Bionano Prep® DLS-G2 Protocol

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E

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bionano

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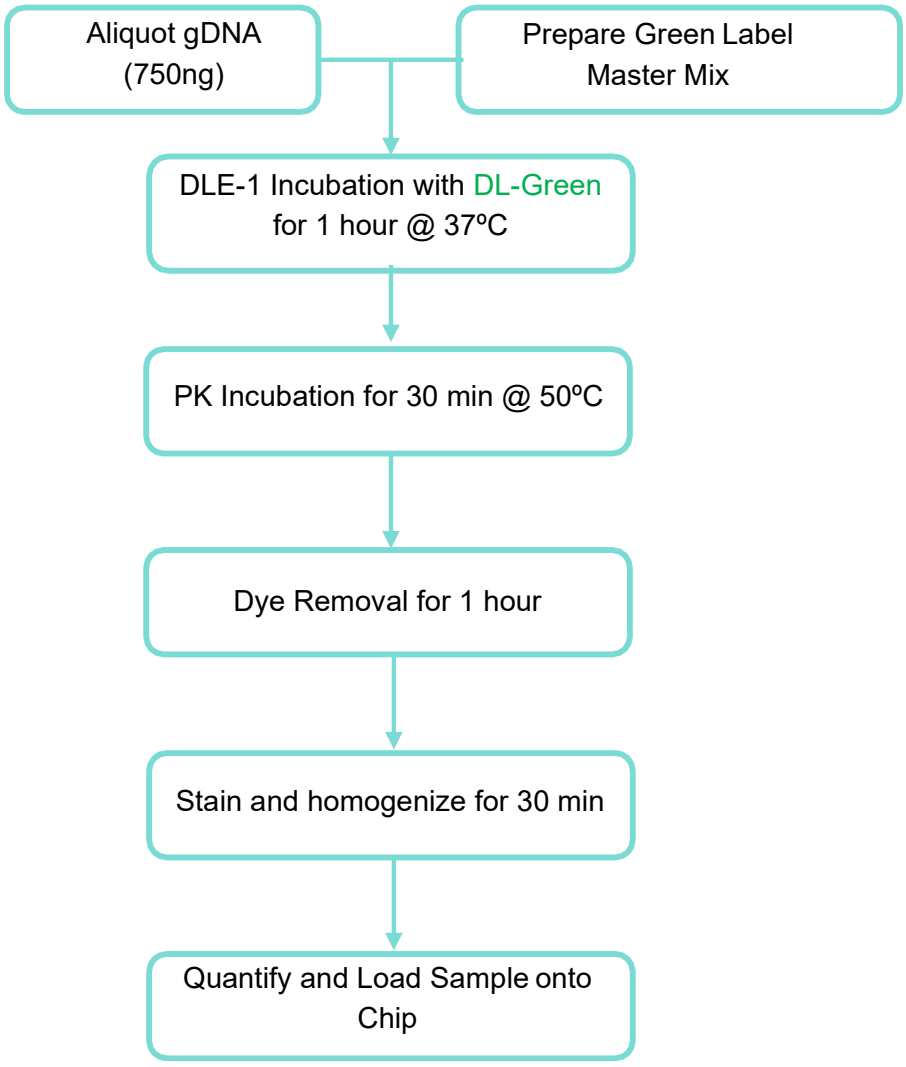
Revision History

REVISION	NOTES
A	Initial release
B	Updated verbiage in steps 9 and 10 for clarification.
C	Removed Troubleshooting Guide/FAQ sections. Now contained in standalone document: CG-30608. Removed time estimates in protocol steps.
D	Updated Prot. K digestion time from 5 minutes 30 minutes throughout document. In user supplied materials, removed standard, unfiltered pipet tips & replaced with standard, filtered pipet tips. Changes made to wording to improve clarity.
E	The DLE-1 Labeling mixing step 3 was updated from mixing a minimum of 5 times to mixing 7 times with a pipet.

Bionano Prep DLS-G2 Overview

The Bionano Prep DLS-G2 (Direct Label and Stain-G2) Labeling Kit and Protocol is a sequence-specific labeling kit and protocol for labeling ultra-high molecular weight (UHMW) genomic DNA (gDNA) for use on the Bionano Optical Genome Mapping (OGM) platform using a Direct Labeling Enzyme (e.g., DLE-1).

