bionano
GENOMICS

GET IT RIGHT

Bionano Advances for Chromosome-level Haplotype-resolved *de novo* Genome Map Assemblies

Bionano Workflow Today

DLS

NLRS

Customer Sample

Blood Cells Tissue Microbes

Isolate High Molecular Weight DNA

Label Specific Sequences Across the Entire Genome

Transfer Labeled DNA into Cartridge for Scanning

saphyr

Load, Linearize & Image Labeled DNA in Repeated Cycling to Scan Whole Genome

HIGH-THROUGHPUT, HIGH-RESOLUTION IMAGING OF MEGABASE LENGTH MOLECULES

Algorithms Convert Images into Molecules

Assembly Algorithms Align Molecules *de novo* to Construct Consensus Genome Maps

Cross-Mapping Across Multiple Samples or to a Reference

Insertion

- Automated SV Detection
- Scaffolding

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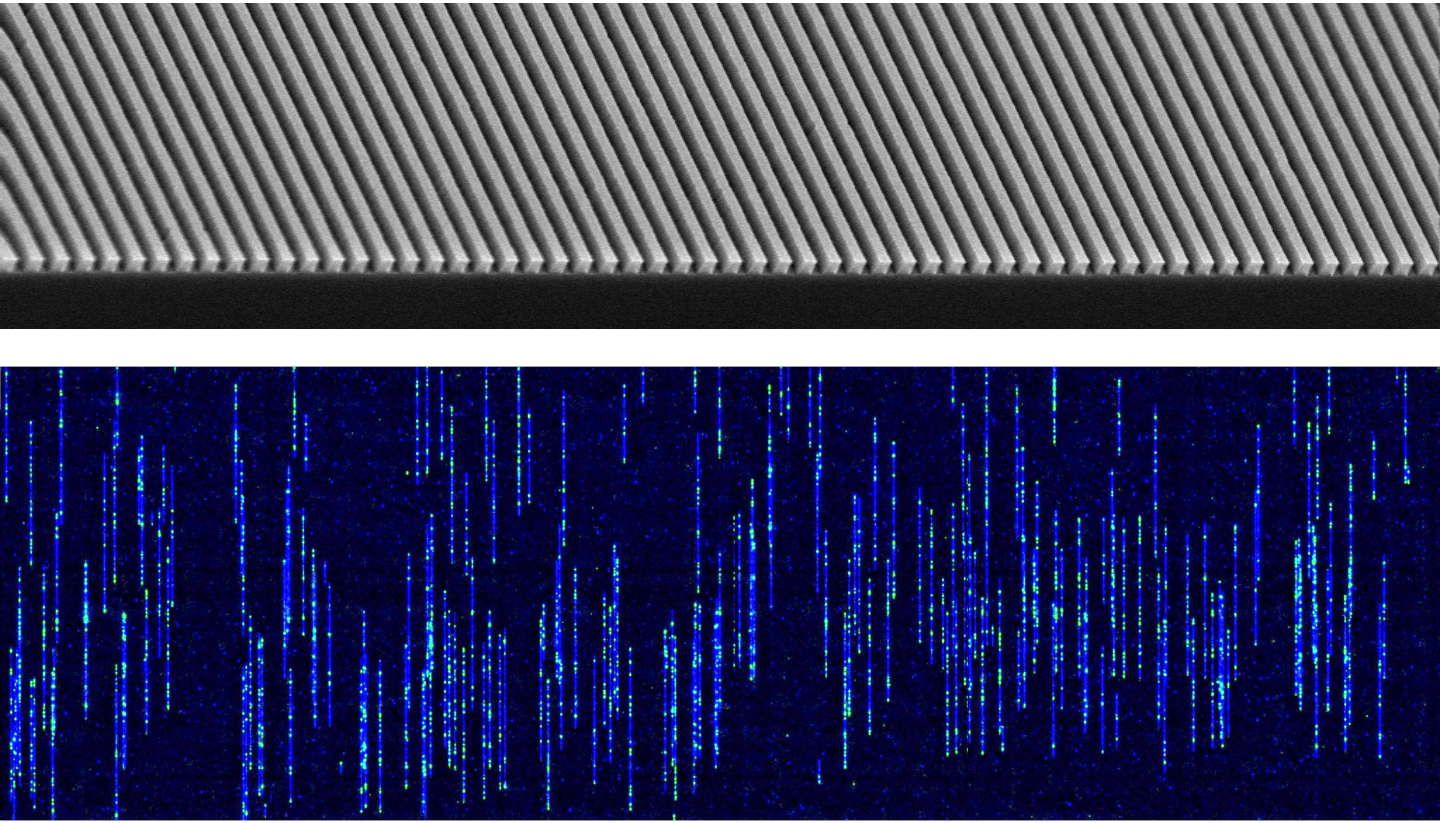
$500 per genome

