# Bionano Saphyr® System Application Specifications

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bionano

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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 |

# 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 tube)<br><br>***Available with Generation 2 Kits*** | 650µL | Aliquot 650µL ~ 1mL fresh blood to each tube.<br><br>Require min. of one tube, two tubes preferred.<br><br>Max. 5 days at 4°C or 66 hours at RT post draw including shipping and handling.<br><br>Ship with cold packs if fresh.<br>*Ship on dry ice if frozen.* |
| Fresh and Frozen Human Blood (Heparin tube + DNA stabilizer)<br><br>***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.<br><br>Max. 3 days at 4°C post draw including shipping and handling.<br><br>Require min. of one tube, two tubes preferred.<br><br>Ship with cold packs if fresh.<br>*Ship on dry ice if frozen.* |
| Cell lines or other purified cells<br><br>***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.<br><br>**NOTE: LCLs or other cell lines are compatible. Not all cell types have been evaluated**. |
| **Bone marrow aspirate (BMA) (EDTA tube)**<br><br>***Available with Generation 2 Kits*** | 0.8 mL | Samples should be frozen within 24 hours of aspiration, keep at 4°C 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. |

| Samples | Minimum Sample Requirements | Sample Prep and Quality Requirements |
|---|---|---|
| Bone marrow aspirate (BMA) (Heparin tube + DNA Stabilizer)<br><br>***Available with Generation 2 Kits*** | 0.8 mL | |
| Tissue Biopsies – Human tumor tissue (breast, liver, lung colon*, kidney*, bladder*, brain*, ovary*, prostate*, thyroid*)<br><br>*Sample type has been tested but not validated | Min. of 10mg required, 30mg preferred | Freshly frozen and stored at -80°C<br><br>Ship on dry ice |
| Amnio/CVS | Min. of 1.0 million variable cells | Fresh or cryopreserved<br><br>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: prepare live cells or frozen cell pellets in DNA Stabilizer for submission to the Services Lab.
- CG-30179 — Blood Shipping Instructions: prepare fresh blood or frozen blood aliquots in EDTA or DNA Stabilizer for submission to the Services Lab.
- CG-30358 — Bone Marrow Aspirate Shipping Instructions: prepare bone marrow aspirates to be frozen in EDTA or Heparin for submission to the Services Lab.
- CG-30186 — Tissue and Tumor Shipping Instructions: prepare 10 mg tissue and tumor portions for submission to the Services Lab.
- TECHN-00008 — Bionano Pre Methanol Glacial Acetic Acid Cell Preparation Tech Note.

**Table 3**. Data Quantity and Quality*

| | 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) | *de novo* Assembly (DN) | Rare Variant Analysis (RVA) |
| **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

# 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

| Application | Constitutional DNA Analysis | | | Somatic Analysis / Low Allele Fraction (LAF) |
|---|---|---|---|---|
| **Analysis pipeline** | *De novo* Assembly | *De novo* Assembly | | *Rare Variant Analysis* |
| **Data collected** | 400 Gbp | 800 Gbp | | 1.5 Tbp |
| **Coverage setting** | 100x | 200x | | 400x |
| **Effective coverage of reference (X)** | 80x | 160x | | 300x |
| **Variant allele fraction** | 50% | 50% | ≥20% | ≥5% |
| **Insertions/ Deletions** | ≥500 bp | ≥500 bp | ≥5 Kbp | ≥5 Kbp |
| **Repeat Expansion/ Contractions*** | ≥500 bp | ≥500 bp | ≥5 Kbp | ≥5 Kbp |
| **Duplications** | ≥30 Kbp | ≥30 Kbp | ≥70 Kbp | ≥70 Kbp |
| **Translocations** | ≥70 Kbp | ≥70 Kbp | ≥70 Kbp | ≥70^ Kbp |
| **Inversions** | ≥50 Kbp | ≥50 Kbp | ≥100 Kbp | ≥70^ Kbp |

All values are based on 90% sensitivity and PPV.
* Performance across the whole genome. See table 8 on EnFocus performance for focused capabilities.
^Confidence filter applied is 0

**Table 5**. 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 6**. 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* and RVA pipelines. Only the CNV pipeline (not the SV pipelines) can find whole chromosome numerical aberrations, terminal deletions, or unbalanced translocations with centromeric breakpoints.
N/A= Not Assessed

**Table 7**. EnFocus™ Analyses for repeat expansion/contraction

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

**Table 8**. Anticipated Typical Computational Time of Each Pipeline

| | *de novo* | *de novo* | RVA |
|---|---|---|---|
| Computation time^ (effective coverage) | ~8 hrs<br>(80x) | ~10 hrs<br>(160x) | ~5 hrs<br>(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 vs. 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 9**. 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 |
| 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 |

| Sample | Disorder | Variant Class | Description |
|---|---|---|---|
| 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 10**. Workflow and Throughput Scenarios

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

| TYPE | CONTACT |
|---|---|
| **Email** | support@bionano.com |
| **Phone** | Hours of Operation:<br>Monday through Friday, 9:00 a.m. to 5:00 p.m., PST<br>US: +1 (858) 888-7663 |
| **Website** | www.bionano.com/support |
| **Address** | Bionano, Inc.<br>9540 Towne Centre Drive, Suite 100<br>San Diego, CA 92121 |