Workflow



Bionano Prep DLS-G2 Labeling Kit and User-Supplied Materials

Table 1. Bionano Prep DLS-G2 Labeling Kit Contents (P/N 80046)

Component	Part #	Quantity	Storage	Handling Considerations
10x DLE-1	20430	44 µl	-25°C to -15°C	Flick tube three times to mix, and centrifuge briefly. Keep in -20°C enzyme cooler until use.
20x DL-Green	20429	22 µl	-25°C to -15°C	Thaw at room temperature. Vortex and centrifuge briefly. Keep on pre-chilled aluminum block (4°C) until use.
5x DLE-1 Buffer	20428	175 µl	-25°C to -15°C	Thaw at room temperature. Vortex and centrifuge briefly. Keep at room temperature until use.
DNA Stain	20356	65 µl	-25°C to -15°C	Thaw at room temperature. Vortex and centrifuge briefly. Keep at room temperature until use; DMSO in DNA Stain will crystalize on ice.
10x DTT	20432	90 µl	-25°C to -15°C	Thaw at room temperature. Vortex and centrifuge briefly. Keep at room temperature until use.
Proteinase K	20434	60 µl	2°C to 8°C	
4x Flow Buffer	20431	225 µl	2°C to 8°C	Vortex and centrifuge briefly. Keep at room temperature until use.
Ultra-Pure Water	20355	900 µl	2°C to 30°C	May keep at room temperature.
DLS 24 Well Plate	20357	One plate	15°C to 30°C	Keep covered to avoid dust.
DLS Membranes	20358	25 ea.	15°C to 30°C	Avoid excess moisture.
DLS Tape Sheets	20433	12 ea.	15°C to 30°C	
DLS amber tubes (2 ml)	20437	14 ea.	15°C to 30°C	

Table 2. User-Supplied Materials

Item	Description	Catalog #
HulaMixer™ Sample Mixer	Thermo Fisher	15920D
Thermocycler with heated lid	General lab supplier	
PCR tubes, 0.2 ml, thin-walled, nuclease-free	Thermo Fisher or equivalent	AM12225
Microcentrifuge tubes: 0.5 ml amber, nuclease-free.	USA Scientific or equivalent	1605-0007
Pipet tips, wide bore, filtered, 200 µl	USA Scientific or Fisher Scientific (LabCon)	1011-8810, 1152-965-008-9
Pipet tips, standard, filtered; 2, 10, 20 and 200 µl	General lab supplier	
-20°C benchtop enzyme cooler	VWR or equivalent	414004-286
4°C aluminum cooling tube block	Sigma Aldrich or equivalent	Z740270
Forceps, pointed and curved	Electron Microscopy Sciences or equivalent	78141-01
Pipets (2, 10, 20 and 200 µl)	General lab supplier	
Ice bucket and Ice	General lab supplier	
Benchtop Vortexer	VWR or equivalent	10153-838
Microcentrifuge for 0.2 ml, 0.5 ml, and 1.5 ml Tubes	General lab supplier	
Qubit™ Fluorometer	Thermo Fisher	Q33238
Qubit Assay Tubes	Thermo Fisher	Q32856
Qubit HS (High Sensitivity) dsDNA Assay Kit	Thermo Fisher	Q32851
Bath sonicator (optional)	Branson or equivalent	CPX 952-119R
Positive-displacement pipet MR-10 (recommended)**	Rainin or equivalent	17008575
Pipet tips, 10 µl, C-10 for pos. displacement (recommended)**	Rainin or equivalent	17008604

*(Item in blue) We strongly encourage not substituting this equipment and getting the exact item recommended, as using these will give the most successful outcome from the workflow.

+Thermocyclers with either lid temperature regulation or the option to turn lid heating on/off are acceptable for our 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 protocol describes an enzymatic labeling approach for direct fluorescent labeling of UHMW gDNA (hundreds of kilobase pairs to mega base pairs in length) at a specific sequence motif by the Direct Labeling Enzyme (DLE-1). This direct labeling does not introduce nicks into gDNA and allows users to generate highly contiguous genome maps, with N50 values of 20-100 Mbp, depending on the genome and sample quality.