640 Gbp throughput/24hrs

>500 bp all types of SVs

Flow Linearized DNA in a Nanochannel array



Bionano Access Assembles *de novo* Genome Maps

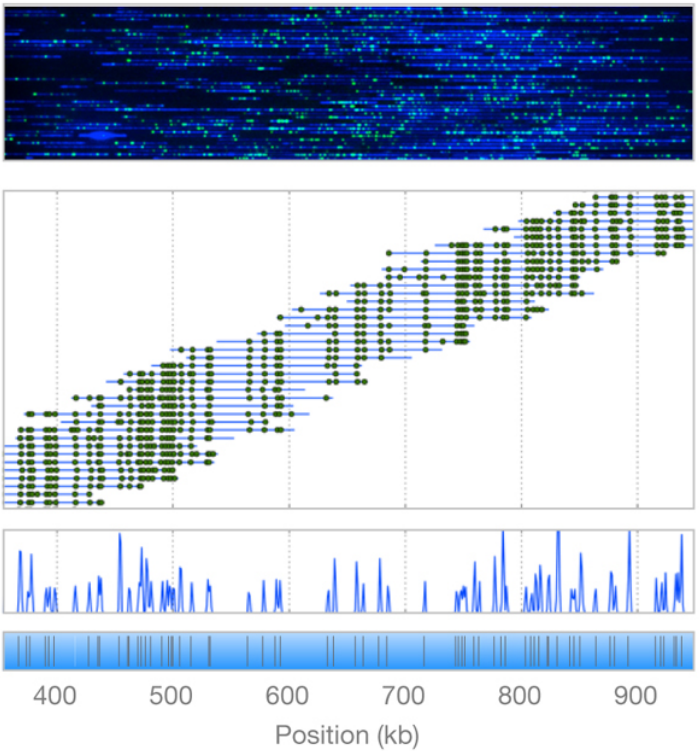
Raw Image Data

Conversion to Molecules

Population of Molecules

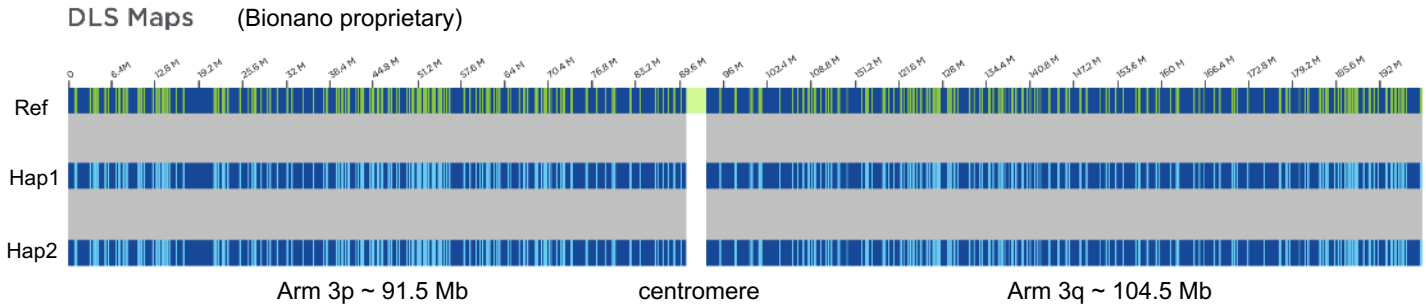
Aggregation & Assembly

Consensus Genome Map



Chromosome Arm to Full Chromosome Length Maps

Human chromosome 3:



De novo Assembly of Diverse Genomes

- A representative subset of genomes mapped on the Saphyr system

Sample	Molecule N50 (kbp)	Bionano Map N50 (Mbp)
NA12878	293	55.9
Human Fresh Blood	307	56.9
Ferret	262	66.1
Mouse	280	101
Pig	335	65.2
Blackbird	243	21.6
Hummingbird	310	38.7
Kakapo	247	69.3
Fish	245	22.3
Brassica	270	12.4
Durum Wheat	364	13
Farro	300	32.7
Maize B73	260	100
Soybean	246	23
Strawberry	241	13.3
Sunflower	317	178.3

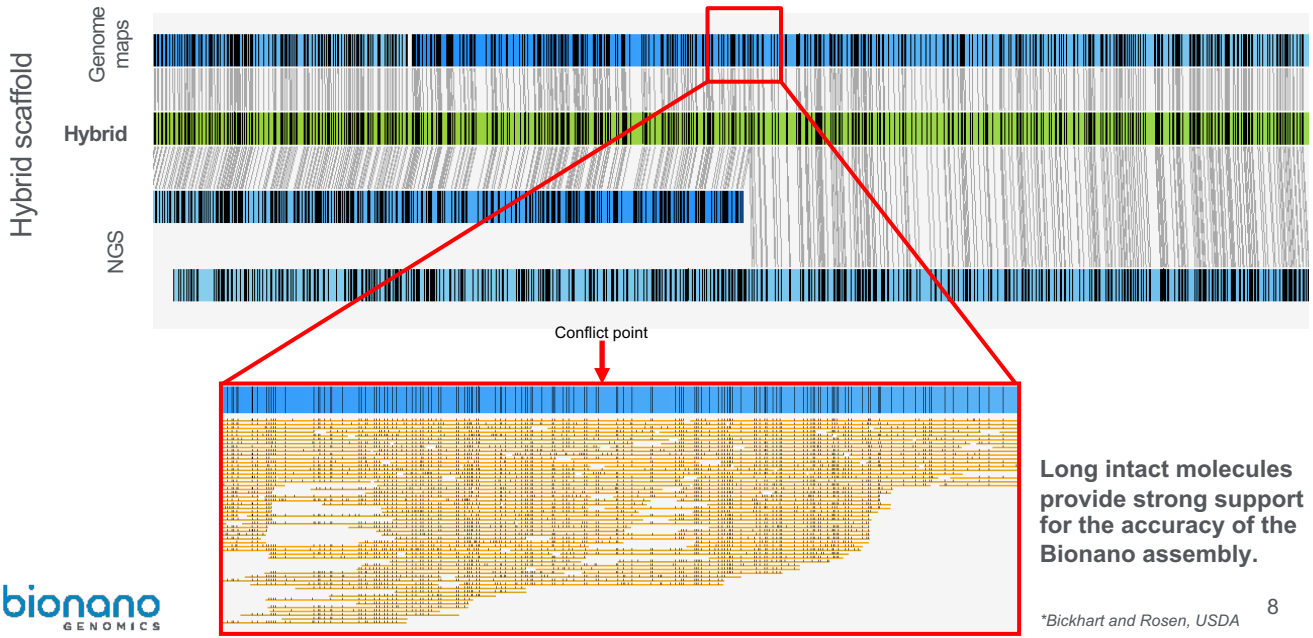
Highest Quality Long Scaffolds

Species	NGS N50 (Mbp)	Scaffold N50 (Mbp)	% NGS Anchored
Maize B73	1.19	100	99.5%
Sorghum	3.05	34	95.9%
Kakapo	4.34	71	95.9%
Blackbird	1.47	42	95.0%

- Enabling projects like the maize pangenome – Corteva; Kelly Dawe
  - High quality
  - High contiguity
  - Low cost
  - Fast turn-around

Highest Quality Hybrid Scaffolding

- Sequence assembly error flagging and correction
  - Chimeric NGS contigs
  - NGS contig overlaps
- Accurate N-gap sizing

