Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide

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Table of Contents

Revision History

REVISION	NOTES
A	Initial Release
B	Updated Options to Resolve Inhibitory substances in the DNA.
C	Updated to include SP-G2 Fresh BMA and Tech Support Europe Contact information.

Introduction

The Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide includes information regarding the use of the Bionano Prep SP-G2 and DLS-G2 Kits. Protocols supported by this guide include the following:

- CG-00003: *Bionano Prep SP-G2 Fresh Cell Pellet DNA Isolation Protocol*
- CG-00004: *Bionano Prep SP-G2 Frozen Cell Pellet DNA Isolation Protocol*
- CG-00005: *Bionano Prep SP-G2 Fresh Human Blood DNA Isolation Protocol*
- CG-00006: *Bionano Prep SP-G2 Frozen Human Blood DNA Isolation Protocol*
- CG-00007: *Bionano Prep SP-G2 Frozen Human BMA DNA Isolation Protocol*
- CG-00062: *Bionano Prep SP-G2 Fresh Human BMA DNA Isolation Protocol*
- CG-30553-1: *Bionano Prep DLS-G2 Protocol*

Important Notes

The intention of this guide is to provide suggestions for resolution of issues that can affect optimum performance regarding a specific sample type or workflow. These suggestions have not been validated by Bionano and instead come from customer feedback and suggested best-practice. Moreover, some recommended options include the use of additional reagent volume, which may result in fewer reactions than each kit was designed for.

It is the responsibility of the user to follow all protocols and to store products according to specified conditions, as improper storage could cause degradation of reagents. For further questions and/or additional support please refer to the Technical Assistance section at the end of this document.

Bionano Prep SP-G2 and DLS-G2 Kit Troubleshooting Guide

Customer Question	Observation	Potential Cause	Options to Resolve
Why is my DNA discolored?	Darkness or discoloration of the DNA bound to the Nanobind disk in the second WB2 wash	The sample is dirty, hemolyzed (blood or BMA), or clotted. More vigorous washes are needed for this sample. Sample may be compromised	Put samples back to HulaMixer and rotate samples on the HulaMixer for another one minute at room temperature at 10 rpm, no shaking or vibration.
Why is my DNA concentration low post-extraction?	DNA concentration is less than 39 ng/µL	***Cells:*** Unable to resuspend cell pellet	Use a standard bore pipette tip to resuspend frozen cell pellets, careful to resuspend pellet no more than 10 times.
		Inaccurate cell counting method	For counting cells, we recommend using a hemacytometer. If using an automated cell counter, verify accuracy by comparison to hemocytometer counts.

Customer Question	Observation	Potential Cause	Options to Resolve
		All Sample types: Sample was not properly stored. Indication of compromised input material Incomplete cell lysis	Ensure samples have not undergone freeze/thaw. Ensure to fully resuspend cells before starting the SP-G2 protocol.
		Compromised Qubit reagents and improper Qubit procedure	Quantify sample with known concentration such as the Bionano SP Large Genome Labelling Control. Verify Qubit standards and the ratio of their measurements per manufacturer's instructions. We recommend using Positive Displacement pipettes and tips for accurate measurement of DNA aliquots for Qubit quantitation.
Why is my DNA not viscous?	Sample consistency is very thin and easily pipetted, but concentration is > 39 ng/µL	The sample is likely not to have ultra-high molecular weight gDNA	Check sample using pulse field gel electrophoresis before labeling to confirm presence of ultra-high molecular weight DNA. Evaluate sample prep method and input material quality/age and repeat DNA isolation from biological sample.
Why is my DNA not homogenous?	Coefficient of variance (CV) of the DNA quantitation measurements >0.3 within 24hrs post gDNA isolation	DNA homogenization was not optimal	Aspirate and dispense the DNA slowly using a wide-bore tip five times. Incubate the DNA at room temperature overnight. Aspirate and dispense the gDNA slowly using a wide-bore tip five times. Repeat quantitation.
Why is my DNA concentration high post-extraction?	Concentration of DNA is greater than 150 ng/µL	Excess source material introduced into the isolation.	Target 1.5 million cells per sample prep. Dilute the DNA. Remove a portion of the DNA. Dilute using 1x TE to 50-150 ng/µL. Pipette mix slowly five times with wide-bore pipette. Let the DNA sit at room temperature overnight. Repeat quantitation. **NOTE:** Increased concentration can also mean excess protein contaminants carried through the DNA isolation process especially concentrations of 250 ng/µL or greater. This can result in low throughput, chip clogging, and short molecule length.
		Compromised Qubit reagents and improper Qubit procedure	Quantify sample with known concentration such as the Bionano SP Large Genome Labelling Control. Verify Qubit standards and the ratio of their measurements per manufacturer's instructions. We recommend using Positive Displacement Pipettes and tips for accurate measurement of DNA aliquots for Qubit quantitation.