The Bionano Prep® DLS-G2 kit provides reagents for sequence-specific labeling of UHMW gDNA for Bionano Optical Genome Mapping (OGM) on the Saphyr® system. After sequence-specific labeling with DLE-1, the labeled gDNA is stained for backbone visualization. DL-Green fluorophores are seen as green labels on a blue molecule when imaged on the Saphyr instrument.

DLE-1 Reaction Size

This protocol yields 60 µl of labeled gDNA. This is sufficient to load on a single flow cell of a Saphyr Chip® consumable, with enough sample remaining for one additional flow cell in cases of low throughput or other failure. Starting material should be at least in the hundreds of kilobases in length. If necessary, the size can be determined via pulsed field gel electrophoresis (PFGE). Labeling metrics are determined on the Saphyr instrument and are measured in labels/100 kilobase pairs (kbp). Additional labeling metrics can be determined by supplying a reference and monitoring map rate, positive label variance (PLV), and negative label variance (NLV). See the “Important Notes” section below for additional details.

Details on expected metrics can be found in the *Molecule Quality Report Guidelines* document (CG-30223).

Important Notes

GENERAL CONSIDERATIONS

- Use of an aluminum tube cooling block, pre-chilled on ice, to hold thawed reaction components and to assemble labeling reactions is recommended.
- Enzymes and buffers should be accurately pipetted out, with no droplets hanging on the outside of the pipet tip. Reagent volumes should be completely delivered into the tube, and bubble formation should be carefully avoided to ensure reproducible results. This is best achieved by holding reagent tubes at eye level when aspirating or dispensing, to visualize the process.
- Slow and thorough pipet mixing of DLE-1 master mix with gDNA is a critical step and promotes gDNA homogeneity and enzyme accessibility for efficient labeling of highly viscous gDNA.
- This protocol involves the handling of light-sensitive fluorescent molecules. It is important to minimize the exposure to light while working. Protect both the reactions and light-sensitive reagents from light during storage.
- Labeled gDNA is quantitated at the end of the protocol same day (or on a subsequent day) after labeling, cleanup, homogenization, staining and prior to loading on a chip. gDNA homogeneity is assessed by quantitating in duplicate. Homogeneity of the labeled DNA allows for an accurate estimate of concentration and

more uniform loading of gDNA onto the chip. The labeled gDNA concentration should be between 4 and 16 ng/µl with a Coefficient of Variation (CV) < 0.30.

Batch Size

- Up to 12 samples can be processed at a time.
- Each Bionano Prep DLS-G2 Kit contains reagents sufficient for twelve samples.

Requirements for Starting gDNA

- The sample should contain megabase pair-length gDNA, typically determined by high viscosity and/or PFGE.
- gDNA concentration should be between 39 and 150 ng/µl.
- gDNA samples > 150 ng/µl should be diluted with TE (pH 8.0) to 50 – 150 ng/µl, mixed 5 times with a wide bore tip, and allowed to relax overnight at room temperature (RT). Verify final gDNA concentration and homogeneity before labeling.
- For gDNA samples < 39 ng/µl, contact Technical Support at support@bionano.com.

Determining Enzyme

- For non-human samples, before starting the DLS-G2 protocol, import the sequence data for the desired sample into either the *in silico* Digestion feature of the Bionano Access® software, or the standalone Label Density Calculator software to ensure that DLS-G2 labeling is an appropriate choice for the sample. Actual label density should be within ± 2 labels of the predicted label density. Contact Technical Support at support@bionano.com for guidance if uncertain.
- For non-human samples, current downstream analysis tools are most successful with genomes that have DLS-G2 label densities between 9 and 25 labels per 100kbp.

Managing Genomic DNA

GENERAL

- This protocol involves the handling of viscous gDNA, which is difficult to pipet accurately. It is critical to follow all steps in the protocol to ensure accurate sampling of gDNA to achieve proper enzyme-to-gDNA and gDNA-to-Stain ratio, and to minimize unnecessary handling of the gDNA, which can result in molecules of insufficient size for analysis.