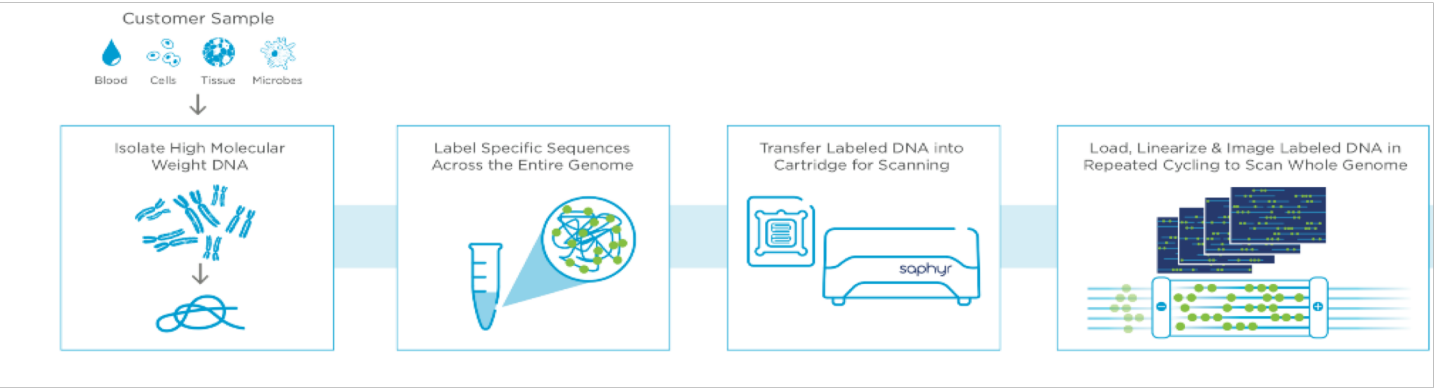


Long intact molecules provide strong support for the accuracy of the Bionano assembly.

**Bickhart and Rosen, USDA*

Bionano Advancements Coming Soon

Tissue preservation and
small input amounts (10mg)



Automatable in-solution UHMW DNA isolation for 12 samples/day

- 1st: blood and cells

Significant increase in data throughput and yields

- Scale to handle the wheat pangenome

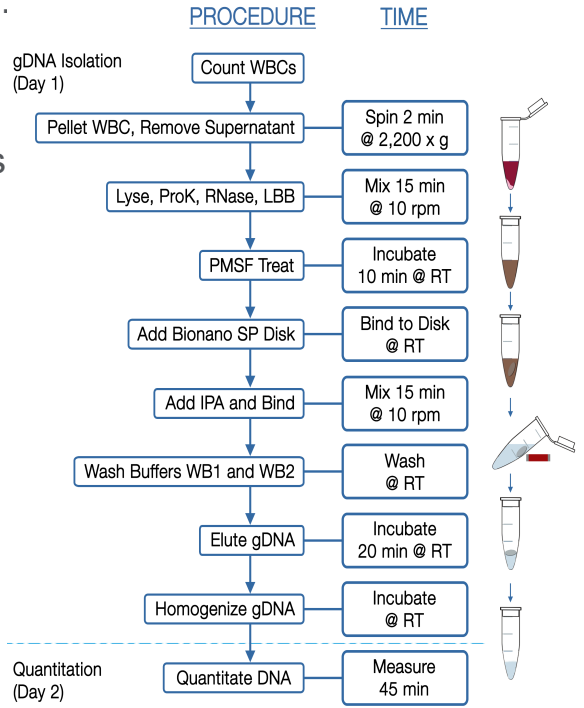
BionanoPrep for low input and chemically preserved tissue

Tissue Type	Storage Method	Prep Method	Mass [mg/prep]	# of Plug	DNA Con. [ng/µl]	DNA Yield [µg/10mg tissue]	N50 >20kbp	N50 >150kbp	Avg PLV [%]
BN-Liver	Freshly frozen, -80°C	BNG Soft Tissue SOP	~10	1	88.1	5.7	194	265	6.7
BN-Liver	Freshly frozen, -80°C	BNG New Prep Kit	~10	2	64.1	8.3	198	256	6.8
BN-Lung	Freshly frozen, -80°C	BNG New Prep Kit	~10	1	204.5	13.3	198	261	6.3
BN-Colon	Freshly frozen, -80°C	BNG New Prep Kit	~10	1	88.1	5.7	122	226	6.0
BN-Bladder	Freshly frozen, -80°C	BNG New Prep Kit	~10	1	185.0	12.0	195	283	4.8
BN-Prostate	Freshly frozen, -80°C	BNG New Prep Kit	~10	1	62.3	4.0	150	258	7.0
HST1-1	Freshly frozen, -80°C	BNG New Prep Kit	~10	1	50.9	3.3	170	246	3.1
HST1-2	AllProtect, RT	BNG New Prep Kit	~10	1	155.5	10.1	150	248	3.5
HST1-3	PAXgene, RT	BNG New Prep Kit	~18	1	42.3	2.7	179	267	4.1
HST2-1	Freshly frozen, -80°C	BNG New Prep Kit	~10	1	72.3	4.7	172	279	2.9
HST2-2	AllProtect, RT	BNG New Prep Kit	~10	1	52.3	3.4	150	276	4.3
HST2-3	PAXgene, RT	BNG New Prep Kit	~18	1	32.0	2.1	133	239	4.8
HST3-1	Freshly frozen, -80°C	BNG New Prep Kit	~10	1	38.7	2.5	153	214	2.6
HST3-2	AllProtect, RT	BNG New Prep Kit	~10	1	73.4	4.8	184	299	3.9
HST3-3	PAXgene, RT	BNG New Prep Kit	~18	1	33.2	2.2	157	253	3.4

