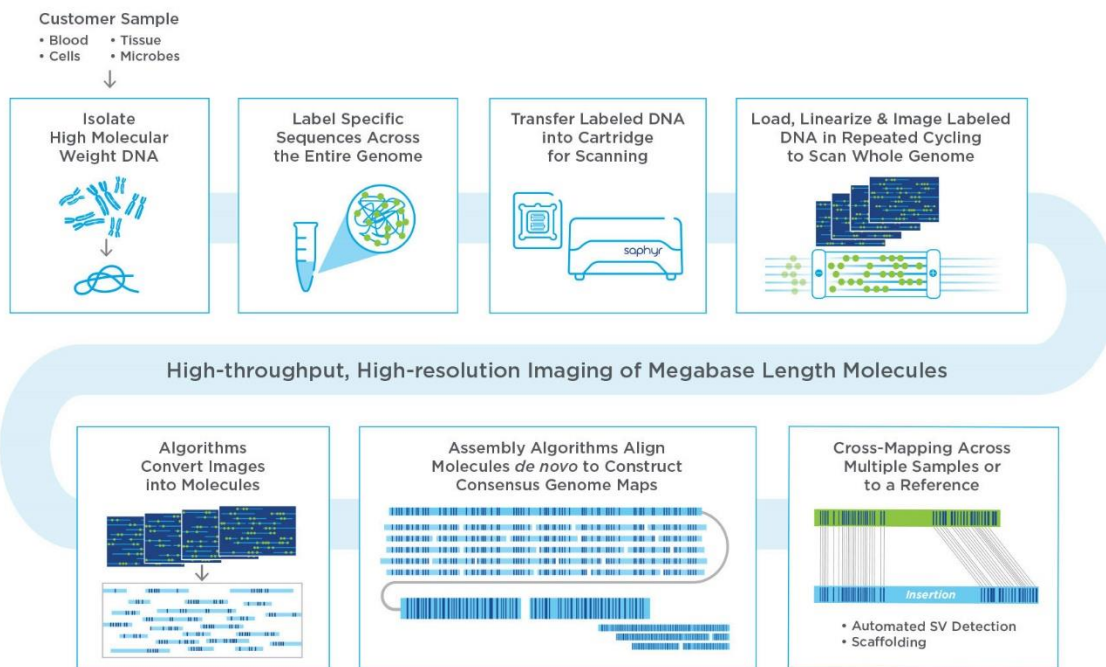# BioNano Submission Instructions
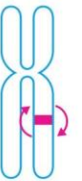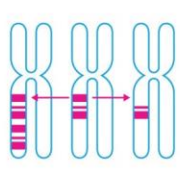
Technology Review:

Bionano Saphyr instrument offers optical mapping technology that uses DLE-1 enzyme based labeling of specific sequences in the genome and then mapping the pattern of the labels on the long DNA strands (up to Mbp in size). This allows creation of Bionano genome maps that enable a variety of analyses, including hybrid scaffolding for *de novo* assembly of genomes and structural variation detection.

Next-Generation Sequencing (NGS) technologies are essential for nucleotide-level information but are limited beyond that resolution as the fragmented reads are too short to infer the underlying genome structure. When constructing *de novo* genomes or looking at tandem duplication regions short-read (Illumina) or long-read (Oxford Nanopore or PacBio) sequencing alone is not sufficient to construct a contiguous and accurate assembly of repetitive structures in the genome. To detect variation and architecture on a larger scale, use Bionano genome mapping, the technology that directly measures the genome with a resolution matching the variation in question—hundreds of kilobases, not hundreds of

base pairs. Adding Bionano maps to sequencing data enables one to view a whole genome, with all its features in context and functional relationships described—across megabases.

Homozygous insertions/deletions larger than 500 bp	Balanced and unbalanced translocations larger than 50 kbp	Inversions larger than 30 kbp	Duplications larger than 30 kbp	Copy number variations larger than 500 kbp
99% sensitivity	95% sensitivity	99% sensitivity	97% sensitivity	97% sensitivity

False positives below 2%

Read more at the Bionano website – https://bionanogenomics.com/

Submission Requirements

Right now, HTSF offers the preparation of samples for Bionano Saphyr from cells and blood. Bionano technology requires long, unbroken DNA strands, larger than can be achieved with most HMW DNA extraction methods. We use Bionano made Blood and Cell kit to extract DNA. The kit is made for mammalian cells but any cells can be used if they can be lysed by denaturation e.g. protoplasts.