ADDING gDNA TO LABELING REACTION

- To ensure accurate sampling from the viscous gDNA stock, first maximize stock gDNA homogeneity by gently pipet mixing the room-temperature, equilibrated gDNA solution with a wide-bore tip five times and follow the guidelines below for proper pipetting into and out of a standard filtered pipet tip, or positive displacement pipet tip, for complete delivery.
- Before drawing viscous gDNA into a standard filtered tip, pipet an identical volume of water and mark the solution level on the tip with a fine tipped marker to serve as a guide when pipetting gDNA. Save the marked tip as a guide and use a new one for gDNA retrieval. Alternatively, the use of a positive displacement pipet can improve consistency when pipetting viscous gDNA.

- To draw viscous gDNA into a standard filtered tip, hold the stock gDNA tube for close-up visualization, depress the pipet plunger until the first stop, submerge the pipet tip toward the middle of the viscous solution, and carefully release the plunger, as slowly as possible while moving the tip in a circular motion, to draw the viscous gDNA into the tip, while carefully monitoring gDNA uptake in the process. Keep the tip submerged even after the viscous gDNA solution stops moving upward and levels off (use the marked tip as rough guide to see if viscous solution levels off at the appropriate mark). Viscous gDNA can take up to 30 seconds to fill the tip to the appropriate level. Releasing the plunger too quickly can produce a bubble in the tip, resulting in under-sampling and the user must start over if this occurs. After the solution in the pipet tip has leveled off and while the tip is still submerged in the gDNA solution, scrape the tip against the bottom of the tube five times using a circular motion. Remove the tip from the gDNA solution and visually inspect to confirm that it is filled to the appropriate level, by comparing it to the marked tip. Removing the pipet tip from the gDNA solution too early, or improperly scraping the tip on the bottom of the tube, can produce a bubble at the end of the pipet tip, indicating under-sampling, and the user must start over if this occurs. Accurate pipetting of viscous gDNA is possible with practice and patience.
- To deposit the entire volume of viscous gDNA into a tube or master mix, manually hold the reaction tube for close-up visualization and deliver the gDNA by inserting the pipet tip in the solution and gently pressing the plunger until the first stop, then to the second stop, while monitoring gDNA release, until all the gDNA has been delivered from the tip. Immediately remove the tip as soon as the last of the gDNA has left the pipet tip while maintaining constant pressure to avoid uptake of fluid or introduction of air bubbles. Visually inspect the tip after removing from solution to confirm it is empty.

Bionano Prep® DLS-G2 Protocol

Setup

1. Thaw 20x DL-Green at room temperature (RT). Vortex well and pulse spin briefly. Keep on 4°C aluminum block until use.
2. Thaw 5x DLE-1 Buffer at RT. Vortex well and pulse spin briefly. Keep at RT until use.
3. Flick 10x DLE-1 enzyme 3 times to mix, and pulse spin briefly. Keep in -20°C enzyme cooler until use.
4. Remove Ultra-Pure water tube from 4°C (if necessary) and keep at RT.

DLE-1 Labeling

DILUTE gDNA AND COMBINE WITH LABELING MIX

1. If gDNA quantitation was performed according to Bionano Prep SP DNA Isolation Protocol instructions immediately prior to labeling, then proceed to **Step 2**. If not, pulse-spin the gDNA for 2 seconds, and repeat quantitation prior to proceeding to **Step 2**.

2. In a thin-walled PCR tube, add 750 ng gDNA (a) to Ultra-Pure water and (b) to a total volume of 19.5 µl. Use **Table 3** to record the volumes of gDNA and water for each sample.

   a. 750 ng/ [gDNA concentration (ng/µl)] = **µl of gDNA**

   b. 19.5 µl – (**µl of gDNA**) = µl of Ultra Pure Water.

