Bionano Saphyr® System Application Specifications

DOCUMENT NUMBER:
CG-00008

DOCUMENT REVISION:
D

Effective Date:

12/12/2023

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Revision History

REVISION	NOTES
A	Initial release.
B	Revised to integrate updates with Access 1.8/ Solve 3.8 / VIA 7.0 and inclusion of the SNP FASST3 CNV algorithm.
C	Revised to include updates with SP-G2 Fresh BMA and European Technical Assistance contact information.
D	Revised to integrate updates with Access 1.8.1/ Solve 3.8.1 and introduce Guided Assembly pipeline

Overview

The purpose of this document is to describe specifications of Bionano's Saphyr® System and associated consumables used in clinical research. Technical information, such as performance specifications and data quality, is included in outline or tabular form for ease of use. These statistics are supported by validation data produced for the Solve 3.8 release, summarized in the *Bionano Solve Theory of Operation: Structural Variant Calling* (CG-30110). Solve 3.8 introduces a new CNV/LOH pipeline, SNP FASST3 with VIA software.

Table 1. Validated Sample Types: Sample and Quality Requirements

Samples	Minimum Sample Requirements	Sample Prep and Quality Requirements
Fresh and Frozen Human Blood (EDTA) ***Available with Generation 2 Kits***	650µL	Aliquot 650µL ~ 1mL fresh blood to each tube. Require min. of one tube, two tubes preferred. Max. 5 days at 4°C or 66 hours at RT post draw including shipping and handling. Ship with cold packs if fresh. *Ship on dry ice if frozen.*
Fresh and Frozen Human Blood (Heparin tube + DNA stabilizer) ***Available with Generation 2 Kits***	650µL (with Bionano DNA stabilizer added)	Aliquot 650µL ~ 1mL fresh blood to each tube and add DNA stabilizer as soon as possible. Aliquot, add DNA stabilizer and store frozen blood at -80°C. Max. 3 days at 4°C post draw including shipping and handling. Require min. of one tube, two tubes preferred. Ship with cold packs if fresh. *Ship on dry ice if frozen.*
Cell lines or other purified cells ***Available with Generation 2 Kits***	≥ 1.5 million cells	Cell lines can be shipped/prepped from live cell cultures or frozen cell pellets at -80°C before shipping. Cells should be counted and aliquoted, frozen at -80°C as dry cell pellets and shipped on dry ice. **NOTE: LCLs or other cell lines are compatible. Not all cell types have been evaluated**.
Frozen Bone marrow aspirate (BMA) (EDTA tube, Heparin Tube + DNA stabilizer) ***Available with Generation 2 Kits***	0.8 mL	Samples should be frozen within 24 hours of aspiration, keep at room temperature until aliquoting and storing at -80°C. Require min. of one tube of a 0.8 mL aliquot, two tubes of 0.8 mL aliquots preferred. Ship on dry ice.
Fresh Bone marrow aspirate (BMA) (EDTA tube, Heparin Tube + DNA Stabilizer) ***Available with Generation 2 Kits***	0.5 mL	Samples should be kept at room temperature during aspiration, storage, shipment, and processing. Samples should be processed within 72 hours of collection. Requires a min. of one tube of a 0.5 mL aliquot, two tubes of 0.5 mL aliquots preferred.

Samples	Minimum Sample Requirements	Sample Prep and Quality Requirements
Tissue Biopsies – Human tumor tissue (breast, liver, lung colon*, kidney*, bladder*, brain*, ovary*, prostate*, thyroid*) *Sample type has been tested but not validated	Min. of 10mg required, 30mg preferred	Freshly frozen and stored at -80°C Ship on dry ice
Amnio/CVS	Min. of 1.0 million variable cells	Fresh or cryopreserved Cells can be shipped/prepped from live cell cultures or cryopreserved cells at -80°C before shipping on dry ice

Table 2. Unsupported Sample Types

Contact support@bionano.com
Buccal/Saliva
Formalin Fixed Paraffin Embedded (FFPE)
MeOH-Acetic acid pellets

Sample Collection, Shipping Instructions, and Document Part Numbers

- CG-30180 — *Cell Line Shipping Instructions*
- CG-30179 — *Whole Blood Collection, Storage and Shipping Instructions*
- CG-30358 — *Frozen Bone Marrow Aspirate Collection, Storage and Shipping instructions*
- CG-00073 — *Fresh Bone Marrow Aspirate Collection, Storage, and Shipping Instructions*
- CG-30186 — *Tissue and Tumor Collection, Storage and Shipping Instruction*
- TECHN-00008 — *Bionano Prep Methanol Glacial Acetic Acid Fixed Cell Preparation Tech Note*

