Bionano Prep Direct Label and Stain (DLS) Protocol

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Table of Contents

Legal Notice 3
Revision History 4
Bionano Prep DLS Overview (750 ng) 5
Workflow Overview – Label and Stain on Day 1, Quant and Load on Day 2 5
Bionano Prep DLS Kit and User-Supplied Materials 6
Introduction and Important Notes 7
Introduction 7
Important Notes 7
Bionano DLS Labeling Protocol 10
Protocol Start, Day 1 10
Protocol Start, Day 2 13
Troubleshooting 16
Frequently Asked Questions 19
Technical Assistance 20

Legal Notice

For Research Use Only. Not for use in diagnostic procedures.

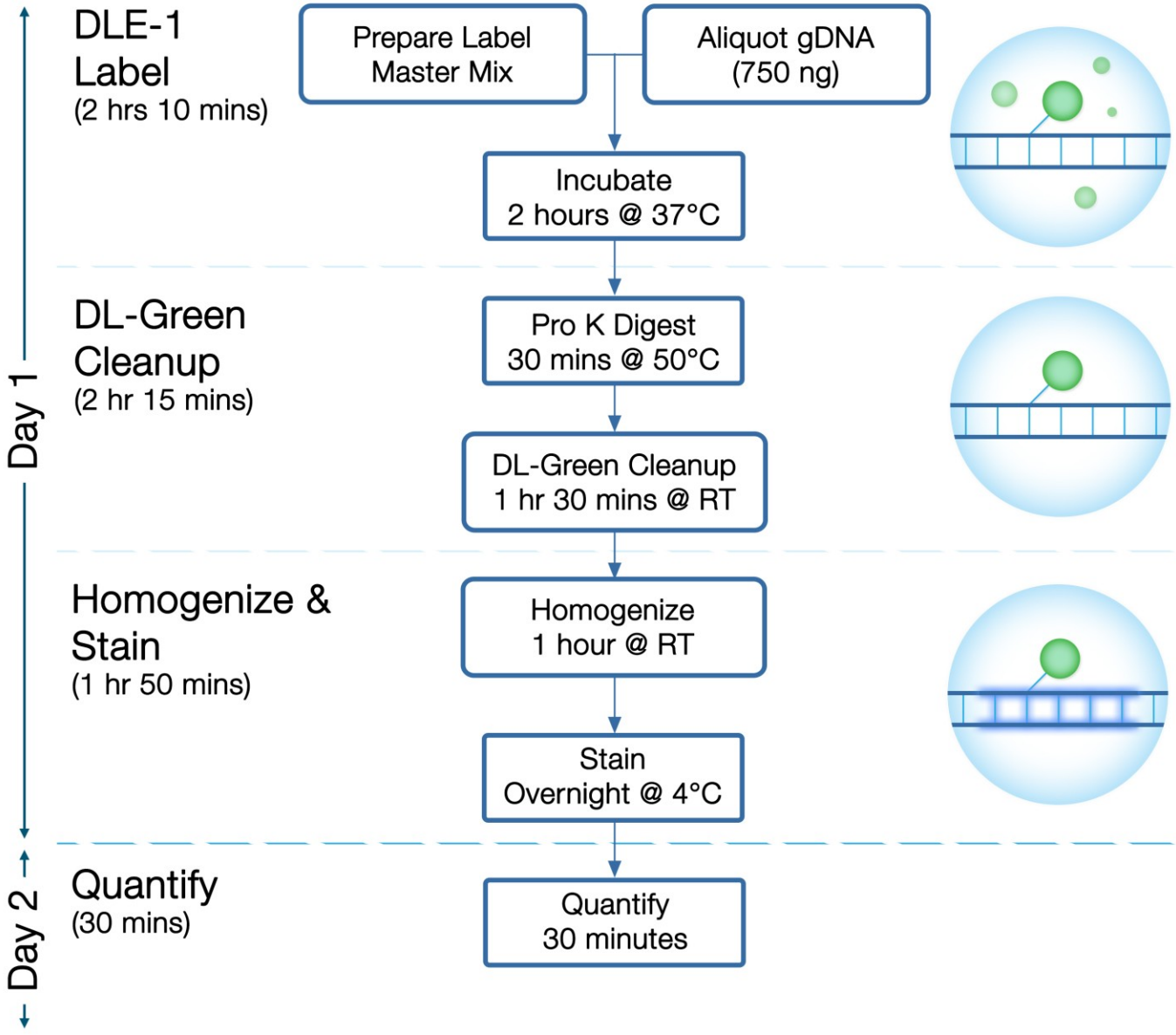
Revision History

Revision	Notes
E	Decrease DTT in staining reaction
F	Include note indicating change in Staining Master Mix table. Update table formatting.

