Bionano Prep Direct Label and Stain (DLS) Protocol

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Table of Contents

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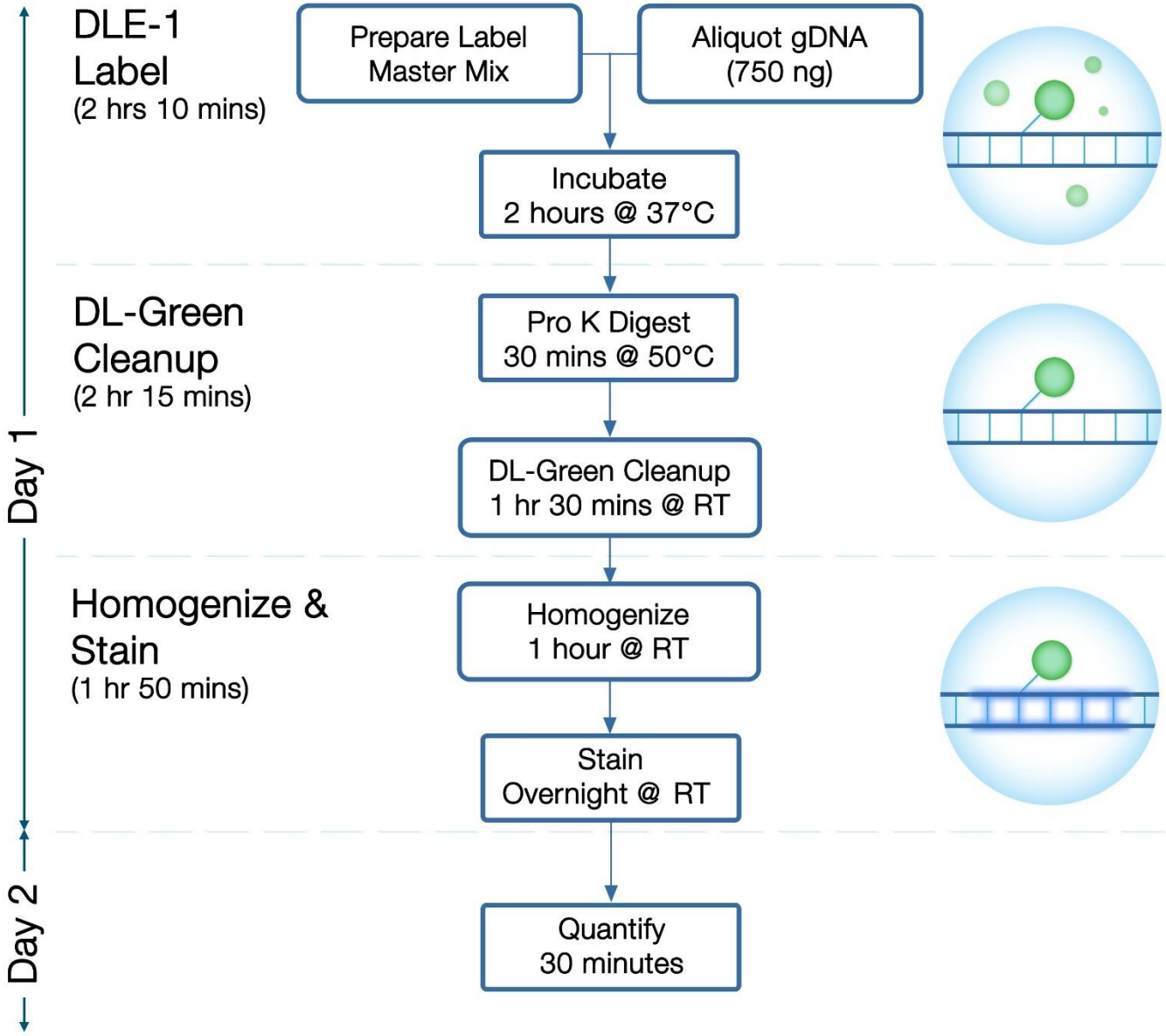
Revision History

Revision	Notes
E	Decrease DTT in staining reaction
F	Include note indicating change in Staining Master Mix table. Update table formatting.
G	Update workflow diagram and user-supplied materials, modify membrane wetting step, edits to text for clarity.