Table 3. Data Quantity and Quality*

Application	Constitutional/Germ Line Including FSHD and Fragile X**	Constitutional/Germ Line including mosaicism	Somatic 5% Variant Allele Fraction**
Data collection target	400Gbp	800Gbp	1500Gbp
N50 (molecules ≥ 150kbp)	≥200 kbp*	≥230 kbp*	≥230 kbp*
Map rate to reference	≥70%	≥70%	≥70%
Effective coverage of reference (X)	≥80x	≥160x	≥300x
Variant Allele Fraction (VAF)	50%	20%	5%
Structural variant (SV) pipelines	*de novo* Assembly (DN) Guided Assembly (GA)	*de novo* Assembly (DN) Guided Assembly (GA)	Rare Variant Analysis (RVA) Guided Assembly (GA)
Copy Number Variant (CNV) pipelines	*Fractional CNV (fCNV)* *SNP FASST3*	*Fractional CNV (fCNV)* *SNP FASST3*	*Fractional CNV (fCNV)* *SNP FASST3*

*Using only internally verified data

**Reference: Bionano Theory of Operations

Structural Variant Pipelines

There is a new pipeline for structural variant calling, Guided Assembly, parameterized for germline and low allele fraction applications available in versions of Access 1.8.1 / Solve 3.8.1 and later. Guided Assembly is like *de novo* assembly but uses a reference genome instead of pairwise alignments to initiate molecule seeding molecules for iterative consensus map alignment, extension, and merging to generate final consensus maps for SV calling. Compared to the legacy SV pipelines, Guided Assembly provides advantages of improved confidence with SV calls, more accurate estimation of variant allele frequency, and improved performance for structural variant detection. Detailed descriptions of the informatic pipelines are provided in the *Bionano Solve Theory of Operation Structural Variant Calling* (CG-30110).

The legacy DN and RVA pipelines continue to be available in Access to apply to informatic jobs. It is recommended to adopt the new Guided Assembly pipeline to take advantage of the latest advancements with informatics and maximize insights from your OGM data. A summary of performance metrics observed for each SV pipeline is provided the following section.

Performance Metrics by Coverage and Bioinformatic Pipeline

Summary results from performance studies of the Bionano SV pipelines with recommended confidence filters for each variant type to achieve 90% sensitivity and PPV.

Table 4. High Confidence Variant Performance Specifications for Somatic / Low Allele Fraction (LAF) Analysis

Somatic Analysis / Low Allele Fraction (LAF)		
Analysis pipeline	*Rare Variant Analysis*	*Guided Assembly LAF*
Data collected	1.5 Tbp	1.5 Tbp
Coverage setting	400x	400x
Effective coverage of reference (X)	300x	300x
Variant allele fraction	≥5%	≥5%
Insertions/ Deletions	≥5 Kbp	≥3 Kbp
Repeat Expansion/ Contractions*	≥5 Kbp	≥3 Kbp
Duplications	≥70 Kbp	≥30 Kbp
Translocations	≥70^ Kbp	≥70 Kbp
Inversions	≥70^ Kbp	≥50 Kbp

* Performance across the whole genome.
^Confidence filter applied is 0

Table 5. High Confidence Variant Performance Specifications for Constitutional Analysis

Analysis pipeline	***De novo* Assembly *Guided Assembly***	***De novo* Assembly Guided Assembly**	
Data collected	400 Gbp	800 Gbp	
Coverage setting	100x	200x	
Effective coverage of reference (X)	80x	160x	
Variant allele fraction	50%	50%	≥20%
Insertions/ Deletions	≥500^ bp	≥500^ bp	≥100 Kbp
Repeat Expansion/ Contractions*	≥500^ bp	≥500^ bp	≥100 Kbp
Duplications	≥30 Kbp	≥30 Kbp	≥70 Kbp
Translocations	≥70 Kbp	≥70 Kbp	≥70 Kbp

Analysis pipeline	*De novo* Assembly *Guided Assembly*	*De novo* Assembly Guided Assembly	
Inversions	≥50 Kbp	≥50 Kbp	≥100 Kbp

* Performance across the whole genome. See **Table 8** on EnFocus performance for focused capabilities.
^No confidence filter applied

