Performance of Second-Generation (G2) Sample Preparation Kits for Saphyr® OGM Analysis

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bionano

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Revision History

REVISION	NOTES
A	Initial release.

Introduction

The Bionano Genomics Saphyr® Optical Genome Mapping (OGM) system images ultra-long, linearized gDNA molecules labeled at specific sequence motifs. Comparative analysis of the label patterns over long contiguous reads across the whole genome reveals Structural Variants (SV) >500 base pairs (bp). All major types of large SVs can be detected at variant allele fractions of 5%.

The Bionano Sample Preparation (SP) DNA Isolation Kits are capable of purifying Ultra-High Molecular Weight (UHMW) gDNA in as little as four hours using an improved lyse, bind, and wash process and novel paramagnetic disks. The resulting purified megabase-sized gDNA is optimal for downstream use with the Saphyr® systems.

Starting with UHMW gDNA purified using the appropriate Bionano Prep™ SP Kit, fluorescent labels are attached to a 6 bp sequence motif, occurring, on average, twenty times per 100kbp. Fluorescent labels are attached via the direct label and stain (DLS) technology which is nondestructive and leaves gDNA samples intact. The result is uniquely identifiable genome-specific label patterns that enable alignment of molecules to consensus genome maps, anchoring sequencing contigs, and discovery of structural variations starting at 500 bp.

The Generation 2 SP-G2 and DLS-G2 workflows (**Figure 1**) are similar to the previous Generation 1 workflows, with a few specific updates in Steps 1 to 3 as reflected in the updated protocol steps below.

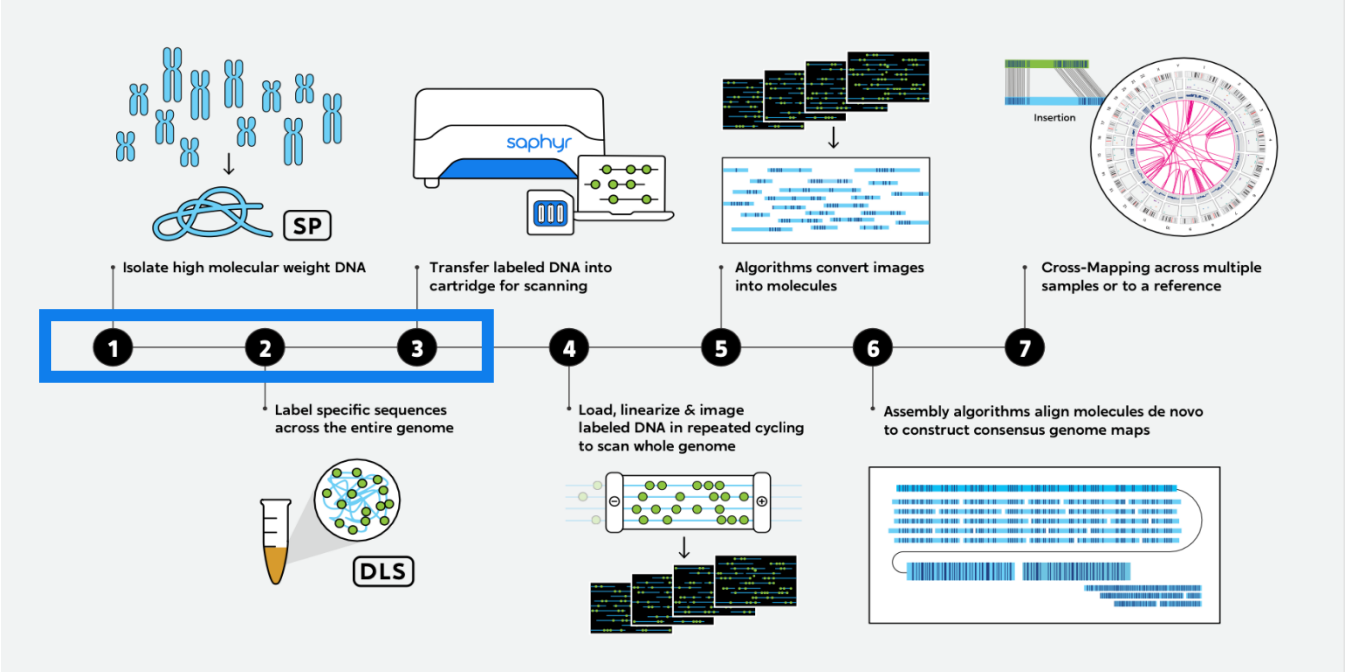

Figure 1. Demonstrates the Optical Genome Mapping workflow from sample prep, staining, loading a Saphyr® instrument through analysis. Boxed in blue contains the version-specific products demonstrated in this white paper.

