aspirate entire supernatant and discard into the 50 ml conical tube containing bleach. Use a 200 µl pipette to remove any residual liquid from cell pellet.
    g. Keep samples on ice until all supernatants have been removed.
13. Add 40 µl cold Stabilizing Buffer/RNase A Cocktail Master Mix on top of each pellet. Place on ice.
14. Processing one sample at a time, and using a 200 µl standard bore pipette tip, gently scratch pellet in a circular fashion three to five times to dislodge the pellet into solution. Then using the same tip, slowly pipette mix the sample ten times to resuspend the pellet. Place sample on ice. Change tips between samples.
    **NOTE:** Aspirate the entire sample volume into the tip and visually inspect the tube while mixing to ensure that 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.

15. Proceed to “Lyse, Digest, and Inactivate Thermolabile Proteinase K” (**Step 27**).

**PREPARING CRYOPRESERVED CELLS FOR gDNA ISOLATION**

16. Calculate the volume of original stock cell culture containing 1.2 million live cells. If the live cell density is <0.8 million live cells/ml, proceed to **Step 17**. If live cell density is ≥ 0.8 million live cells/ml, proceed to **Step 18**.

17. Concentrate cells (if cell density is < 0.8 million live cells/ml)
    a. Transfer appropriate volume equivalent to the 1.2 million live cells of stock cell culture into a 15 ml conical tube.
    b. Centrifuge 15 ml conical tube at room temperature at 500 x g for 5 minutes in a swinging bucket rotor to pellet cells.
    c. Using a sterile serological pipette, remove supernatant, discard into the 50 ml conical containing bleach, and resuspend cells with a smaller volume of growth medium to obtain a live cell concentration of at least 0.8 million live cells/ml.
    d. Count number of live cells with a cell counting device using AO/PI staining solution.
    e. Calculate concentrated stock cell culture volume to yield 1.2 million live cells per pellet.
18. Aliquot cells
    a. Pipette mix stock cell culture suspension to ensure homogenous cell suspension.
    b. Aliquot the calculated volume of concentrated stock cell culture containing 1.2 million live cells (calculated in **Steps 17d** and **17e**) into each pre-labeled, pre-chilled 1.5 ml Protein LoBind tube. Place on ice.
19. Using a fixed angle centrifuge, centrifuge cells at 500 x g for 5 minutes at room temperature. While cells are in centrifuge, prepare freezing medium (growth medium with 5% DMSO).
20. Remove supernatant and discard into bleach waste. Based on the AO/PI live cell count from **Step 17**, resuspend the pellet in freezing medium at a concentration of 1.2 million live cells/ml. Aliquot the 1 ml suspension into a 1 ml cryovial.
21. Freeze overnight at -1°C/min in a -80°C freezer using a freezing chamber designed for cell cryopreservation. Follow manufacturer’s protocol for use instructions.
22. After the cells have completely frozen, it is recommended to transfer cryovials to a liquid nitrogen tank for long-term storage.
23. To isolate gDNA from cryopreserved cells, retrieve cryovials from liquid nitrogen tank and transfer to a 37°C water bath. Continuously swirl the vials in the water bath, checking every 20 seconds to see if any vials have fully thawed.
24. Once a vial of cells has thawed, immediately remove it from water bath and place on ice.
    **NOTE**: Depending on the volume in each vial, some vials may thaw sooner than others. If a vial has thawed, but others are still frozen, place the thawed vial on ice, and continue swirling the remaining vials in the 37°C water bath.

25. Using a 1000 µl pipette, transfer the entire volume of thawed cell culture into a pre-labeled, pre-chilled 1.5 ml Protein LoBind tube. Place the 1.5 ml Protein LoBind tube back on ice.

26. Perform **Steps 11-14** for each sample.

**LYSE, DIGEST, AND INACTIVATE THERMOLABILE PROTEINASE K**

27. 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 order of component addition listed in **Table 2**. Do not add TLPK to the Cocktail Master Mix yet. Cap the tube, invert mix fifteen times, and place the tube on a tube rack at room temperature.
**NOTE:** Do not vortex.

**Table 2.** 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 | 180.0 | | 1.2 | | 1 |
| Nuclease-free Water | 40.8 | | 1.2 | | 2 |
| LBB* | 53.0 | | 1.2 | | 3 |
| DE Detergent* | 2.5 | | 1.2 | | 4 |
| TLPK** | 10.0 | | 1.2 | | 5 |
| Total | **286.3** | | | | |

*Pipette LBB and DE Detergent slowly due to high viscosity and risk of bubble formation.
**Add right before use at **Step 28** in gDNA Isolation.