Bionano Prep DLS Overview (750 ng)

DLS is a sequence-specific labeling of ultra-high molecular weight (UHMW) genomic DNA (gDNA) for Bionano Optical Genome Mapping (OGM) using a Direct Label Enzyme (e.g., DLE-1), followed by staining of the DNA backbone.

Workflow Overview – Label and Stain on Day 1; Quantify on Day 2, Load onto chip after quantification



Note: Dashed lines (-- -- -- -- --) denote potential pausing/stopping points.

Bionano Prep DLS Kit and User-Supplied Materials

Table 1: Bionano Prep DLS Kit Contents (Part # 80005)

Component	Part #	Amount	Storage	Handling Considerations
20x DLE-1	20351	18 µl	-20°C	Flick tube 3 times to mix, and centrifuge briefly. Keep in -20°C enzyme cooler until use.
20x DL-Green	20352	18 µl	-20°C	Thaw at room temperature (RT). Vortex and centrifuge briefly. Keep on pre-chilled aluminum block until use.
5x DLE-1 Buffer	20350	200 µl	-20°C	Thaw at room temperature. Vortex and centrifuge briefly. Keep at room temperature until use.
4x Flow Buffer	20353	190 µl	4°C	Vortex and centrifuge briefly. Keep at room temperature until use.
DNA Stain	20356	65 µl	-20°C	Thaw at room temperature. Vortex and centrifuge briefly. Keep at room temperature until use; DMSO in DNA Stain will crystalize on ice.
1M DTT	20354	75 µl	-20°C	
Ultra Pure Water	20355	900 µl	4°C	May keep at room temperature.
DLS 24 Well Plate	20357	1 plate	RT	Keep covered to avoid dust.
DLS Membranes (13 mm)	20358	25	RT	Avoid excess moisture.
DLS Plate Sealing Strips	20361	10	RT	
DLS Amber Tubes, Round Bottom	20362	12	RT	

Note: RT = 18 - 25°C

Table 2: User-Supplied Materials

Item	Description	Catalog #
HulaMixer Sample Mixer	Thermo Fisher	15920D
Thermocycler with heated lid	General lab supplier	
Puregene Proteinase K	Qiagen	158918 or 158920
PCR tubes, 0.2 ml, thin-walled, flat cap, nuclease-free	Thermo Fisher or equivalent	AM12225
Microcentrifuge tubes, 0.5 ml, Amber, nuclease-free	USA Scientific or equivalent	1605-0007
Pipet tips, unfiltered, 200 µl	USA Scientific or equivalent	1111-1810
Pipet tips, wide-bore, filtered, 200 µl	VWR or Rainin equivalent	46620-642
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	78141-01
Pipets (2, 10, 20 and 200 µl)	General lab supplier	
Ice bucket and Ice	General lab supplier	
Vortexer	General lab supplier	
Microcentrifuge for 0.2, 0.5, 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 (recommended)	Branson or equivalent	CPX 952-119R
Positive-displacement pipet MR-10 (optional)	Rainin or equivalent	17008575
Pipet tips, 10 µl, C-10 for pos. displacement (optional)	Rainin or equivalent	17008604

Introduction and Important Notes

Introduction

This protocol describes an enzymatic labeling approach for direct fluorescent labeling of UHMW gDNA by the Direct Label Enzyme (DLE-1). The DLE-1 enzyme attaches the DL-Green fluorophore via covalent modification at a specific-sequence motif. This labeling process is non-damaging to the gDNA, allowing the generation of highly contiguous genome maps and providing high sensitivity to detection of structural variants. After sequence-specific labeling with DLE-1, the labeled DNA is stained for backbone visualization. When imaged on the Saphyr, the labeled samples are visualized as green dots on blue lines. The Bionano Prep Direct Label and Stain (DLS) kit (Catalog # 80005) provides the necessary reagents for this protocol.