Methods and Study Design

G1 Verification Methods

Designated Sample Preparation (previous version kits, Generation 1) kits from development lots (previous SP kits blood and cell, BMA version 2), produced by manufacturing, in conjunction with DNA Labeling and Staining kits (previous version kits, Generation 1), ICS 5.1 software, and Saphyr G2.3 Chips were chosen for testing.

Two operators prepared replicates of fresh cells, frozen cells, fresh blood, frozen blood, and frozen BMA samples to demonstrate reproducibility and repeatability.

Ten replicates of each supported sample type (five per operator) were produced.

G2 Verification Methods

Designated SP-G2 kits from development lot, produced by manufacturing and evaluated by Assay Development, in conjunction with DLS-G2 labeling kits, ICS 5.3 software, and G3.3 Saphyr Chips.

A kit inspection and documentation review confirmed design and manufacturing process met requirements.

Two operators prepared replicates of fresh cells, frozen cells, fresh blood, frozen blood, and frozen BMA samples to demonstrate reproducibility and repeatability.

Twelve replicates of each supported sample type (six per operator) were produced.

Alpha Study Design

Samples were chosen based on data/sample availability and sample type. DNA criteria was that no sample used was older than one year. Thirty samples included twenty-seven human and three mouse specimens. The sample types were frozen cell pellets, cryopreserved cells, frozen blood, and frozen bone marrow aspirates (BMA). Some samples which originally failed to meet Molecular Quality Report passing thresholds were chosen to evaluate whether they could be “rescued” or not.

Four operators used between six and twelve prepared samples each. Consumables included development lot reagents and commercial Saphyr® Second Generation chips.

Data analysis was conducted by evaluating downstream Copy Number Variant (CNV) quality metrics and Structural Variant calling concordance for different project types (solid tumor, cell QC/manufacturing, post-natal constitutional, heme, and human research) using Bionano Solve 3.7. The dual Variant Annotation Pipeline (VAP) was run for a subset of samples.

G1 vs. G2 Verification Results

Decrease in Turnaround Time for DNA Labeling and Staining

When compared to previous labeling protocols, Generation 2 protocols improve overall sample TAT on average by 40%. Previously, DLS (generation 1 protocols) TAT ranged between 7 - 8 hours as well as an overnight incubation (**Figure 2**), which now is reduce to under 4 hours. A large contribution to this reduction of the total sample TAT reduction is this new protocol where almost 3 full hours are reduced compared to the current DLS protocol. In addition, with increased labeling efficiency and higher gDNA purity, an observed scan time range of 1 - 2 hours is reduced across all five sample types **(Figure 3).**