28. 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.

29. Add 286.3 µl of Complete Lysis and Digestion Cocktail Master Mix to each sample. Cap the tube and place sample at room temperature. Change tips between samples.

30. Invert mix each sample fifteen times.

31. Rotate sample on HulaMixer for fifteen minutes at room temperature at 10 rpm, with no shaking or vibration.

32. During the rotation return the TLPK back to -20°C storage. Discard any remaining unused Complete 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 of the contents down the sink.

33. Remove sample from HulaMixer, and pulse spin sample for two seconds.
34. Incubate sample in a Thermomixer pre-set to 55°C for ten minutes, with no shaking.
35. Remove sample and place at room temperature.

**gDNA BIND, WASH AND ELUTE**

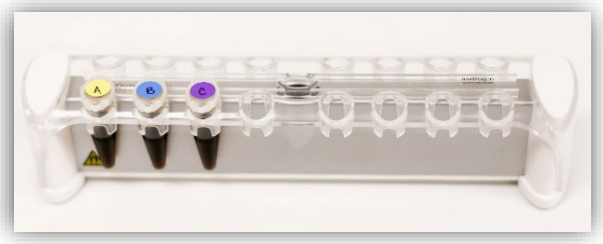
36. Using pointed forceps, carefully add a single 4 mm Nanobind Disk to the lysate.
    **NOTE:** Disks can sometimes stick together.
37. Add 320 µl of 100% IPA to each sample.
38. Invert mix each sample five times.
39. Rotate sample on HulaMixer for fifteen minutes at room temperature at 10 rpm, no 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 the HulaMixer and invert the 1.5 ml Protein LoBind tube until the Nanobind Disk goes back into the solution. Replace the tube on the HulaMixer and resume mixing.
40. Remove sample from HulaMixer.
41. Combine Dynamag clear rack with the magnetic base as outlined below, making sure Nanobind Disk is secured by the magnet near the top of the liquid level. If not secured, re-rack (See Training Video).

    a. Invert clear Dynamag tube rack and place upside down with sample lids touching the work surface. The tubes will be on the same row of the rack, and in the row furthest from the front.

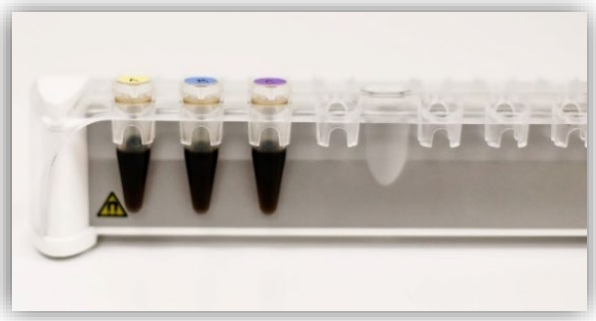
    b. Invert Dynamag magnetic base and lower onto clear rack.

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

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



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



42. Set one 1000 µl  Pipette to 1,000 µl and a second to 700 µl.
43. Remove supernatant as outlined below, being careful not to aspirate the gDNA and changing tips between samples (See Training Video, at minute 1:15):
    a. Angle entire rack at 45° by holding it in one hand (grasping the entire apparatus from below with tubes visible and lids towards user's other hand).
    b. Wait two seconds for gDNA to lay on the Nanobind Disk.
    c. Gently remove all liquid with a 1,000 µl extra-long tip angled away from the Nanobind Disk and/or gDNA to avoid disruption.
    d. Dispense supernatant into the 50ml conical 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 Training Video at minute 8:50.
44. Perform Wash with 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 1 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 41a** through **41e**.
g. Remove supernatant as described in **Step 43**.
**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 Troubleshooting section.