DLE-1 Reaction Size (750 ng)

This protocol yields 60 µl of labeled DNA. This is sufficient to load on a single flowcell of a Saphyr Chip, with enough sample remaining for one additional flowcell in cases of low throughput or other failure. Starting material should be at least in the hundreds of kilobase in length; if necessary, this can be determined via pulsed-field gel electrophoresis (PFGE). Labeling metrics are determined on the Bionano Saphyr, 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 Document 30223, Saphyr Molecule Quality Report Guidelines.

Important Notes

General Considerations

- We recommend using an aluminum tube cooling block pre-chilled on ice to hold thawed reaction components and to assemble labeling reactions.
- Enzymes and buffers should be accurately pipetted out, with no droplets hanging on the outside of the pipet tip. The enzyme should be completely delivered into the reaction tube, and bubble formation should be carefully avoided to ensure reproducible reactions. 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 DNA homogeneity and enzyme accessibility for efficient labeling of highly viscous DNA.
- This protocol involves the handling of light-sensitive fluorescent molecules. It is important to minimize the exposure to light of both the reactions and the light-sensitive reagents while working. Additionally, protect

from light the light-sensitive reagents during storage.

- Labeled DNA concentration is measured on Day 2, after labeling, cleanup, homogenization, and staining. DNA homogeneity is assessed by quantitating in duplicate (Coefficient of Variation (CV) < 0.30). Homogenous labeled DNA allows for an accurate estimate of concentration and more uniform loading of DNA onto the chip. The labeled DNA concentration should be between 4 and 12 ng/µl.

Batch Size

- Up to 12 samples can be processed at a time.
    - Each Bionano Prep DLS Kit contains reagents sufficient for 10 samples.

Requirements for Starting DNA

- The sample should contain megabase-length gDNA, typically determined by high viscosity and/or PFGE of the sample.
- gDNA concentration should be between 36 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. Verify final DNA concentration and homogeneity before labeling.
    - For gDNA samples < 36 ng/µl, contact Technical Support at Support@bionanogenomics.com.

Determining Enzyme

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

Handling Genomic DNA

General:

- This protocol involves the handling of viscous genomic gDNA, which is difficult to pipet accurately. It is critical to follow all steps in this protocol carefully to ensure accurate sampling of DNA in order to achieve

proper enzyme-to-DNA and DNA-to-Stain ratio, and to minimize unnecessary handling of the gDNA, which can result in insufficiently long molecules for analysis.

Adding gDNA to Labeling Reaction:

- To ensure accurate sampling from the viscous gDNA stock, first maximize stock DNA homogeneity by gently pipet mixing the room temperature-equilibrated DNA solution with a wide-bore tip 5 times and follow guidelines below for proper pipetting into and out of a standard pipet tip, or positive displacement pipet tip, for complete delivery.

- Before drawing viscous gDNA into a standard 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 the guide and use a new one for DNA retrieval. Alternatively, the use of a positive displacement pipet can improve consistency when pipetting viscous gDNA.