Customer Question	Observation	Potential Cause	Options to Resolve
Why does buffer LBB have precipitate inside the tube/on the rim of tube opening?		Temperature fluctuation, improper storage condition	Make sure the cap is tight. Put the tube in the 37C water bath for 30 minutes. Invert them to mix and see if the precipitate goes away. Leave them on the bench and allow the tube and LBB to come to room temp, inspect for precipitate before using. Contact Support if precipitants persist.
Can I use blood that has not been collected in EDTA tubes?	Blood collected in heparin or using other methods of prevention of coagulation have been used.		Heparinized blood is not recommended for our protocol. Bionano DNA Stabilizer can be added after collection to store such samples. Refer to the instructions in the Blood Shipping Instructions – 30179, Rev D document on collection and storage of blood samples. For Blood collected using other methods, contact Bionano Support for guidance.
I am using cryopreserved cells. How can I proceed?	Cells have been frozen in a cryopreservant as opposed to a pellet containing 1.5 million cells	NA	Refer to the Bionano Prep SP Frozen Cryopreserved Cell DNA Isolation Technote – TECHN-00001, Rev 01 and follow the steps outlined therein for DNA isolation from Cryopreserved cells.
Why am I getting low DNA yield from the DLS-G2 labeling protocol?	DLS-G2 Membrane does not wet within 3 minutes	Issue with the membrane that is preventing proper wetting	Discard membrane and perform wetting step with a new membrane. If multiple membranes from the same box show improper wetting issues, contact Bionano Support.
	Low DNA recovery from membrane as determined by post-stain Qubit HS quantitation	DNA is too viscous to fully remove from membrane using the recommended pipette setting in the protocol	Set the pipette to 50 µL (or up to 100 µL) and move the tip back and forth across the entire (pink-colored) area of the membrane to ensure all DNA is removed. Use an unfiltered pipette tip. Aspirate very slowly. Ensure good suction, employing a 90-degree angle of tip to membrane surface (see DLS-G2 Membrane Demo video).
		DNA is non-homogenous before beginning DLS-G2 protocol	Perform additional mixing of the DNA with a wide-bore pipette tip (five times up and down). Allow DNA to homogenize at room temperature overnight. Repeat quantitation with the Qubit Broad Range assay and then repeat DLS-G2 if the concentration is within 39 – 150 ng/µL.
		Qubit BR/HS dsDNA reagents are not performing optimally	Repeat quantitation with new Qubit kit reagents. Quantify sample with known concentration such as the Bionano Biological Control A (pre-labeled). Verify Qubit standards and the ratio of their measurements per manufacturer's instructions.

Customer Question	Observation	Potential Cause	Options to Resolve
			We recommend using Positive Displacement Pipettes and tips for accurate measurement of DNA aliquots for Qubit quantitation.
What do I do when the 1M DTT from the DLS-G2 kit does not go into solution?	White particles or precipitates are observed in the 1M DTT upon thawing that do not go into solution upon thawing or vortexing.	Improper storage of reagent or degradation of DTT.	Vortex the 1M DTT to bring the precipitates into solution. If precipitates remain, heat at 37°C for 5 minutes, vortex for 30 seconds, and spin at 16,000 x g for 10 minutes. To avoid any remaining microprecipitates, transfer the 1M DTT into a new tube, pipetting from the top and leaving behind a small volume (approximately 10 µL) at the bottom of the tube. Leave DTT on the bench and allow it to come to room temperature. Inspect for precipitate before using. Contact Support if precipitants persist