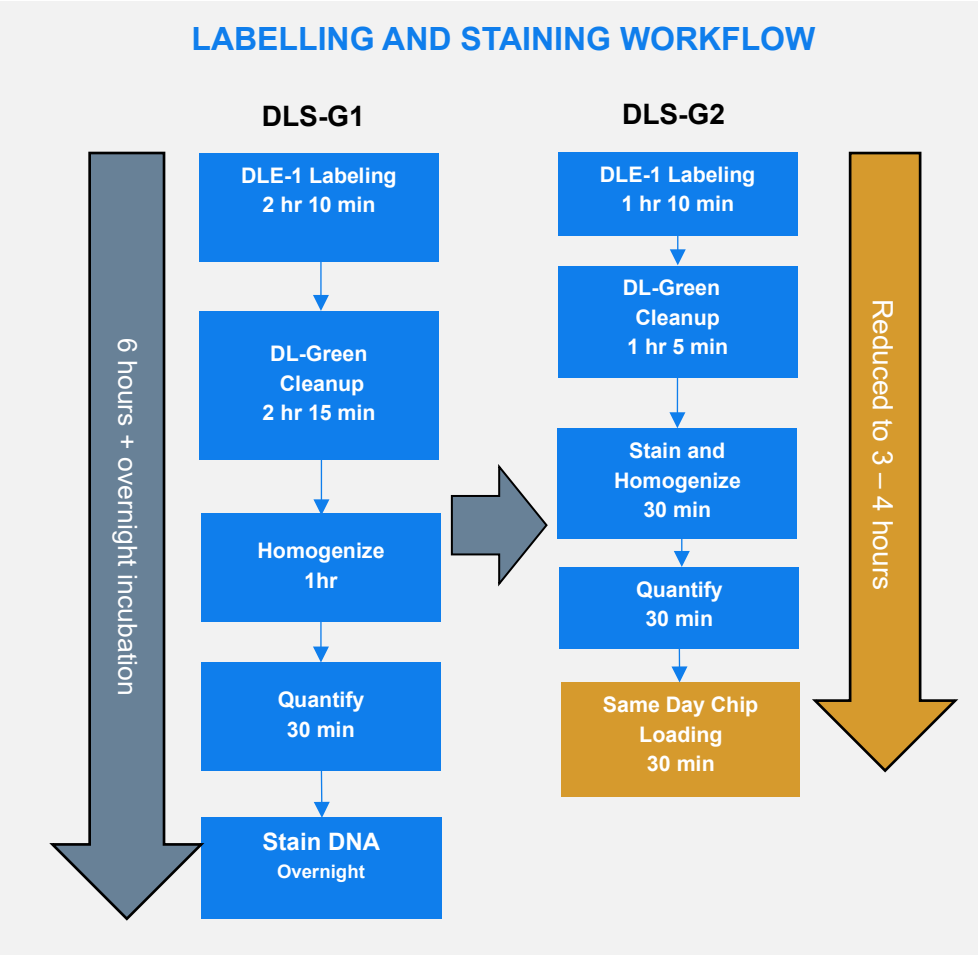

Figure 2. DLS Workflow improvements. The new DLS-G2 workflows depict shortened turnaround time (TAT) compared to previous versions by two hours. Same day chip loading added to the DLS-G2 workflow which previously was not possible with DLS-G1.

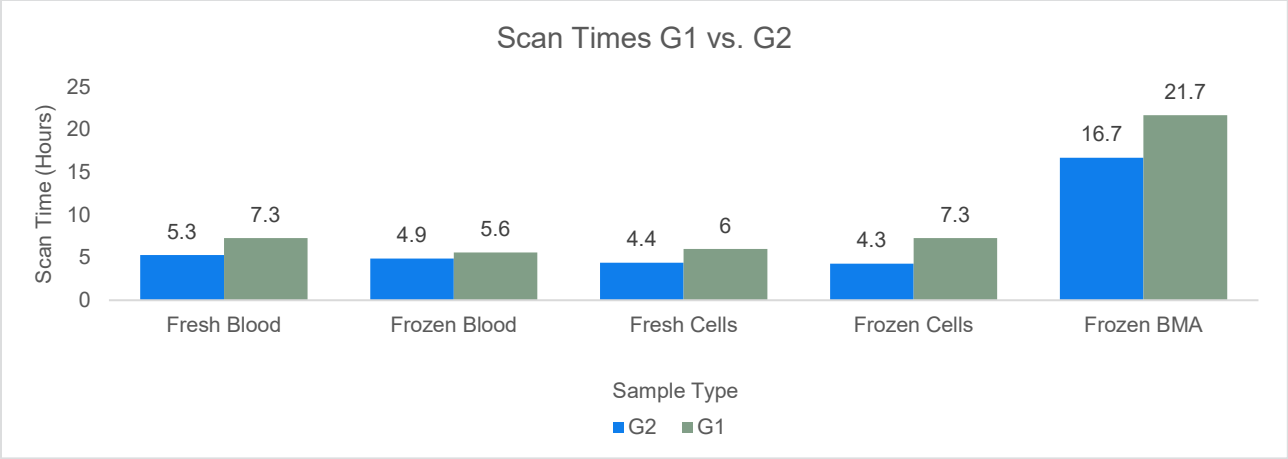

Figure 3. Scan time (hours) for on-market vs. Generation 2 kits. Fresh and Frozen Blood as well as Fresh and Frozen cells targeted 400Gbp throughput; Frozen BMA targeted 1.5 Tbp throughput.