- To draw viscous gDNA into a standard tip, hold the stock DNA 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 DNA into the tip while carefully monitoring DNA uptake. Keep the tip submerged even after the viscous DNA solution stops moving upward and levels off (use the marked tip as rough guide to see if viscous solution levels off at the appropriate level). Viscous DNA 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 (start over if this occurs). After the solution in the pipet tip has leveled off, and while the tip is still submerged in the DNA solution, scrape the tip against the bottom of the tube 5 times using a circular motion. Remove the tip from the DNA solution and visually inspect to confirm that it is filled to the appropriate level, by comparing 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 (start over if this happens). **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, hold the reaction tube for close-up visualization and deliver the DNA by inserting the pipet tip in the solution and gently pressing the plunger until the first stop, then to the second stop, while monitoring DNA release, until the last bit of DNA has left the tip. Immediately remove the tip a3Ws soon as the last bit of DNA has left the pipet tip while maintaining a constant pressure to avoid uptake of fluid or introduction of air bubbles. Visually inspect the tip after removing from solution to confirm that it is empty.

Bionano DLS Labeling Protocol

Protocol Start, Day 1

See *Important Notes* section for proper handling of gDNA. gDNA concentration should be between 36 ng/µl and 150 ng/µl.

See *Kit Contents* and *User-Supplied Materials* sections for proper handling and storage of reagents.

Setup

1. Thaw 20x DL-Green. Vortex well and pulse spin. Hold on ice in 4°C aluminum block.
2. Thaw 5x DLE-1 Buffer. Vortex well and pulse spin. Hold at room temperature until use.
3. Flick 20x DLE-1 Enzyme three times and pulse spin. Hold on bench in -20°C enzyme block.
4. Remove Ultra Pure Water tube from 4°C (if necessary) and keep at room temperature.

DLE-1 Labeling (30 µl Reaction, 2 hours 10 minutes)

Dilute gDNA and Combine with Labeling Mix (10 minutes)

5. If gDNA quantitation has already been performed immediately prior to labeling, then proceed to Step 6. If not, pulse spin and then repeat quantitation prior to proceeding to Step 6.
6. In a thin wall PCR tube, add 750 ng gDNA (a) to Ultra Pure Water (b) to a total volume of 21 µl.
    a. 750 ng/ [gDNA concentration] = µl of gDNA
    b. 21 µl – (µl of gDNA) = µl of Ultra Pure Water.

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

7. If processing more than one sample, prepare a Labeling Master Mix in a 0.5 ml amber tube. Add the components in the order outlined in the table below. Pipet the entire volume of the Labeling Master Mix up and down with a standard pipet tip, five times, taking care not to generate bubbles. Pulse spin and keep in aluminum block on ice until use. Use within 30 minutes of mixing the components.

   Note: After making the master mix, leave 5x DLE-1 Buffer at room temperature to use in Step 12.

Labeling Master Mix Calculation Table

Labeling Reaction	1 Sample	# of Samples	Master Mix Excess	Master Mix Total
gDNA (750 ng) + Ultra Pure Water	21 µl			
5x DLE-1 Buffer	6.0 µl		× 1.2	µl
20x DL-Green	1.5 µl		× 1.2	µl
20x DLE-1	1.5 µl		× 1.2	µl
Final Reaction Volume	30 µl			µl

8. Using a standard pipet tip, add 9 µl Master Mix on top of the 21 µl gDNA + Ultra Pure Water, with no mixing. Then, using a new standard 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. Protect from light. ⚠

   See the video titled DLS Master Mix Mixing under the DLS Labeling Kit section of the Support website.

   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 pipette tip to the tube) to maximize the mixing.

Labeling (2 hours)

9. Incubate in a thermocycler using a heated lid set at 47°C (or "On" if no temperature choice is available):
   a. 2 hours at 37°C
   b. Hold at 4°C until next step. After removing from thermocycler, proceed quickly to the next step. Pulse spin briefly.

Proteinase K Digestion and DL-Green Cleanup (2 hours 15 minutes)

Proteinase K Digestion (35 minutes)

10. Dispense 5 µl Puregene Proteinase K (Qiagen) directly into the central bulk of the sample contained in the PCR tube. To avoid inadvertently removing DNA that may adhere to the tip, do not mix.