Table 3. gDNA Quantity Calculation

gDNA Sample ID	gDNA Concentration (ng/µl)	Volume of gDNA(µl)	Volume of Ultra- Pure Water (µl)

Table 4. Labeling Master Mix Calculation Table

Labeling Master Mix Components	Volume for 1 Sample	# of Samples	Master Mix Excess	Master Mix Total
5x DLE-1 Buffer	6.0 µl		× 1.2	µl
20x DL-Green	1.5 µl		× 1.2	µl
10x DLE-1	3.0 µl		× 1.2	µl
Total Labeling Master Mix Volume	**10.5 µl**			**µl**

3. Prepare Labeling Master Mix in a 0.5 ml amber tube. Add the components in the order outlined in **Table 4**. Mix the Labeling Master Mix (before adding to the sample tube) by pipetting the entire volume up and down with a standard filtered pipet tip, a *minimum of 7* times (sample batches of 6 or more may require 10+ times of pipet mixing) to ensure that the Labeling Master Mix is homogenous, taking care not to generate bubbles. Pulse-spin for 2 seconds and keep in an aluminum block on ice until use. Use as soon as possible after mixing the components.

4. Using a standard filtered pipet tip, add 10.5 µl Labeling Master Mix on top of the 19.5 µl [gDNA + Ultra-Pure Water], with no mixing. Then, using a new standard filtered pipet tip, with pipet set to 28 µl, mix sample slowly up and down 5 times (1 up + 1 down = 1 time). Pulse-spin tube for 2 seconds.

WARNING: Protect the samples from light. ⚠

NOTE: See the video titled “DLS Master Mix Mixing” on the Bionano Support website for guidance on proper mixing technique.

NOTE: A carefully and thoroughly mixed sample is necessary to efficiently label all molecules. Draw the sample from the bottom and dispense near the top (without touching the pipet tip to the tube) to maximize mixing.

LABELING REACTION

5. Incubate in a thermocycler with the heated lid set to 47°C, or “On” if no temperature choice is available:

    a. 1 hour at 37°C (thermocycler temperature)

    b. Hold at 4°C until next step.

WARNING: Protect the samples from light. ⚠

NOTE: After placing the samples into the thermocycler, prepare the microplate for DL-Green cleanup (**Step 8**) while the labeling reaction is incubating.

PROTEINASE K DIGESTION

6. Dispense 5 µl Proteinase K directly into the central bulk of the sample contained in the PCR tube. To avoid inadvertently removing gDNA that may adhere to the tip, do not mix.

7. Incubate in a thermocycler with the heated lid set to 60°C, or "On" if no temperature choice is available:

    a. 30 minutes at 50°C (thermocycler temperature)

    b. Hold at 4°C until next step. Once sample is removed from the thermocycler, pulse-spin briefly and proceed quickly to the next step.

WARNING: Protect the samples from light.⚠

DL-Green Cleanup

PREPARE DLS 24-WELL PLATE FOR DL-GREEN CLEANUP

NOTE: Membranes can be wetted immediately after setting up the labeling reaction. Make sure to seal the plate with DLS Tape Sheet until time of use.

NOTE: For all membrane steps, please see the video Bionano Prep DLS-G2 Membrane Workflow (CG-00013) on the Bionano Support Website.

8. For each sample, wet the underside of 1 DLS Membrane with 1x DLE-1 Buffer in the DLS 24-Well Plate:

    a. For each sample, prepare 30 µl of 1x DLE-1 Buffer (6 µl 5x DLE-1 Buffer + 24 µl Ultra Pure Water). Vortex to mix. Pulse-spin for 2 seconds.

    b. Dispense 25 µl of 1x DLE-1 Buffer into the center of one well of the DLS 24-Well Plate. Repeat into a new well for each sample.

    c. Use forceps to place a DLS Membrane on top of the 1x DLE-1 buffer in each well. Repeat for all wells prepared in **Step 8b**. See **Note** below.

    d. Seal wells immediately with a DLS Tape Sheet to prevent evaporation until ready to proceed.

NOTE: Ensure that membranes are completely wetted after 3 minutes. Wetted membranes will have a uniform, translucent blue appearance across the membrane. If the membranes are not wetted after 3 minutes, please discard the membrane, and wet a new one from the membrane pack. Contact support@bionano.com with any questions or concerns.