Increased Average DNA Throughput and N50 Metrics

An observed improvement in metrics between Generation 1 verification and Generation 2 verification are the significantly increased DNA throughput (Gbp/scan) seen in **Figure 4** as well as N50 (≥ 20kbp and ≥ 150kbp) seen in **Figure 5.** The differences can be seen routinely across the five distinct sample types utilized in OGM analysis between the 2 generations of Bionano products. DNA throughput and N50 metrics are demonstrated as specific measures supporting the argument for G2 comparability and superiority, as seen by the data below. Seen in **Figure 5,** Generation 2 achieved equivalency or increased average N50 metrics; N50 (filtered for molecules ≥ 20 kbp) increased by average of 36kbp and equivalency achieved for N50 (filtered for molecules ≥ 150 kbp).

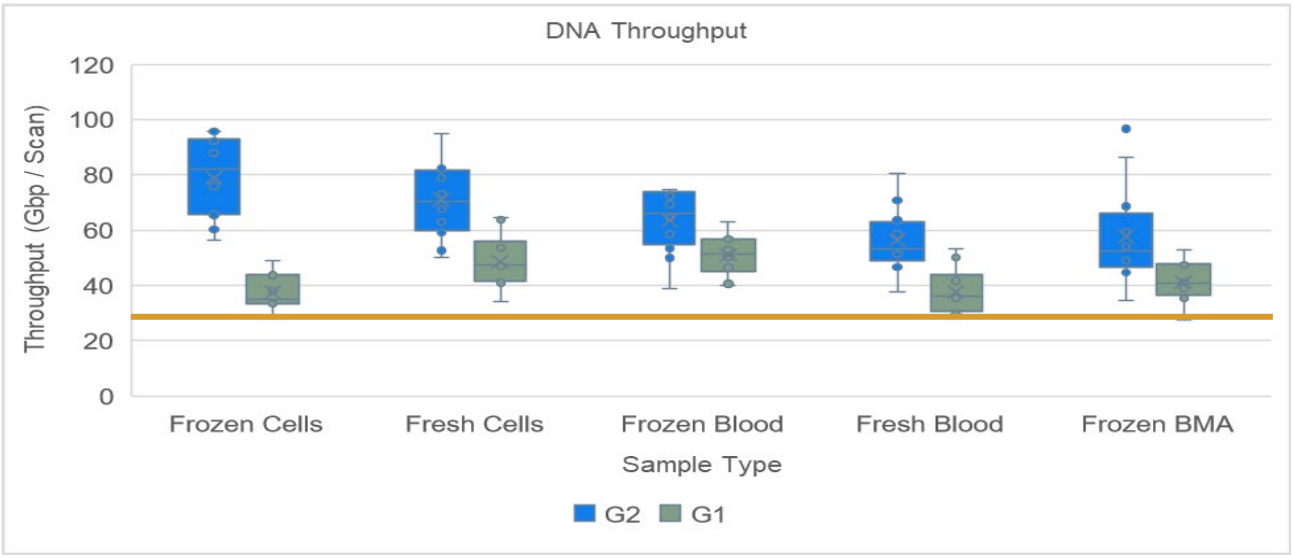

Figure 4. Comparison of G1 and G2 throughput (Gbp/scan). Recommended throughput thresholds increase from 30 Gbp/scan to 40 Gbp/scan for Fresh and Frozen cells as well as Fresh and Frozen blood. Frozen BMA remains at a 30 Gbp/scan threshold. Mean Throughput (Gbp/scan) of each group of samples is labeled by a “X” on the graph.