11. Incubate in a thermocycler using a heated lid set at 60°C (or "On" if no temperature choice is available):

a. 30 minutes at 50°C

b. Hold at 4°C until next step. After removing from thermocycler, proceed quickly to the next step. Pulse spin briefly.

Membrane Adsorption (1 hour, 40 minutes)

For Steps 12-18, please see the video titled DLS Membrane Demo under the DLS Labeling Kit section of the Support website.

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

    a. For each sample, prepare 60 µl of 1x DLE-1 Buffer (12 µl 5x DLE-1 Buffer + 48 µl Ultra Pure Water). Vortex to mix and pulse spin.

    b. Dispense 25 µl of 1x DLE-1 Buffer into the center of one well of the DLS 24 Well Plate.

    c. Use forceps to place a DLS Membrane on top of buffer.

    d. Seal wells immediately with a DLS Plate Sealing Strip to prevent evaporation. While holding the plate, apply pressure to secure the DLS Plate Sealing Strip to the top rim of the wells.

    e. Allow the membrane to fully wet for 10 minutes.

    Note: Membrane should change to a bluish color once fully wet. See around the 0:55 mark of the DLS Membrane Demo video. If membrane doesn't fully wet after 10 minutes, use a new membrane. Additional 1x DLE-1 buffer may need to be prepared for the new membrane.

13. Perform DL-Green cleanup by dispensing labeled DNA sample onto the center of the wetted membrane:

    a. Hold the plate securely and carefully remove the DLS Plate Sealing Strip. Using a standard pipet tip with the pipette set to 38 µl, aspirate the entire volume (~35 µl) of labeled DNA.

    b. Carefully dispense the labeled DNA onto the middle of the wetted DLS Membrane.

    c. Seal wells immediately with a DLS Plate Sealing Strip to prevent evaporation. While holding the plate, apply pressure to secure the DLS Plate Sealing Strip to the top rim of the wells.

    d. Protect the DLS 24 Well Plate from light (cover) and incubate at room temperature for 1 hour. Ensure that the plate stays undisturbed, with no inadvertent movement of the plate during incubation. ⚠

    e. 10 minutes before the incubation is complete, wet a second membrane in an unused well of the DLS 24 Well Plate, following steps 12b - 12e above.

    f. After 1 hour, hold the plate securely and carefully remove the DLS Plate Sealing Strip.

    g. Using an unfiltered standard pipette tip, with pipet set to 38 µl, slowly aspirate the entire labeled sample while making contact perpendicularly with the membrane and move the tip across the DNA area while aspirating to collect the DNA.

14. Repeat steps 13b - 13d, but dispense onto the second (unused) membrane prepared in Step 13e and incubate at room temperature for 30 minutes.

15. During the 30-minute incubation period, bring 1M DTT, 4x Flow Buffer, and DNA Stain to room temperature. Once thawed, vortex all tubes well, and pulse-spin briefly to collect contents. Keep all tubes at room

temperature until ready to use.

16. After 30 minutes, hold the plate securely and carefully remove the DLS Plate Sealing Strip.
17. Using an unfiltered standard pipette 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 DNA area while aspirating to collect the DNA. Transfer into a new PCR tube or 0.5 ml amber tube. Pulse spin for 2 seconds. Protect tubes from light. ⚠
18. Using a standard 200 µl pipette tip, aspirate 20 µl of the labeled sample from the PCR tube or 0.5 ml amber tube, and dispense into the bottom of the DLS Round Bottom Amber Tube (2 ml). Proceed to the next step (**DNA 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.

DNA Staining & Homogenization (1 hour 10 minutes)

Staining and Homogenization (1 hour, 10 minutes)

19. In a new 0.5 ml amber tube, prepare Staining Master Mix according to table below. Vortex to mix, then pulse-spin to collect contents.