Bionano Prep DLS Overview (750 ng)

Sequence-specific labeling of megabase-size genomic DNA (gDNA) for Bionano mapping using a Direct Label Enzyme (i.e. DLE-1) and subsequent staining.

Workflow Overview – Label and Stain on Day 1, Quant and Load on Day 2



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, centrifuge 3 sec. Keep on -20°C enzyme block until use.
20x DL-Green	20352	18 µl	-20°C	Vortex and centrifuge briefly. Keep on pre-chilled aluminum block until use.
1M DTT	20354	75 µl	-20°C	
5x DLE-1 Buffer	20350	200 µl	-20°C	Vortex and centrifuge briefly. Keep at room temperature (RT) until use.
4x Flow Buffer	20353	190 µl	4°C	
DNA Stain	20356	65 µl	-20°C	Thaw at RT. Vortex and centrifuge briefly. Keep at RT until use as DMSO will crystalize on ice.
Ultrapure Water (Nuclease Free)	20355	900 µl	RT	None
DLS 24 Well Plate and Cover	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	

Table 2: User-Supplied Consumables

Item	Description	Catalog #
Puregene Proteinase K	Qiagen	158920
Qubit® HS (High Sensitivity) dsDNA Assay Kit	Thermo Fisher	Q32851
Qubit® Assay Tubes	Thermo Fisher or Axygen	Q32856 or 10011-830
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 wide-bore, filter, aerosol, 200 µl	VWR or Rainin equivalent	46620-642
Pipet tips, aerosol-resistant, 2, 10, 20 and 200 µl	General lab supplier	
Pipet tips, 10 µL, C-10 for pos. displacement (optional)	Rainin or equivalent	17008604

Table 3: User-Supplied Equipment

Item	Description	Catalog #
HulaMixer Sample Mixer	Thermo Fisher	15920D
-20°C enzyme block	General lab supplier	
4°C aluminum cooling tube block	General lab supplier or Sigma Aldrich	Z740270
Forceps for membrane placement	TDI International or equivalent	TDI-2A-SA
Bath sonicator (recommended)	Branson or equivalent	CPX 952-119R
Pipet (2, 10, 20 and 200 µl)	General lab supplier	
Ice bucket and Ice, or 4°C tube block	General lab supplier	
Vortexer	General lab supplier	
Microcentrifuge for 0.2, 0.5, and 1.5 ml Tubes	General lab supplier	
Thermocycler with heated lid (+10°C above block temp)	General lab supplier	
Fluorometer, Qubit 3.0	Thermo Fisher or equivalent	Q33216
Positive-displacement pipet MR-10 (optional)	Rainin or equivalent	17008575

Introduction and Important Notes

Introduction:

This protocol describes an enzymatic labeling approach that allows for direct fluorescent labeling of native double-stranded gDNA at a specific sequence motif. The Direct Label Enzyme (DLE-1) shows very high sequence specificity and efficiency. This direct labeling does not introduce nicks into DNA and allows users to generate genome maps with N50s of 20-100 Mbp, depending on the genome and sample quality.

The Bionano Prep Direct Label and Stain (DLS) kit provides reagents for sequence-specific labeling of megabase-length gDNA for Bionano mapping. Sequence specificity is provided by DLE-1. The labeled DNA 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 (750 ng)

This protocol produces approximately 60 µl of labeled gDNA sample and is designed for tackling genomes that can be interrogated on a single flowcell of a Saphyr Chip, with sufficient sample for one additional flowcell in cases of low throughput or other failure. Starting material (gDNA) should be megabase-length gDNA. This can be determined via Pulsed-Field Gel Electrophoresis (PFGE). DNA and labeling quality is determined on the Bionano Saphyr System by supplying a reference and monitoring map rate, positive label variance, negative label variance, and label density. See the “Important Notes” section.

Table 4: Typical Results for Plug Lysis gDNA from Human Cells, Fresh & Frozen Blood, and Frozen Tissue when hg19 is supplied as reference.

N50 (> 150 kbp)	Labels/ 100 kbp	Map Rate	Positive Label Variance	Negative Label Variance	Effective Throughput/Scan
240 - 300 kbp	14 - 17	70 - 90%	3 - 10%	6 - 15%	9 - 20 Gbp

For additional information, please refer to **Data Collection Guidelines** (PN 30173) on the Support page.

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 and bubble formation should be

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 while working. Protect both the reactions and light-sensitive reagents from light during storage.
- Labeled DNA concentration is measured on Day 2, after labeling, cleanup, homogenization and staining. DNA homogeneity is assessed by quantitating in duplicate (CV < 0.25). Homogenous labeled DNA allows for an accurate estimate of concentration and more uniform DNA loading. The DNA concentration should be between 4 and 12 ng/µl.