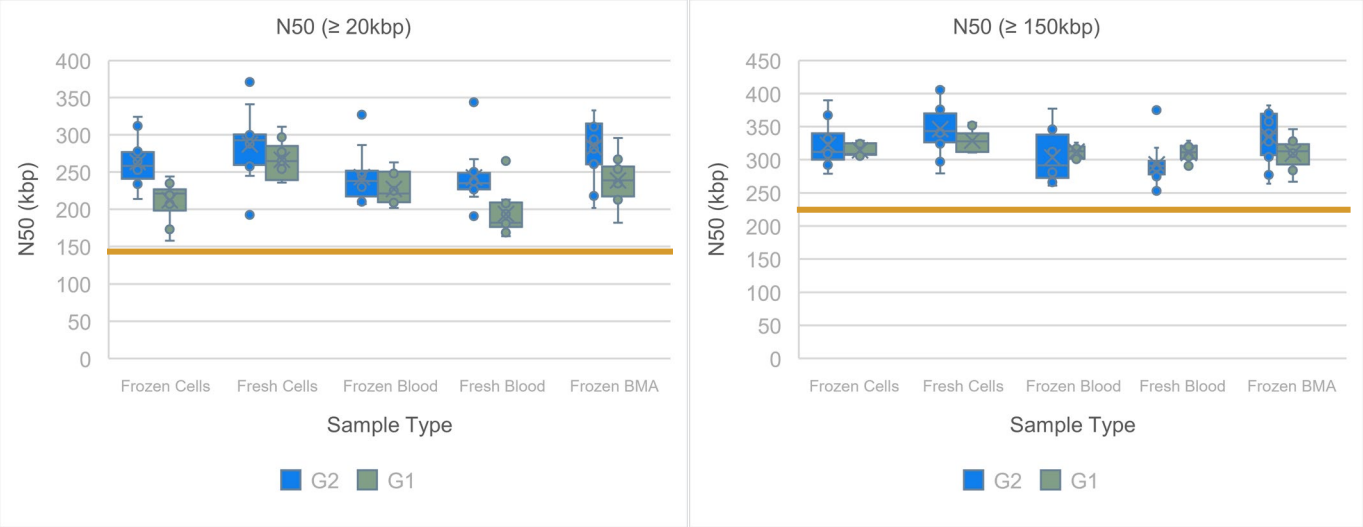

Figure 5. G1 versus G2 N50 for the 20kbp and 150kbp cutoffs. N50 is a proxy for size that indicates a weighted average length of DNA molecules in the dataset. The threshold for N50 (≥ 20kbp) and N50 (≥ 150 kbp) is respectively 150 kbp and 230 kbp. Mean N50s of each group of samples is labeled by a "X" on the graph.

Equivalency in Map Rate and Label Density Metrics

An observed equivalency in metrics between Generation 1 verification and Generation 2 verification for percentage map rate seen in **Figure 5** as well as label density (LD) seen in **Figure 6.**