Staining Master Mix Calculation Table

Staining Reaction	1 Sample	# of Samples	Master Mix Excess	Master Mix Total
Labeled Sample (Step 19)	20 µl			
4x Flow Buffer	15 µl		× 1.25	µl
1M DTT	6 µl		× 1.25	µl
DNA Stain	3.5 µl		× 1.25	µl
Ultra Pure Water	15.5 µl		× 1.25	µl
Total	**60 µl**			**µl**

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

20. For each labeled DNA, add 40 µl Staining Master Mix on top of the labeled sample (20 µl) contained in the DLS Round Bottom Amber Tube (2 ml). Do not mix.

    Note: Master mix is dispensed on top of the labeled sample in order to avoid inadvertently drawing out DNA that may stick to the pipet tip.

21. Place DLS Round Bottom Amber Tubes containing samples into HulaMixer (Thermo Fisher) with speed set to 5 rpm. Tube holder surface should be flat and parallel to the work surface. Mix for 1 hour at room temperature with all options other than rotation turned off.

22. After 1 hour, remove samples from the HulaMixer. Pulse spin to collect contents.

    Note: Do not allow the rotation to proceed for longer than 1 hour, as this may decrease molecule N50.

23. Store overnight at room temperature, protected from light.

Protocol Start, Day 2

See list of User Supplied Consumables and Equipment to make sure they are all available.

Quantitation of Labeled and Stained DNA (30 minutes)

DNA Quantitation (30 minutes)

Determine the final concentration of the labeled and stained DNA. Best results will be obtained if the DNA concentration (average of two measurements) is between 4 and 12 ng/µl. Variation in the final concentration is due to the difficulties in accurately sampling the viscous starting gDNA and variation in gDNA recovery from DL-Green removal step. If your sample concentration does not fall within this range, see Troubleshooting section for recommendations.

Qubit dsDNA HS (High Sensitivity) Assay Kit & Qubit Fluorometer:

Note: The standard Qubit dsDNA HS Assay protocol will not provide accurate measurements of concentration due to the extremely long lengths of the labeled DNA. We have modified the Qubit protocol to include a sonication step to fragment an aliquot of the labeled DNA 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 at 50 µl, mix labeled and stained DNA 5 times. Pulse-spin.
2. Let Qubit HS Standards and labeled DNA come to room temperature (at least 30 minutes).
3. Prepare 0.5 ml Qubit Assay Tubes:
    a. 2 separate Assay Tubes for the HS Standard measurement, each containing 10 µl of Qubit HS Buffer.
    b. 2 separate Assay Tubes per labeled sample, each containing 18 µl of Qubit HS Buffer.
4. Using a standard pipet tip or positive displacement pipet, remove two separate 2 µl aliquots from each sample and dispense into 18 µl HS Qubit buffer in Qubit Assay tube, rinsing tip. Place Qubit tubes in a floating rack and sonicate in a bath sonicator for 10 minutes. During sonication, prepare Working Solution as described below.

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

    a. If a bath sonicator is not available, vortex for at least 30 seconds at maximum speed, then spin down briefly for 2 seconds.
5. Prepare Working Solution by diluting the Dye Assay Reagent into HS Dilution Buffer (1:200):
    a. Prepare 200 µl Working Solution for each of the two standards (400 µl total).
    b. 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 DNA and Qubit DNA Standard plus HS Buffer. Vortex for 5 seconds, and centrifuge briefly to collect solution at the bottom of tubes.
9. Incubate samples in the dark for 2 minutes before quantitation on the Qubit Fluorometer.

   Note: The labeled DNA concentration should ideally fall between 4 - 12 ng/µl with a CV (standard deviation ÷ mean) between the samplings < 0.30. If both samplings are outside of 4 - 12 ng/µl, see **Troubleshooting** section below. If one sampling is between 4 - 12 ng/µl and the other is outside of this range, follow these guidelines:

   - If one sampling is between 4 - 12 ng/µl and the other is above 12 ng/µl, proceed to load chip.
   - If one sampling is between 4 - 12 ng/µl and the other is below 4 ng/µl, repeat HulaMixer mixing for 30 minutes and repeat the quantitation.

