Bionano Prep SP and DLS Kit Troubleshooting Guide

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Revision History

Revision	Notes
A	Initial Release

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Introduction and Important Notes

Introduction:

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

- 30206, Bionano Prep Direct Label and Stain (DLS) Protocol
- 30339, Bionano Prep SP Tissue and Tumor DNA Isolation Protocol
- 30395, Bionano Prep SP Frozen Human Blood DNA Isolation Protocol v2
- 30396, Bionano Prep SP Fresh Cells DNA Isolation Protocol v2
- 30397, Bionano Prep SP Fresh Human Blood DNA Isolation Protocol v2
- 30398, Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol v2
- 30399, Bionano Prep SP BMA DNA Isolation Protocol v2
- 30400, Bionano Prep SP Brain Tissue DNA Isolation Tech Note
- 30403, Bionano Prep SP Amnio and CVS Culture DNA Isolation Protocol

Important Notes:

The intention of this guide is to provide suggestions for resolution of errors that can affect optimum performance regarding a specific sample type or workflow. These suggestions have not been validated by Bionano and some recommended options include the use of additional reagent volume, which will result in less than the 10 standard reactions the 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.

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Bionano Prep SP and DLS Kit Troubleshooting Guide

Customer Question	Observation	Potential Cause	Options to Resolve
Why am I not getting enough DNA from the labeling reaction?	Bionano Prep DLS: Membrane does not wet within 10 minutes	Issue with the membrane that is preventing proper wetting	Discard membrane and perform wetting step with a new membrane.
	Bionano Prep DLS: 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 ul (or up to 100 ul) and move the tip back and forth across the membrane to ensure all DNA is removed.
		DNA is non-homogenous before beginning DLS	Perform additional mixing of the DNA with a wide-bore pipette tip (5x up and down). Allow DNA to homogenize at room temperature overnight. Repeat quantitation with the Qubit BR assay and then repeat DLS if the concentration is within 36 – 150 ng/ul with CV < 0.3.
		Qubit BR/HS reagents are not performing optimally	Repeat quantitation with new Qubit kit reagents.

Customer Question	Observation	Potential Cause	Options to Resolve
Why is my label density low?	Average Label Density per 100kbp is < 14	Inadequate mixing of highly viscous DNA with labeling reagents at the start of DLS	Increase the number of up and down pipetting cycles (up to 8 cycles) or increase the speed of pipetting. Refer to the training video on the Bionano Support page for detailed instructions: https://bionanogenomics.com/videos/dls-master-mix-mixing/
		DNA isolated from tissue is not sufficiently purified during SP isolation (can be identified by a hazy appearance)	Add an additional 0.5 ul DLE-1 to the labeling reaction and increase the incubation time in Proteinase K to 1 hour during DLS. If label density remains low, then DNA isolation should be repeated.
		Inhibitory substances in DNA	Increase incubation time in Proteinase K and LBB to 1 hour (15 minutes end-over-end, 45 minutes on benchtop) during SP isolation and/or add an additional 0.5 ul DLE-1 to the labeling reaction and increase labeling time up to 4 hours and increase the incubation time in Proteinase K to 1 hour during DLS.
		Post-stain DNA concentration is > 12 ng/ul	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 BR assay and then repeat DLS if the concentration is within 36 – 150 ng/ul with CV < 0.3.
		PMSF has degraded	Repeat SP isolation with new aliquot from stock bottle of PMSF or use a new bottle of PMSF (confirm that it is not expired).

Customer Question	Observation	Potential Cause	Options to Resolve
Why are my N50s low?	Average N50 ≥ 150kbp is < 230 kbp	Low cell viability in fresh or frozen cell pellets - dead or dying cells are pelleted down while performing SP isolation	Centrifuge cells at 300 x g for 10 min at 4C. Remove supernatant with a pipette and count pelleted cells to determine input into SP isolation.
		Size reduction of DNA during homogenization at end of SP isolation	Skip the shearing steps after elution but proceed with end over end homogenization.
		Size reduction of DNA during centrifugation step to remove DNA from the Nanobind after incubation in Elution Buffer	Increase DNA elution time from 20 minutes to 2 h or overnight at Room Temperature to facilitate more effective "passive" DNA elution.
		Additional shearing is required due to high eluate viscosity	Elute in a larger volume, up to 100 ul.
		Nuclease contamination	Ensure that only nuclease-free water is used for all protocol steps. Note: water provided in Bionano Kits is nuclease-free.
		Compromised starting sample quality	1. Minimize time from tissue isolation to flash freezing. 2. Post-mortem isolation of tissues should be done ASAP as DNA degradation continues over time. 3. Blood samples should be collected in EDTA tubes. 4. Blood samples should be processed within 4 days post-draw. 5. DNA stabilizer needs to be added to heparin BMAs or blood before freezing
		Freeze-thaw cycles	Take care to avoid any additional freeze-thaw cycles of starting sample.

Customer Question	Observation	Potential Cause	Options to Resolve
Why is my DNA throughput low?	Average DNA collection rate is < 40 Gbp per scan	Old version of Instrument Control Software (ICS) was used	Update Saphyr system to ICS version 5.2 or above.
		Average N50 ≥ 150kbp is < 230 kbp	Refer to troubleshooting instructions for low N50s above.
		Post-stain Qubit HS DNA concentration is < 4 ng/ul	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 BR assay and then repeat DLS if the concentration is within 36 – 150 ng/ul with CV < 0.3.
		Post-stain Qubit HS DNA concentration is > 12 ng/ul indicating underestimate of original SP DNA concentration, resulting in Saphyr Chip clogging	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 BR assay and then repeat DLS if the concentration is within 36 – 150 ng/ul with CV < 0.3.
		DNA throughput decreases progressively with each scan, which may indicate that material in the SP 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 isolation and/or increase the incubation time in Proteinase K to 1 hour during DLS.

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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