45. Set the second pipette to 500 µl (previously at 700 µl).
46. 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 41a** through **41e**.
    g. Remove supernatant as described in **Step 43**.
    **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 Training video minute 8:50.
47. Repeat Wash WB2, **Step 46**.
48. 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.
49. Fully insert the Bionano Prep SP Magnetic Retriever into a clean Magnetic Retriever Plastic Sheath until the Magnetic Retriever comes into full contact with the bottom of the sheath.
50. 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.
51. 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

52. Add 65 µl of EB to 0.5 ml Protein LoBind tube containing the Nanobind Disk and cap the tube.
53. Spin the tube on benchtop microcentrifuge for 5 seconds.
54. Using a 10 µl standard tip, gently nudge the Nanobind Disk towards the bottom of the tube, ensuring that it is fully submerged in liquid. The disk should remain parallel to the bench surface (see Training Video).
55. Incubate submerged Nanobind Disk in EB at room temperature for twenty minutes.
56. Collect extracted gDNA by transferring eluate to the labeled 2.0 ml microcentrifuge tube with a 200 µl standard tip.
57. Spin the tube with the Nanobind Disk on a benchtop microcentrifuge for five seconds to separate the residual eluate from the Nanobind Disk.
58. 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.
59. Pulse spin samples for two seconds.

### Homogenization of gDNA Solution (70 minutes)

#### gDNA HOMOGENIZATION

60. Slowly pipette the entire gDNA volume into a standard 200 µl tip, then slowly dispense the gDNA. Avoid creating bubbles.
    Repeat this process 3 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.
61. Place 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 gDNA solution remains in the bottom of the tube during initial rotations, turn off the HulaMixer and position the rack so that the microcentrifuge tube is oriented upside down. Gently flick the bottom of the microcentrifuge tube until the gDNA is drawn into the lid and resume mixing.
62. 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.

63. Allow the gDNA to equilibrate overnight at room temperature to homogenize.
    **NOTE:** Most samples can be labeled the next day or within forty-eight hours post gDNA isolation using the *DLS-G2 protocol* (CG-30553).

## gDNA Quantitation (45 minutes)

### QUBIT QUANTITATION – BROAD RANGE (BR) dsDNA ASSAY

Refer to the Qubit dsDNA BR Assay Kit user manual for kit details and follow the methods described in the **Important Notes** "Pipetting Viscous Genomic DNA (gDNA)" section, to ensure accurate pipetting of viscous gDNA.

1. Equilibrate Qubit BR Assay Kit Standards to room temperature.
   **NOTE:** If the gDNA has been stored at 4ºC, equilibrate at room temperature and pulse spin before moving to the next step.
2. Add Qubit BR Buffer to 0.5 ml Qubit Assay Tubes:
   a. For each sample, add 18 µl of Qubit BR Buffer to three separate Qubit Assay Tubes.
   b. For the Qubit Standards, add 10 µl Qubit BR Buffer to two separate Qubit Assay Tubes.
3. Using a 200 µl pipette with a wide bore tip, gently mix the entire gDNA sample volume by pipetting up and down five times, being careful not to generate bubbles.
4. Using a new standard pipette tip or positive displacement pipette tip for each draw:
   Remove 2 µl aliquots from the left side, middle, and right side of each sample and dispense into BR Buffer of corresponding Qubit Assay Tube, rinsing tip when dispensing. Place Assay Tubes in a floating rack and sonicate for ten minutes. Perform **Steps 5** and **6** during sonication.
   **NOTE:** If a bath sonicator is not available, vortex for at least thirty seconds at maximum speed, then spin down briefly for two seconds.
5. Prepare Working Solution by diluting the Dye Assay Reagent into BR Dilution Buffer (1:200):
   a. 200 µl Working Solution for each of the two standards (400 µl total).
   b. 200 µl Working Solution for each sample aliquot (600 µl for each sample).
6. For the Qubit DNA standards, add 10 µl of Standards 1 and 2 to the Assay Tubes containing Qubit 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 Qubit BR 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-G2 labeling the next day.

**Table 3.** gDNA Quantitation (BR dsDNA) Worksheet

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

## LABELING

gDNA samples are ready for DLS-G2 labeling within 48 hours post isolation. See the “Kits and Consumables” section at https://bionano.com/support/ for applicable kits and protocols and the *DLS-G2 Protocol* (CG-30553).

# 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., PT<br>US: +1 (858) 888-7663<br><br>Monday through Friday, 9:00 a.m. to 5:00 p.m., CET<br>UK: +44 115 654 8660<br>France: +33 5 37 10 00 77<br>Belgium: +32 10 39 71 00 |
| **Website** | www.bionano.com/support |
| **Address** | Bionano, Inc.<br>9540 Towne Centre Drive, Suite 100<br>San Diego, CA 92121 |

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