9. Perform DL-Green cleanup by dispensing labeled gDNA sample onto the center of the wetted membrane:
    a. Hold the DLS 24-Well Plate securely and carefully remove the DLS Tape Sheet.

b. Using a 200 µl standard filtered pipet tip with the pipet set to 37 µl, aspirate the entire volume (~35 µl) of labeled gDNA.

c. Carefully dispense the labeled gDNA onto the middle of the wetted DLS Membrane.

d. Repeat **Steps 9b** and **9c** for all samples.

e. Seal all sample wells with DLS Tape Sheets to prevent evaporation. While holding the DLS 24- Well Plate in place on the bench, apply pressure to secure the DLS Tape Sheet to the top rimof the wells.

WARNING: Protect the DLS 24-Well Plate from light. ⚠ Incubate at RT for 1 hour. Ensure that the plate stays undisturbed and there is no inadvertent movement of the plate during incubation.

10. During the 1-hour incubation period, bring 10x DTT, 4x Flow Buffer, and DNA Stain to RT. Once thawed, vortex all tubes well, and pulse-spin briefly to collect contents. Keep all tubes at RT until ready to use.

11. After 1 hour, hold the plate securely, and carefully remove the DLS Tape Sheet.

12. Using a 200 µl standard filtered pipet tip, with the pipet set to 35 µl, slowly aspirate the entire labeled sample while making contact perpendicularly with the membrane and move the tip across the gDNA area while aspirating to collect the gDNA. Transfer into a new PCR tube or an amber 0.5 ml microfuge tube. Pulse-spin for 2 seconds.

WARNING: Protect the samples from light. ⚠

13. Using a standard filtered 200 µl pipet tip, aspirate 20 µl of the labeled sample from the PCR tube or 0.5 ml amber tube and dispense into the bottom of a DLS amber tube (2 ml). Proceed to the next step (gDNA Staining and Homogenization).

    a. If sample volume recovered is < 20 µl, bring the volume up to a total of 20 µl using 1x DLE-1 Buffer.

gDNA STAINING AND HOMOGENIZATION

14. If labeling 6 or fewer samples use a 0.5 ml amber tube. If labeling 7 or more samples use a 2 ml amber tube. Prepare Staining Master Mix according to **Table 5** below. Vortex to mix, then pulse-spin to collect contents.

Table 5. Staining Master Mix Calculation Table

Staining Master Mix Components	Volume for 1 Sample	# of Samples	Master Mix Excess	Master Mix Total
4x Flow Buffer	15 µl		x 1.25	µl
10x DTT	6 µl		x 1.25	µl
DNA Stain	3.5 µl		× 1.25	µl
Ultra-Pure Water	15.5 µl		× 1.25	µl
Total Staining Master Mix Volume	**40 µl**			**µl**

NOTE: Flow Buffer is viscous, so pipet solutions containing it slowly to increase accuracy.

15. For each labeled gDNA, add 40 µl Staining Master Mix on top of the labeled sample (20 µl) contained in the DLS amber tube (2 ml). Do not mix.

NOTE: Staining Master Mix is dispensed on top of the labeled gDNA to avoid inadvertently drawing out gDNA that may stick to the pipet tip.

16. Place DLS amber tubes containing samples into a HulaMixer (Thermo Fisher) with the speed set to 5 rpm. The tube holder surface should be flat and parallel to the work surface. Mix for 30 minutes at RT with all options other than rotation turned off.

17. After 30 minutes, remove samples from the HulaMixer. Pulse-spin for 2 seconds.

NOTE: Do not allow the rotation to proceed for longer than 30 minutes, as this may decrease molecule N50.

18. If data collection is desired on the same day, proceed with Quantitation immediately before loading samples on the Saphyr Chip consumable. Otherwise, this is a potential stopping point, and users can store the samples protected from light at 4°C until ready to proceed with quantitation.

WARNING: Protect the samples from light.

QUANTITATION OF LABELED AND STAINED gDNA

NOTE: Before proceeding, verify list of User-Supplied Materials (**Table 2**) to ensure all required materials are available.

Determine the final concentration of the labeled and stained gDNA prior to loading on the Saphyr chip consumable. Best results will be obtained if the gDNA concentration (average of two measurements) is between 4 and 16 ng/µl. Variation in the final concentration is due to the difficulties in accurately sampling the viscous

starting gDNA and variation in labeled gDNA recovery from the DL-Green Cleanup step. If the sample concentration does not fall within this range, see the *Bionano Prep SP-G2 and DLS-G2 Troubleshooting Guide* (CG-30608) for recommendations.