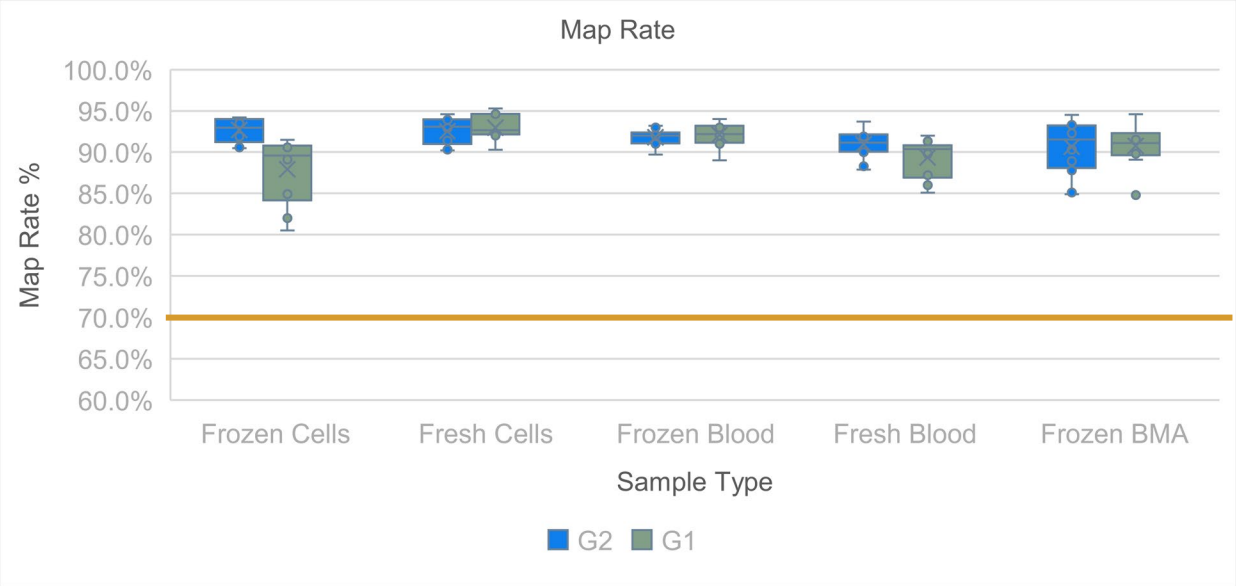

Figure 6. G1 versus G2 map rates by percentage map rate. Map rate is defined by the percentage of Bionano molecules that align to the reference, with a target percentage map rate is 70%. Mean of map rate percentage across samples is labeled by a "X" on the graph.

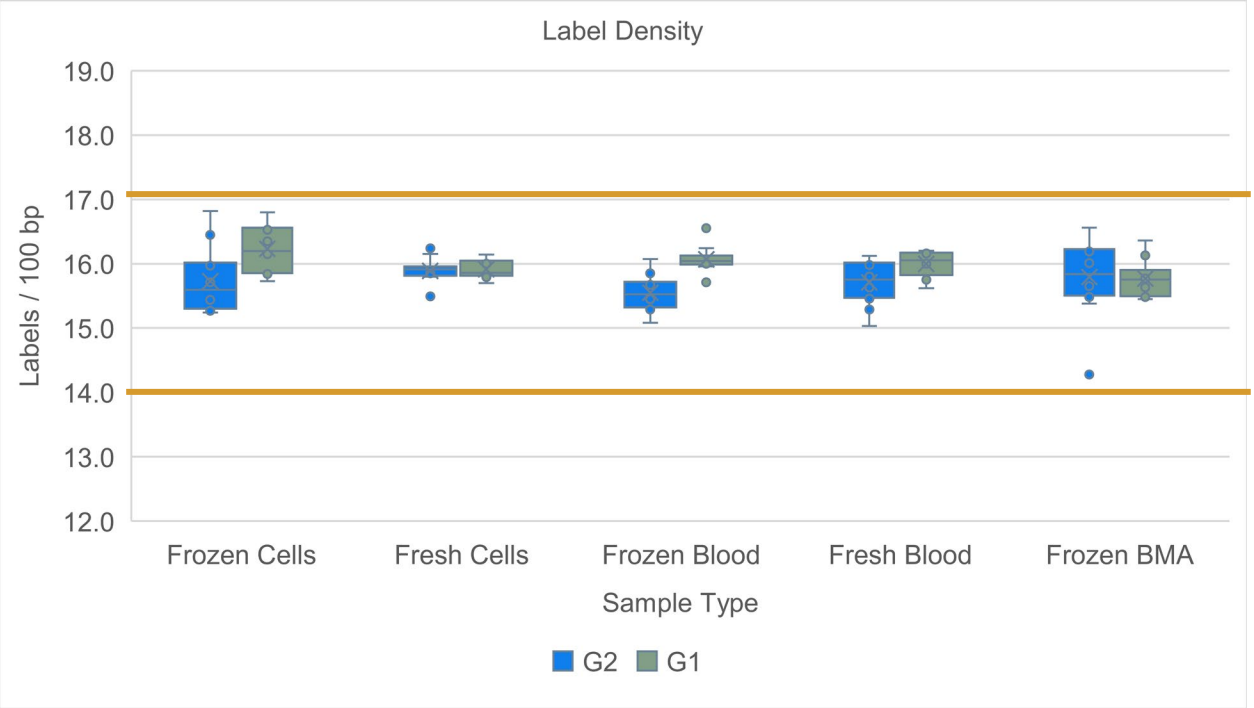

Figure 7. Comparison of label density between G1 and G2. Label density is defined by the number of labels detected during an OGM Saphyr run per 100 kbp of DNA. The threshold lies between fourteen labels and seventeen labels, each per 100 kbp. Mean label density of each group of samples is labeled by a “X” on the graph.