Table 6. CNV Performance Specifications for Cancer Analysis

CNV Algorithm	Fractional CNV	SNP FASST3
Effective coverage of reference (X)	300x	300x
CNV size (at 90% sensitivity & PPV)	gains & losses >2.5 Mbp at 20% VAF	gains & losses >2.5 Mbp at 20% VAF
CNV size (at 90% sensitivity)	gains and losses >500 Kbp at 50% VAF >2.5 Mbp at 20% VAF	gains and losses >400 Kbp at 50% VAF >850 Kbp at 20% VAF
Chromosomal aneuploidy	95% sensitive at 20% VAF	93% sensitive at 20% VAF
Absence of Heterozygosity (AOH)*	Not Detected	> 20 Mbp at 92% sensitivity 25% Aberrant Cell Fraction
Triploidy	Triploidy unable to be called but can be visualized. Genome recentering capability available with VIA software	

*Measured as Aberrant Cell Fraction (ACF), the percent mosaic cellularity of cells harboring the aberration

Table 7. Performance Specifications of CNV calling for Constitutional Analyses

CNV Algorithm	fCNV†	fCNV†	SNP FASST3	SNP FASST3
Effective coverage of Reference (X)	80x	160x	80x	160x
CNV size (90% sensitivity & PPV)	gains and losses >0.6 Mbp at 50% VAF	gains and losses >0.6 Mbp at 50% VAF >3.5 Mbp at 20% VAF	>0.6 Mbp for loss, ›1.0 Mbp for gain at 50% VAF	gains and losses >0.6 Mbp at 30% VAF

†fCNV is run as part of *de novo* RVA, and GA pipelines. Only the CNV pipeline (not the SV pipelines) can find whole chromosome numerical aberrations, terminal deletions, or unbalanced translocations with centromeric breakpoints.

Table 8. EnFocus™ Analyses for repeat expansion/contraction

Application	EnFocus Analysis
EnFocus FSHD	> 1 unit
EnFocus FXS	97% sensitivity 100% PPV
Repeat expansions	Repeat expansions (e.g., *DMPK*, *CNBP*, *ATXN10*) can be inferred and calculated >~600 bp
Computation time^	EnFocus ~1 h, other repeat expansions will be a part of *de novo* and GA pipeline

Table 9. Anticipated Typical Computational Time of Each Pipeline

	de novo	*de novo*	GA	GA LAF	RVA
Computation time^ (effective coverage)	~10 hrs (80x)	~13 hrs (160x)	~8.5 hrs (160x)	~10 hrs (300x)	~5 hrs (300x)

^Single Saphyr Compute, Gen4 with good quality data: Map Rate > 80%, molecule N50 (>20kbp) > 180kbp

Variant Detection Limitations

Important limitations include single nucleotide variants (SNV). In addition, balanced Robertsonian translocations and other balanced translocations where breakpoints are in hundreds of kbp-long, non-unique regions of the genome, cannot be detected. Performance for the detection of terminal deletions and duplications are limited. Simulated datasets indicating high sensitivity for detecting homozygous deletions >100 Kbp in size.

Limit of Detection

The limit of detection of variants is a function of two parameters: depth of usable coverage (estimated by data volume * mapping rate) and the structural variant pipeline that is being utilized.

For all constitutional cases, *de novo* Assembly-based Structural Variant (SV) calling combined with Copy Number Variant (CNV) pipeline calling is recommended. This is run with 400 Gbp of raw data to assure at least 80x effective coverage depth.

For somatic variation, the Rare Variant Analysis (RVA) pipeline is recommended and can be run with 1.5 Tbp of input data to assure at least 300x effective coverage depth.

Masked Regions of the Genome

Parts of the genome are complex and not uniquely assayable by Bionano Optical Genome Mapping (OGM) due to ambiguous alignments, high control sample noise or incorrectly assembled reference genomes. These regions are masked from CNV and/or SV calling and reporting but can be found in certain bed files. The hg19/38/T2T_CHM13_v2.0 CNV Masks and the hg19/38/T2T_CHM13_v2.0 DLE-1 SV Mask are preloaded in Bionano Access and are plotted in Appendix B of *Bionano Solve Theory of Operation Structural Variant Calling* (CG-30110).