QUBIT dsDNA HS (HIGH SENSITIVITY) ASSAY KIT AND QUBIT FLOUROMETER:

NOTE: The standard Qubit dsDNA HS Assay protocol will not provide accurate measurements of concentration due to the extremely long lengths of the labeled gDNA. The Qubit protocol has been modified to include a sonication step to fragment an aliquot of the labeled gDNA to ensure accurate concentration measurements. Refer to the Qubit dsDNA HS Assay Kit user manual for kit details.

1. Using a wide bore tip on a 200 µl pipet set to 50 µl, mix labeled and stained gDNA 5 times. Pulse-spin.
2. Let Qubit HS Standards and labeled gDNA come to room temperature for at least 30 minutes.
3. Prepare 0.5 ml Qubit Assay Tubes:
    a. Two separate Assay Tubes for the HS Standard measurements, each containing 10 µl of Qubit HS Buffer.
    b. Two separate Assay Tubes per labeled sample, each containing 18 µl of Qubit HS Buffer.
4. Using a standard filtered pipet tip or positive displacement pipet, remove two separate 2 µl aliquots from each sample and dispense into 18 µl HS Qubit buffer in a Qubit Assay Tube, rinsing the tip. Place Qubit tubes in a floating rack and sonicate in a bath sonicator for 10 minutes. If a bath sonicator is not available, vortex for at least 30 seconds at maximum speed, then spin down for 2 seconds. During sonication, prepare the Working Solution as described in **Step 5**.

NOTE: If a long string of gDNA is attached to the tip when removing the tip from the tube, dispense the sample back into the tube and repeat aliquot removal with a new tip.

5. Prepare Working Solution by diluting the Dye Assay Reagent into HS Dilution Buffer (1:200): Prepare 200 µl Working Solution for each of the two standards (400 µl total). Prepare 200 µl Working Solution for each sample aliquot (400 µl for each sample).
6. For the Qubit DNA standards, add 10 µl of Standards 1 and 2 to separate, labeled Qubit Assay Tubes containing 10 µl of Qubit HS Buffer from **Step 3a**.
7. Once sonication is complete, retrieve assay tubes and centrifuge briefly to collect solution at the bottom of the tubes. Vortex tubes for 5 seconds at maximum speed, then spin down tubes for 2 seconds.
8. Add 180 µl of Working Solution (prepared in **Step 5**) to each tube of sonicated labeled gDNA and Qubit DNA Standard plus HS Buffer. Vortex for 5 seconds, and centrifuge briefly to collect solution at the bottom of the tubes.
9. Incubate samples in the dark for 2 minutes. Quantitate on the Qubit Fluorometer.
10. Record Qubit measurements in **Table 6**. Calculate Mean and CV.

NOTE: The labeled gDNA concentration should ideally fall between 4 - 16 ng/µl with a CV (standard deviation ÷ mean) between the measurements < 0.30. If both measurements are outside of 4 - 16 ng/µl, see the *Bionano Prep SP-G2 and DLS-G2 Troubleshooting Guide* (CG-30608). If one measurement is between 4 - 16 ng/µl and the other is outside of this range, follow these guidelines:

- If one measurement is between 4 - 16 ng/µl and the other is above 16 ng/µl, proceed to load the chip.
- If one measurement is between 4 - 16 ng/µl and the other is below 4 ng/µl, repeat HulaMixer mixing for 30 minutes and repeat the quantitation.

Table 6. gDNA Qubit Measurements

Sample ID	Measurement 1 (ng/µl)	Measurement 2 (ng/µl)	Average (Mean) (ng/µl)	CV (Standard deviation ÷ mean)

11. Potential stopping point: If the samples will not be loaded onto a chip immediately, store protected from light at 4°C until use.

Loading Saphyr Chip

Refer to the *Saphyr System User Guide* (CG-30247) for complete instructions on chip loading and instrument operation.

NOTE: When aspirating DLS-labeled sample for chip loading, draw from the middle of the tube.

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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