Alpha Data

Alpha data is represented in **Table 1** below. SP-G2/DLS-G2 passed N50 ≥ 20kbp, N50 ≥ 150kbp, Map Rate, PLV, and NLV metrics (≥ 90%). SP-G2/DLS-G2 had a 20% higher first-pass success rate (sixteen of twenty-six samples) compared to DLS-G1 (eleven of twenty-six samples).

Table 1: Alpha study data with pass rates compared to required thresholds

QC Metric	Threshold	G2 Pass Rate*
DNA Concentration	≥ 39 ng/uL	89% (24/27)
N50 (filtered for molecules ≥ 20kbp)	≥ 150kbp	96% (23/24)
N50 (filtered for molecules ≥150kbp)	≥ 230kbp	100% (24/24)
Label Density	≥ 14 and ≤ 17 labels per 100kbp	88% (21/24)
Map Rate	≥ 70%	92% (22/24)
PLV	≤ 10%	100% (24/24)
NLV	≤ 15%	92% (22/24)
Throughput	≥ 40Gbp per scan for Blood/Cell l≥ 30Gbp per scan for BMA	83% (20/24)

*G2 sample "passes" if it either meets the specification, or if it is the same or better than the G1 matched pair +/- 10%.

SP-G2/DLS-G2 had high concordance (>99% of all SVs) with SP-G1/DLS-G1 (see **Table 2**). Over half of the missed calls not found in the Bionano control database (7/12) were categorized as “low coverage,” meaning that either the G1 or G2 SV call was +/- two molecules of the VAP coverage threshold, and three other calls were small (~6 kbp) insertions/deletions. Putative false calls are those which are not found in the Bionano control database (e.g., polymorphic), not overlapping segmental duplication regions (e.g., spurious calls), and not at the borderline of our limit of detection (e.g., +/- two molecules would not change the Variant Annotation Pipeline result). CNV noise statistics were comparable between SP-G2/DLS-G2 and SP-G1/DLS-G1.

Table 2: Alpha data structural variant calls

Clustered and Unmasked SV calls						
n=13 samples	Insertion	Deletion	inversion	Duplication	Intra-Fusion	Inter-Fusion
Putative false negative calls	2	2	0	1	0	0
Total missed in G2 dataset	91	50	4	37	1	3
Total clustered G1 calls	12741	9847	2845	2198	93	62
Percent concordant in G2 dataset	99.29%	99.49%	99.86%	98.32%	98.92%	95.16%

DLS-G2 vs. DLS-G1 First Pass Success

Increased activity and concentration of the DLE-1 enzyme in the DLS-G2 protocol can "rescue" previously DLS-G1 samples that originally failed due to poor label metrics according to current quality control standards. See **Table 3**.

- DLS-G2 rescued 72% of samples that originally failed previous generation 1 kits, including tissue samples.
  - Of the rescued samples, 72% (14/21) had improved label density, 71% (15/21) had improved map rate and 38% (8/21) had improved N50 ≥ 150kbp.
- DLS-G2 was successful for 90% of the samples that originally passed G1 (10% DLS-G2 failure rate)
  - Of the four samples (shown in **Table 3**, table on the right) that originally passed DLS-G1 but failed DLS-G2, 75% had low map rate, 75% had high NLV, 50% had low LD and 25% had high label density.

Table 3. First pass data

	DLS-G2 Pass	DLS-G2 Fail
DLS V1 Pass	36 (52%)	4 (6%)
DLS V1 Fail	21 (30%)	8 (12%)

Sample Type	DLS-G2 Failure Mode
Frozen soft tissue (1) Frozen cells (1)	Low mapping rates Low label density High NLV
Frozen cells dry pellet (1)	Low map rate High label density
Frozen buffy coat (1)	High NLV

Conclusion

A summary of G2 robustness is depicted in **Table 4** in **Appendix A**. Average standard deviations for manual throughput, N50 for filtered molecules, negative label variance (NLV) and positive label variance (PLV) are included as additional information and lend a holistic picture of Bionano's second generation products.

Manufactured development lots of kits, assessed and evaluated by Assay and Reagent Development, were controlled under strict conditions to enhance data quality by in-house laboratory technicians during verification. Results demonstrated reproducibility and repeatability.