Structural Variant versus Copy Number Variant Calls

Every case is run through a computation protocol that includes SV calling (fusions) and CNV calling (coverage depth). SV calling refers to the detection of changes in the structure of the genome by detecting abnormal fusions and truncations (terminal deletions), which includes CNVs when they occur with an abnormal fusion (i.e., interstitial deletions and duplications). For CNVs involving whole chromosomes (aneuploidy), no abnormal fusion will be present; only a dosage change will be displayed, therefore, it cannot be detected as an SV. In these cases, the abnormality will be detected using the CNV calling tool.

There are also some cases where an abnormal fusion is not detectable by the SV pipeline because it occurs in an unmappable region such as the centromere, the short arm of acrocentric chromosomes, or exceptionally long low copy repeats (LCRs). In these cases, deletions and duplications will usually still be called with the CNV tool only.

Difficult to Detect Regions/Variants

Some multicopy genes or homologous genes may be difficult to unambiguously interpret. These may include CYP21A2, HBA1/2, SMN1/2, PMS2/CL, and STRC. Deletions and duplications may be associated with specific genes based on location, but gene conversion could be undetectable. Other loci affected by segmental duplications include 16p11.2 distal deletion/duplication, 16p12.1 deletion, 15q11.2 BP1-BP2 deletion/duplication, KANSL1, CHRNA7 (intragenic), NPHP1 carrier, regions completely within PAR1/PAR2, and 1q21.1 distal deletion/duplication. These may need to be manually assessed.

Table 10. Example Cell Lines Used to Evaluate the System

Sample	Disorder	Variant Class	Description
Benchmark Cell Lines			
GM24385	n/a	n/a	
HG00733	n/a	n/a	
Cell Lines Relating to Constitutional Disorders			
GM04403	carrier Emmanuel syndrome	translocation	Balanced carrier of a recurrent translocation t(11;22), mother of GM04370
GM16736	Deafness with DNA repair deficiency	translocation	46, XY, t(9;22)(p22;q11.2)
GM21074	Developmental delay	inversion	Inv(2p23-q31)
GM01695	DMD	translocation	46, X,t(X;11)(Xqter>Xp21::11q13>11qter;11pter>11q13::Xp21>Xpter)
GM05113	DMD	intragenic deletion	46, XY.arr Xp21.1(31869808-32028005)x0
GM04370	Emmanuel syndrome	translocation - unbalanced	47,XX,+der(22)(22pter>22q11:: 11q23>11qter)mat, affected daughter of GM04403
GM14266	Micrognathia	inversion	Inv(4q34.2-35.2)
GM21891	Prader Willi	translocation	46,XY,t(4;15)(q27;q11.2)
GM04927	Down syndrome	Trisomy	47,XY,+21[24].arr(21)x3
GM50192	Cri-du-chat syndrome	Terminal deletion	46,XX,del(5)(:p13>qter).ish del(5)(D5S23-,D5S721-)
GM04376	Hydrocephalic; stillborn	Triploidy	69, XXX
Repeat Related Cell Lines			
ND07669	ALS	repeat expansion	ALS - C9orf72 expansion

Sample	Disorder	Variant Class	Description
GM04025	Fragile x	repeat expansion	FMR1 Coriell-645 repeat units
GM07861	Fragile x	repeat expansion	FMR1 Coriell-351-400 repeat units
GM09237	Fragile x	repeat expansion	FMR1 Coriell-931-940 repeat units
GM20232	Fragile x	repeat expansion	FMR1 Coriell-46 repeat units
GM20233	Fragile x	repeat expansion	FMR1 Coriell-117 repeat units
GM20239	Fragile x	repeat expansion	FMR1 Coriell-20/183-193 repeat units
GM16250	FSHD	repeat contraction	FSHD
GM17868	FSHD	repeat contraction	FSHD

Table 11. Workflow and Throughput Scenarios

Constitutional/Germline (Human Blood) 400 Gbp	Somatic SV Calling (Human Heparin BMAs) 1.5 Tbp
• 1 Saphyr® System • Typical 5-day weekly workflow with one laboratory technician generates 400Gbp coverage/sample, leading to an average of twenty samples/week (estimate). • Typical 7-day weekly workflow with three laboratory technician, 24 hours per day, generates 400Gbp coverage/sample, up to thirty-four samples/week.	• 1 Saphyr® System • Typical 5-day weekly workflow with one laboratory technician generates 1.5Tbp coverage/sample, average of estimated fifteen samples/week (after week one). • Typical 7-day weekly workflow with three laboratory technician, 24 hours per day, generates 1.5Tbp coverage/sample, maximum of twenty four samples/week.

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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