1. Fresh cells

Samples should be submitted in 1.7 ml Eppendorf tubes with 2 million cells suspended in 1 ml of growth medium. If possible, make sure the cells are actively growing with high viability as this maximizes quality and size of isolated gDNA.

Fresh cells must be processed immediately and the drop-off needs to be scheduled beforehand. Please contact HTSF so the date and time can be coordinated.

2. Frozen cells.

Before preparing frozen cells please contact HTS to receive Cell Buffer and DNA Stabilizer from the kit. Follow the Bionano procedure for freezing cells in the file -> Preparing Frozen Cell Pellets

a. Put cells in a -80°C freezer for long term storage.

b. Do not thaw aliquot from -80°C until proceeding with gDNA isolation.

c. Bring to HTSF on dry ice.

3. Fresh blood

This protocol requires blood drawn into EDTA tubes stored at 4°C and processed within 4 days of the draw date. This protocol was tested using human blood so it's based on WBC count. It requires 2 million WBC cells. If you do not have a way to measure number of WBC HTSF can do it. Generally, 650 µl of human blood should be more than enough but other organisms may differ.

4. Frozen blood.

The gDNA content is obtained from the white blood cells (WBC). The volumes and amounts used for this process assume normal patient human whole blood drawn into an EDTA tube. 2 blood aliquots (650 µl) should be frozen (-80°C) in separate tubes within 4 days from draw date and stored without thawing until gDNA isolation. Typically, only one aliquot will be required for this protocol, with the second serving as a backup for any failure.

a. Mix fresh human whole blood thoroughly at room temperature to ensure for good uniformity (15 minutes on a rocker at room temperature).

b. Transfer two 650 µl aliquots into DNAse/RNAse free 1.5 ml tubes.

c. Cap tubes and immediately move aliquots to -80°C for long term storage.

d. Do not thaw aliquot from -80°C until proceeding with gDNA isolation.

e. Bring to HTSF on dry ice.

5. All samples - If the samples are non-human or if you want to use a different reference than standard hg19 or hg38 please submit the reference genome you want to use in fasta format before the first run. The samples can be run without reference but even for de novo genome scaffolding it is recommended to use reference for the run even if it is poor quality. Please also submit genome size for your sample.

6. Please specify the number of flowcells to be run per sample. Maximum output of one flowcell is currently 1300 Gbp. Generally, for human sized genomes 1 flowcell of 600 Gbp is recommended and should be enough for most analysis. The full capacity 1300 Gbp run is only recommended for highly

heterogenous samples (mixed genomes expected) like mixed cancer samples. Please mark in the submission form if your sample is the latter.

Submission form -> HTSF-Bionano-Submission-Form_april202

Please send electronic version of submission form before bringing samples. Bring printed out copy with the samples when submitting them for processing.

Data output

The Bionano Access software is available for download and installation by any user - https://bionanogenomics.com/support/software-downloads?_sft_download-type=saphyr. It allows for manipulating and visualizing the data. HTSF has also Bionano Solve installed and we can run analysis of the data.

1. The run creates sample molecule files for each flowcell (our current system uses 2 flowcell chips). If you request your samples to be run on more than one flowcell (or if run needs to be stopped and restarted) multiple molecule files are generated for each sample. Those files can be merged creating merged molecule file. The molecule files can be available by request.

2. By default molecule files are run by through De Novo Assembly pipeline. The result of that analysis is the main data out exported for delivery. You can import it into your version of Bionano Access.

3. We can also run Variant Annotation Pipeline for the human samples that allows to compare the sample to one (e.g. mutant vs wt) or two reference (child vs parents) samples. If you want this analysis to be run contact us to specify which samples you want to compare.

4. Let us know if you need any other analysis - https://bionanogenomics.com/products/bionano-data-solutions/