Alpha data relinquished a higher pass rate for SP-G2 and concordance with G1 kits, an improvement in the quality of the metrics. Turnaround time for labeling was decreased when staining sample type DNA, and N50 metrics illustrated quality for both 150 and 20kbp measurements. Additionally, average DNA throughput was increased with the G2 kits, a distinct improvement over G1.

Overall, data suggests equivalent or improved performance between the Generation 2 and Generation 1 reagents, as well as improved times savings for the Optical Genome Mapping workflow.

References

30206, Bionano Prep Direct Label and Stain (DLS) Protocol

30339, Bionano Prep SP BMA DNA Isolation Protocol v2

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

CG-30553-1, Bionano DLS-G2 Protocol

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 Bone Marrow Aspirate (BMA) DNA Isolation Protocol

30247, Saphyr System User Guide

30175, Bionano Molecule Quality Report Guidelines

30190, Bionano Solve Theory of Operation: Variant Annotation Pipeline

30110, Bionano Solve Theory of Operations: Structural Variant Calling

Appendix A

Table 4. Summary of Data for On Market kits versus G2 launch kits

	Fresh Blood		Frozen Blood		Fresh Cells		Frozen Cells		Frozen BMA	
	On Market Average (Std. Dev)	**G2 Kit Launch** Average (Std. Dev)	**On Market** Average (Std. Dev)	**G2 Kit Launch** Average (Std. Dev)	**On Market** Average (Std. Dev)	**G2 Kit Launch** Average (Std. Dev)	**On Market** Average (Std. Dev)	**G2 Kit Launch** Average (Std. Dev)	**On Market** Average (Std. Dev)	**G2 Kit Launch** Average (Std. Dev)
Throughput (Gbp/Scan)	38 (+/- 8)	56 (+/- 12)	51 (+/- 7)	63 (+/- 11)	48 (+/- 10)	71 (+/- 14)	37 (+/- 6)	79 (+/- 15)	41 (+/- 7)	58 (+/- 18)
N50 (filtered for molecules `≥ 20 kbp)	194 kbp (+/- 29)	243 kbp (+/- 37)	227 kbp (+/- 22)	243 kbp (+/- 34)	266 kbp (+/- 26)	287 kbp (+/- 45)	212 kbp (+/- 27)	263 kbp (+/- 31)	239 kbp (+/- 31)	285 kbp (+/- 35)
N50 (filtered for molecules ≥ 150 kbp)	310 kbp (+/- 13)	293 kbp (+/- 32)	312 kbp (+/- 9)	303 kbp (+/- 37)	329 kbp (+/- 16)	346 kbp (+/- 38)	314 kbp (+/- 9)	322 kbp (+/- 32)	310 kbp (+/- 22)	334 kbp (+/- 33)
Label Density (Labels per 100kbp)	16.0 (+/- 0.2)	15.7 (+/- 0.3)	16.1 (+/- 0.2)	15.5 (+/- 0.3)	15.9 (+/- 0.1)	15.9 (+/- 0.2)	16.2 (+/- 0.4)	15.7 (+/- 0.5)	15.8 (+/- 0.3)	15.8 (+/- 0.7)
Map Rate	89.4% (+/- 2.4)	90.9% (+/- 1.7)	92.1% (+/- 1.5)	91.8% (+/- 1.0)	93.0% (+/- 1.5)	92.6% (+/- 1.6)	87.9% (+/- 4)	92.7% (+/- 1.4)	90.7% (+/- 2.6)	90.9% (+/- 2.6)
NLV	8.7% (+/- 1.8)	7.1% (+/- 0.4)	8.1% (+/- 0.8)	7.1% (+/- 0.4)	8.4% (+/- 1.8)	6.6% (+/- 0.4)	8.7% (+/- 2.1)	7.5% (+/- 4.4)	9.4% (+/- 1.6)	7.9% (+/- 1.8)
PLV	5.9% (+/- 0.4)	5.8% (+/- 0.8)	6.8% (+/- 1.3)	4.8% (+/- 0.6)	4.3% (+/- 0.6)	4.3% (+/- 0.5)	5.5% (+/- 0.6)	3.7% (+/- 0.6)	5.4% (+/- 0.5)	5.5% (+/- 1.8)

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.

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