10. Record Qubit measurements in the table on the next page.
11. If you will not be running the samples on the same day, store in the dark at 4°C until use.

    Note: Labeled samples show no reduction in performance if used within one month.

gDNA Qubit Measurements

Sample ID	Measure 1 (ng/µl)	Measure 2 (ng/µl)	Average (ng/µl)	CV (stdev/mean)

Loading Bionano Chip (40 minutes; 30 minutes to bring chip to room temp and 10 minutes to load)

Refer to **Saphyr System User Guide** (for Saphyr P/N 60325 or 60239) 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.

Troubleshooting

Labeled sample should be stored at 4°C in a light-protected box when not in use. Labeled sample should be brought to room temperature prior to quantitation and/or chip loading.

Expected metrics based on internal experience at Bionano Genomics with human samples:

N50 (> 150 kbp)	Labels/ 100 kbp	Map Rate	Positive Label Variance	Negative Label Variance
> 230 kbp	14 - 17	> 70%	< 10%	< 15%

A. The gDNA is not homogeneous before labeling

Evidence: CV of the quantitation measurements does not meet requirements of the DNA isolation protocol (e.g., CV > 0.30 between left, middle, and right measurements in the SP protocols).

Steps to Follow:

1. Aspirate and dispense the sample slowly using a wide-bore tip 5 times.
2. Incubate the gDNA at room temperature for 1 to 3 days.
3. Aspirate and dispense the sample slowly using a wide-bore tip 5 times.
4. Quantitate with Qubit Broad Range Assay.

B. The gDNA is not viscous

Evidence: Sample consistency is very thin (non-viscous) and easily pipetted, but concentration is > 35 ng/µl.

It is likely that the sample does not contain high molecular weight gDNA.

Evaluate size of starting gDNA by pulsed-field gel electrophoresis (PFGE) before labeling.

Evaluate sample prep method and input material quality and age, and repeat DNA isolation from biological sample.

C. The gDNA concentration is < 36 ng/µl

Evidence: The concentration of gDNA measured at the end of the DNA isolation protocol is less than 36 ng/µl.

Contact Bionano Genomics Support at Support@bionanogenomics.com

D. The labeled sample is too viscous

Evidence: The sample takes an abnormally long time (greater than 30 seconds) to fill the fingers of the chip.

Contact Bionano Genomics Support at Support@bionanogenomics.com.

E. Label density is lower than expected

Evidence: The average detected label density will always be lower than the expected average site density. This is due to a combination of site clustering, DNA stretch, and optical resolution. For example, the average site density for DLE-1 in humans is 20.7 labels per 100 kbp, but the detected label density can vary from just above 14 to just below 17 labels per 100 kbp.

Low label density can be the result of suboptimal enzymatic labeling, photobleaching of fluorophores, or detection issues.

Potential causes of low label density during the protocol:

- Inhibitory substances in the DNA prep.
- Inadequate mixing of viscous gDNA and master mix.
- Mishandling of DLE-1 (exposure to elevated temperature, vortexing, etc.).
- Exposure of labeling reaction to light and photobleaching DL-Green.
- Prolonged exposure of DL-Green to the pH of the master mix (> 30 min).
- Hardware issue.

F. Labeled DNA is < 4 ng/µl for both measurements

Evidence: Both measurements are outside the concentration range of 4 -12 ng/µl using the Qubit High Sensitivity Assay Kit.

Steps to Follow:

1. Repeat quantitation of the starting DNA stock.
2. If the sample is 3 - 4 ng/µl, proceed to load at your own risk, but expected throughput will likely not be reached.
3. If the sample is < 3 ng/µl do not load. Check starting DNA concentration and repeat the labeling assay.