Troubleshooting Based on Saphyr Run Metrics

Customer Question	Observation	Potential Cause	Options to Resolve
Why are my N50s low?	Average N50 ≥ 150kbp is < 230 kbp	**Cells:** Low cell viability (<70%) in fresh or frozen cell pellets - dead or dying cells are pelleted down while performing SP-G2 isolation	Centrifuge cells at 500 x g for 5 min at 4ºC. Remove supernatant with a pipette and count pelleted cells to determine input into SP-G2 isolation.
		Blood/BMA: Compromised starting sample quality	Frozen Blood samples should be collected in EDTA tubes and frozen at -80°C or processed within 120 hours at 4ºC or 66 hours at 25ºC of the draw date. DNA stabilizer needs to be added to heparinized BMAs or blood before freezing.
		Isolated DNA using Bionano Protocols: Size reduction of DNA during homogenization at end of SP-G2 isolation	Skip the controlled shearing steps after elution in EB (mixing with a regular p200 tip) but proceed with end over end homogenization on the Hulamixer. Elute in a larger volume, up to 100 µl.
		Additional shearing is required due to high eluate viscosity	Avoid vortexing, rapid pipetting, or excessive pipetting with standard bore tips and use commercial wide-bore tips when appropriate.
		Improper handling of purified DNA	
		Nuclease contamination	Ensure that only nuclease-free water is used for all protocol steps. **NOTE**: Water provided in Bionano kits is nuclease-free.
		LBB Buffer contains precipitates	Refer to the question on what to do if the LBB tube contains precipitates in the SP-G2 kit troubleshooting section.

Customer Question	Observation	Potential Cause	Options to Resolve
		Freeze-thaw cycles	Take care to avoid any additional freeze-thaw cycles of starting sample prior to DNA isolation.
Why is my DNA throughput low?	Average DNA collection rate is < 30 Gbp per scan	Old version of Instrument Control Software (ICS) was used	Prior to attempting additional troubleshooting, ensure Saphyr ICS is updated to latest version.
		Post-stain Qubit HS DNA concentration is < 4 ng/µL	Perform additional mixing of the isolated DNA with a wide-bore pipette tip (5x up and down). Allow DNA to homogenize at room temperature overnight. Repeat quantitation with the Qubit Broad Range assay and then repeat DLS-G2 if the concentration is within 39 – 150 ng/µL with CV<0.3. If concentration is still below 4 ng/µL, ensure DNA recovery from membrane cleanup step and repeat DLS-G2 after verifying Qubit BR concentration of the stock DNA.
		Post-stain Qubit HS DNA concentration is >16 ng/µL and CV>0.3	If one reading for the sample with the Qubit HS Assay is >16 ng/µL but the other is within 4-16 ng/µL, proceed with loading. If one reading for the sample with the Qubit HS Assay is >16 ng/µL but the other is <4 ng/µL, repeat Hulamixer mixing for 30 mins and repeat quantitation. If both readings are > 16ng/µL, dilute labeled DNA with the Staining Master Mix such that 1x DLE-1 Buffer is used in place of the 20µL sample volume in the table on Page 13 of the DLS-G2 protocol (Staining master mix + 1x DLE-1 Buffer is the “diluent" to be used) to reach the target concentration within 4-16ng/µL. Consistent high HS concentrations may indicate incorrect Qubit BR measurements or non-homogeneous DNA taken forward into DLS-G2 labeling process. If re-labeling is needed, perform additional mixing of the isolated DNA with a wide-bore pipette tip (5x up and down). Allow DNA to homogenize at room temperature overnight. Repeat quantitation with the Qubit Broad Range assay and then repeat DLS-G2 if the concentration is within 39 – 150 ng/µL with CV<0.3.R
		DNA throughput decreases progressively with each scan, which may indicate that material in the SP-G2 DNA prep from cells or tissues is clogging the nanochannels of the Saphyr chip	Increase incubation time in Proteinase K and LBB to 1 hour during SP-G2 isolation (15 minutes rotation + 45 minutes without rotation) and/or increase the incubation time in Proteinase K to 1 hour during DLS-G2.