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 4°C. Verify final DNA concentration and homogeneity before labeling.
  - For gDNA samples < 36 ng/µl, contact Technical Support at Support@bionanogenomics.com.

Determining Enzyme

- Before starting the DLS protocol, import the sequence data for your sample into either Bionano Access or the Label Density Calculator software to ensure that DLS labeling is the appropriate choice for your sample. Contact Technical Support at Support@bionanogenomics.com for guidance if uncertain.
- Label Density Calculator Software available at: http://bnxinstall.com/labeldensitycalculator/LabelDensityCalculator.htm

  Note: Because the recognition sequence for DLE-1 is a palindrome, the Label Density Calculator Software double counts the recognition sites. Divide the result by 2 to get the correct value.
- Recommended DLS labeling density is between 8 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 the protocol to ensure accurate sampling of DNA in order to achieve proper enzyme-to-DNA and DNA-to-Stain ratio, and also 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, 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 as 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 bubble. Visually inspect the tip after removing from solution to confirm that it is empty.

Protocol Start, Day 1

See *Important Notes* section, gDNA concentration should be between 36 ng/µl and 150 ng/µl.

See *Kit Contents and Consumables* sections for proper handling and storage of reagents.

Setup

1. Thaw 20x DL-Green. Vortex well, pulse spin, and hold on ice in 4°C aluminum block.
2. Thaw 5x DLE-1 Buffer. Vortex well, pulse spin. Hold at RT until use.
3. Flick 20x DLE-1 Enzyme three times and pulse spin. Hold on bench in -20°C enzyme block.

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

Dilute gDNA and Combine with Labeling Mix (10 minutes)

4. Remove gDNA from 4°C storage, pulse spin briefly if condensate is present, and allow it to equilibrate to room temperature for 30 minutes. Pipet mix 5 times with a wide bore tip.
5. In a thin wall PCR tube, add 750 ng gDNA and nuclease-free H_2O to a total volume of 21 µl.
6. If processing more than one sample, prepare a Labeling Master Mix in a 0.5 ml amber tube in the order outlined in the table below. Be careful not to generate bubbles. Use within 30 minutes.
    a. Master Mix contains an additional 20% excess of each reagent. **Mix by pipetting entire volume five times.** Pulse spin and keep in aluminum block on ice until use.

    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) + H_2O	21 µl			
5x DLE-1 Buffer	6.0 µl		× 1.2	µl
20x DL-Green	1.5 µl		× 1.2	µl
20x DLE-1 Enzyme	1.5 µl		× 1.2	µl
Final Reaction Volume	30 µl			µl

7. Add 9 µl Master Mix on top of the 21 µl gDNA + H_2O. Using a standard pipet tip with pipet set to 28 µl, mix sample slowly up and down 5 times. Pulse-spin tube for 2 seconds. Protect from light. ⚠

    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 to maximize the mixing.

Labeling (2 hours)

8. 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. Proceed quickly to the next step.
9. Before proceeding, pulse spin briefly if any condensation is visible on the tube wall.

DL-Green Cleanup & Pre-Stain Reaction (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. Proceed quickly to the next step.

Membrane Adsorption in Microplate (1 hour, 40 minutes)

Note: Membranes may be wetted up to 10 minutes before sample application.

12. For each sample, wet the underside of 1 DLS Membrane with 1x DLE-1 Buffer in the Bionano-supplied microplate:
    a. For each sample, prepare 60 µl of 1x DLE-1 Buffer (12 µl 5x DLE-1 Buffer + 48 µl H_2O). Vortex to mix.
    b. Dispense 25 µl of 1x DLE-1 Buffer into the center of one well of the DLS Microplate.
    c. Use forceps to place a DLS Membrane on top of buffer.
    d. If not proceeding right away, seal wells immediately with a DLS Plate Sealing Strip to prevent evaporation until ready to proceed.
13. Perform DL-Green cleanup by dispensing labeled DNA sample onto the center of the wetted membrane:
    a. Using a standard pipet tip, dispense entire volume (~35 µl) of labeled DNA onto the middle of the DLS Membrane.
    b. Seal membrane wells with DLS Plate Sealing Strip. While holding the microplate, apply pressure to secure the Sealing Strip to the top rim of the wells to prevent evaporation.
    c. Protect the microplate from light (cover) and incubate at room temperature for 1 hour. ⚠
    d. Up to 10 minutes before the incubation is complete, wet a second membrane in an unused well of the DLS Plate, following steps 12b - 12d above.
    e. After 1 hour, hold the plate securely and carefully remove the Sealing Strip.
    f. Using an unfiltered standard pipette tip, with 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.

Note: See support video at: https://bionanogenomics.com/support-page/dna-labeling-kit-dls/ for demonstration.