G. Labeled DNA is > 12 ng/µl for both measurements

Evidence: Both measurements are outside the concentration range of 4 -12 ng/µl using the Qubit High Sensitivity Assay Kit.

Steps to Follow:

1. If the sample is > 12 ng/µl, contact Bionano Customer Support (Support@bionanogenomics.com) for guidance.
2. You can proceed to load at your own risk, but the labeled sample may clog the chip and have reduced molecule N50.

H. The N50 (≥ 150 kbp) is less than 230 kbp, or the N50 (≥ 20 kbp) is less than 150 kbp

Evidence: The dashboard N50 metrics and Bionano Access Molecule Quality Report (MQR) results do not reach

the specifications listed above.

Steps to Follow:

1. Evaluate size of starting gDNA by pulsed-field gel electrophoresis (PFGE).
2. Evaluate sample prep method if there is no high molecular weight (megabase) gDNA present.
3. If starting gDNA size is large, relabel gDNA, making sure to avoid excessive pipetting or pipetting at high velocity.
4. Contact Bionano Customer Support (Support@bionanogenomics.com) for guidance as to whether you should rerun the sample on a different chip.

I. Map rate is low (human samples)

Evidence: The Bionano Access MQR map rate is < 70%

Steps to Follow:

1. Is the label density low (< 14 labels per 100 kbp)?
    a. If so, repeat quantitation of starting gDNA stock and repeat labeling after considering potential causes listed in Section D.
2. If the label density is within 14 – 17 labels per 100 kbp across all scans, contact Support.
3. Check N50 (≥ 20 kbp) value in the MQR. If this value is low (e.g., less than 100 kbp), it can cause issues with map rate

J. Effective throughput is less than 10 Gbp per scan

Evidence: The throughput after Scan 7 is still less than 10 Gbp per scan in the ICS or Access dashboard.

Steps to Follow:

1. Repeat quantitation of labeled sample.
2. Ensure flowcell is properly hydrated with nuclease-free water.

Potential Causes:

- Low N50 (≥ 20 kbp) and/or low N50 (≥ 150 kbp).
- Evaporation causing an increase in salt concentration, thus changing DNA migration.
    a. Were the Inlet and Outlet wells topped off (rehydrated) with nuclease-free water before placing chip plugs/clips?
- Non-homogenous DNA.
- DNA outside of the 4-12 ng/µl range.

Frequently Asked Questions

1. **How far in advance can the membranes be wetted on the plate?**

   We recommend 10 minutes before adding sample to the membrane. It is possible to wet both membranes at the same time (10 minutes before first membrane use), but this is entirely dependent on the user's ability to create a tight seal with the sealing strip to prevent evaporation. Keep sealing strip on unless applying sample.

2. **How long can labeling reaction sit at 4°C hold step?**

   Overnight, as long as it is protected from light.

3. **How long can sample sit at 4°C after Pro K?**

   Overnight, as long as it is protected from light.

4. **What is the impact of a DNA sample concentration being out of range?**

   We have not had problems loading samples with DNA concentration as high as 12 ng/ul, though samples above 12 ng/ul may clog the chip if homogeneity is poor, or may not be stained properly. In contrast we have found that samples with DNA concentration < 4 ng/ul may not collect enough data for effective throughput.

5. **How long is the sample good for?**

   While we have found that stained DNA samples at optimal conditions can sit at 4°C protected from light for up to one month without degradation of sample metrics, we suggest running the samples within a week to reduce likelihood of sample quality issues that can develop over time.

6. **The green background in DLS-labeled samples is higher than in NLRS-labeled samples. Is that a problem?**

   The leftover DL-Green often will show higher background in the images taken on the Saphyr than what is seen in NLRS-labeled samples. This is normal, and the membrane cleanup steps remove enough DL-Green to not impact data metrics. Positive label variance (PLV) from extra fluorophores occurs randomly and is not a problem if within expected metrics.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

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

Type	Contact
Email	support@bionanogenomics.com
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