Customer Question	Observation	Potential Cause	Options to Resolve
		DTT contains precipitates	Refer to the question on what to do if DTT contains precipitates in the above SP-G2 kit Troubleshooting section.
Why is my map rate low?	PLV>10%	Insufficient adsorption of excess DL-Green	Repeat labeling and membrane adsorption. Allow the membrane to fully wet for 3 minutes. Membrane should change to a bluish color once fully wet. See around the 0:55 mark of the DLS-G2 Membrane Demo video. If membrane does not fully wet after 3 minutes, use a new membrane. Additional 1x DLE-1 buffer may need to be prepared for the new membrane. Protect the DLS-G2 24 Well Plate from light (cover). Ensure that the plate stays undisturbed, with no inadvertent movement of the plate during incubation. Seal wells to prevent evaporation. Follow recommended incubation times.
	Map rate < 70% for human sample labeled with DLS-G2	Low molecule N50 (fragmented DNA)	Refer to section on: “Why are my N50s low?”.
		Lower than expected label density	Refer to section “Why is my label density low?.”
		Low throughput per scan	Refer to section: “Why is my DNA throughput low?.”
	More than 20% decrease in map during a chip run. High N50’s and high label density (>17 labels/100 kbp) may also be observed.	Sticking and streaking (DNA sticks in the nanochannels of the Saphyr chip)	Check for labeled DNA homogeneity (CV<0.3). If DNA is not homogeneous, use a wide bore tip to slowly pipette the labeled DNA up and down 2-3 times. Keep the DNA at room temperature overnight. Rerun the sample on a new chip the following day. If DNA is homogeneous, rerun on a new chip. If sticking and streaking is still observed after the chip rerun, repeat DNA extraction. Label re-extracted DNA side-by-side with the Bionano control DNA (SP Large Genome Labeling Control).
Why is my label density low?	Average Label Density per 100 kbp is < 14	Enzymatic activity is low	Check expiration date and storage conditions of the DLE-1 enzyme.
		Mishandling of DLE-1 (exposure to elevated temperature, vortexing)	Handling considerations for DLE-1 are as follows: Flick tube three times to mix, and centrifuge briefly. Keep in -20°C enzyme cooler until use.
		Prolonged exposure of DL-Green to the pH of the Labeling Master Mix (>30 minutes)	Add the Labeling Master Mix components in the order outlined in the DLS-G2 kit protocol. Keep in aluminum block on ice until use. Use within 30 minutes of mixing the components.

Customer Question	Observation	Potential Cause	Options to Resolve
		Incomplete mixing of DNA with the other components in the Labeling Master Mix	Increase the number of up and down pipetting cycles (up to eight cycles) or increase the speed of pipetting. Refer to the training video on the Bionano Support page for detailed instructions: DLS-G2 Master Mix Mixing
		Incorrect enzyme to DNA ratio in the labeling reaction (i.e., too much DNA and/or too little enzyme)	Ensure correct volumes of both DNA and enzyme are added to the labeling reaction. Re-quantify DNA to confirm Qubit Broad Range reading and ensure 750 ng input DNA is added to the labeling reaction.
		Inhibitory substances in the DNA	At the second WB2 wash during DNA isolation using the SP-G2 protocol, increase the number of total inversions, up to twenty total inversions. Repeat labeling reaction if the overall data quality is poor by extending the labeling time or decreasing input DNA. If the repeat labeling reaction also yields a lower than expected data quality, repeat DNA isolation from the biological sample. If DNA is discolored, see the section “Why is my DNA discolored?”
		Incorrect storage of labeled DNA. Exposure of labeling reaction to light. DL-Green photobleaching.	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 has been tested for stability at 4°C for up to 1 month.
		Carryover of contaminants in the extracted DNA	Refer to the questions above on DNA appearing discolored after isolation and inhibitory substances in the DNA.
		Post-stain DNA concentration is >16 ng/µl	Refer to the question on what to do when DNA throughput is low because of post labeling concentrations >16 ng/µL.

NOTE: Please refer to Appendix A of document *Saphyr System User Guide* (CG-030247) for additional troubleshooting of issues encountered during commencement of Saphyr run or post completion of Saphyr run.

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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