14. Repeat steps 13a - 13c, but dispense onto the second (unused) membrane prepared in Step 13d, and incubate for 30 minutes.
    a. During the second adsorption, prepare Staining Master Mix (see next section, Step 18).
15. After 30 minutes, hold the plate securely and carefully remove the Sealing Strip.
16. 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 (see note below). Transfer into a new PCR tube or an amber 0.5 ml microfuge tube. Pulse spin for 2 seconds. Protect tubes from light. ⚠

    Note: See Support Video for demonstration.
17. Using a 200 µl pipet, dispense 20 µl of the labeled sample from the PCR or 0.5 ml amber tube into the DLS Round Bottom Amber Tube (2 ml) and proceed to DNA Staining and Homogenization. If sample volume recovered is < 20 µl, make up the difference with the appropriate amount of 1x DLE-1 Buffer.

DNA Staining & Homogenization (1 hour 10 minutes)

Staining and Homogenization (1 hour, 10 minutes)

18. Bring 1M DTT, 4x Flow Buffer and DNA Stain to room temperature, vortex well, and pulse spin briefly. Keep at RT to avoid crystallization of the DMSO in the DNA Stain.
19. Prepare Staining Master Mix according to table below. Add 25% excess of each reagent for multiple samples. Vortex to mix then pulse spin.

Staining Master Mix Calculation Table

Staining Reaction	1 Sample	# of Samples	Master Mix Excess	Master Mix Total
Labeled Sample (Step 17)	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
Nuclease Free H_2O	15.5 µl		× 1.25	µl
Total	60 µl			µl

** Denotes change from previous protocol revision.*

Note: Since Flow Buffer is fairly viscous, 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 solution in order to avoid inadvertently drawing out DNA that may stick to the pipet tip.

21. Place DLS 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 sample from the HulaMixer. Pulse spin to collect contents.

23. Store overnight at RT 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 does not meet this criteria, 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 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 sampling < 0.25. If both samplings are outside of 4-12 ng/µl, see troubleshooting section. If one sample is between 4-12 ng/µl and the other is outside of this range see below:

   - 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)

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 sample for chip loading, draw from center or bottom of tube (not top).

Troubleshooting

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

A. The gDNA is not homogeneous before labeling

Evidence: The gDNA quantitation CV of measurements (top, middle and bottom) is > 0.25.

Steps to Follow:

1. Aspirate and dispense sample slowly using a standard 200 µl pipet tip up to 3 times.
2. Aspirate and dispense sample slowly using a wide bore tip for a total of 5 times.
3. Incubate the gDNA at room temperature for 1 to 3 days.
4. Aspirate and dispense the sample slowly using a wide bore tip 5 times.
5. 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.

The sample is likely 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 Customer Support for guidance.

E. Label density is lower than expected

Evidence: The average detected label density will always be lower than the 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 HMW 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 pH in master mix (> 30 min).

F. Labeled DNA is < 4 ng/µL or > 12 ng/µL for both top and bottom 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 > 12 ng/µl, proceed to load at your own risk, but the labeled sample may clog the chip and have reduced molecule N50.
    a. If sample is > 12 ng/µl, contact Bionano Customer Support for guidance.
3. If the sample is 3-4 ng/µl, proceed to load at your own risk, but expected throughput will likely not be reached.
4. If the sample is < 3 ng/µl do not load. Check starting DNA concentration and repeat the labeling assay.

G. Filtered (> 150 kbp) N50 is < 220 kbp, or unfiltered (> 20 kbp) N50 is < 150 kbp

Evidence: The ICS dashboard N50 metrics and Access 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 as to whether you should rerun sample on a different chip.

H. 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.

I. 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 Dashboard.

Steps to Follow:

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

Potential Causes:

- Low center of mass (N50).
- Evaporation causing an increase in salt & changing DNA migration.
    a. Were the wells topped off (rehydrated) with nuclease-free water before clip sealing?
- 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 no more than 10 minutes before adding sample to the membrane. Membranes can be wetted and used immediately. It is possible to wet both membranes at the same time, but this is entirely dependent on the users ability to create a tight seal with the sealing strip to prevent evaporation. Keep Sealing Tape on plate 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. In contrast we have found that samples with DNA concentration ≤ 3 ng/ul rarely reach typical results for effective throughput.

5. How long is the sample good for?

While we have found that stained DNA samples 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.

6. The green background in the DLS is higher than in the NLRS. Is that a problem?

The leftover DL-Green often gives a higher background in the chip than what is seen in the NLRS sample, but it should not impact data quality. Positive label variance from extra fluorophores occurs randomly.

Technical Assistance

For technical assistance, contact Bionano Genomics 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@bionanogenomics.com
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