BN: Brown Norway; HST: Human Solid Tumor

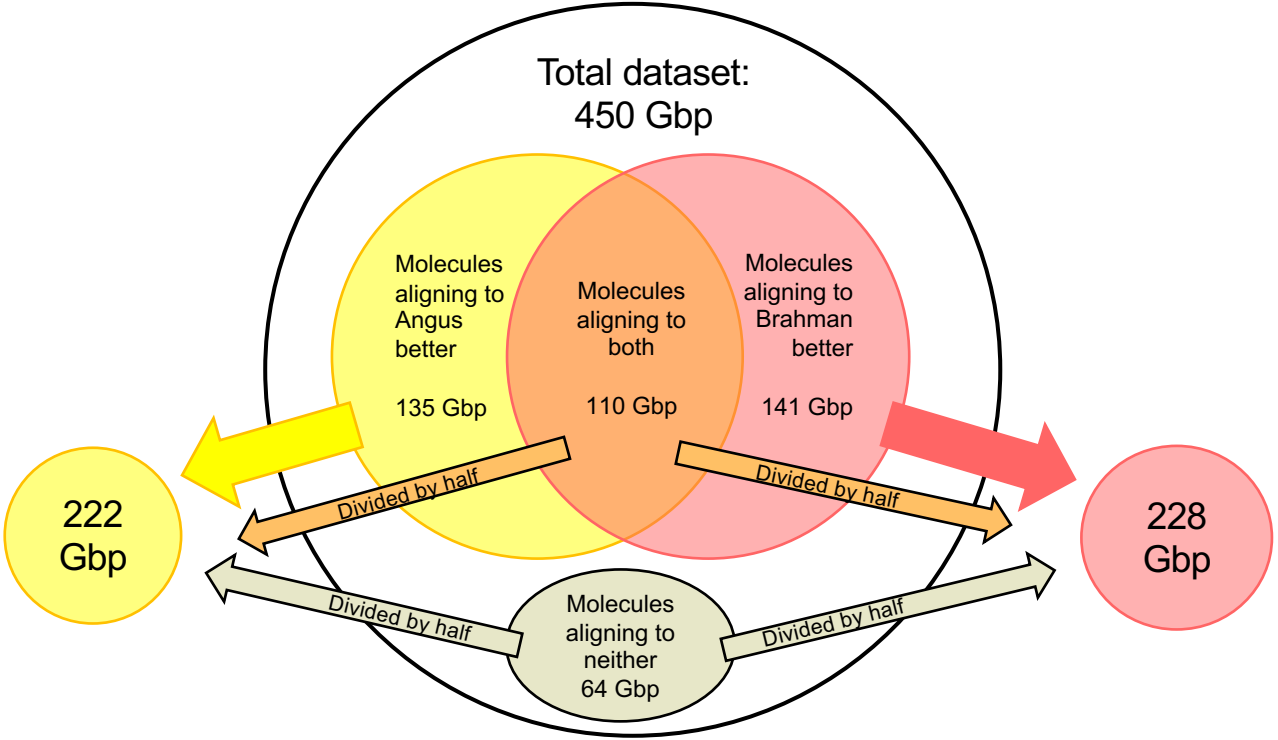
Bionano Prep SP – New (fast) DNA Isolation Kit

- Manually process up to 6 samples in <3hrs.
  - Validated for 12 samples per day
- First available for fresh/frozen blood & cells
  - Will be porting other sample types over time
- Currently at early access sites
- Automatable – future work



Resolving heterozygosity

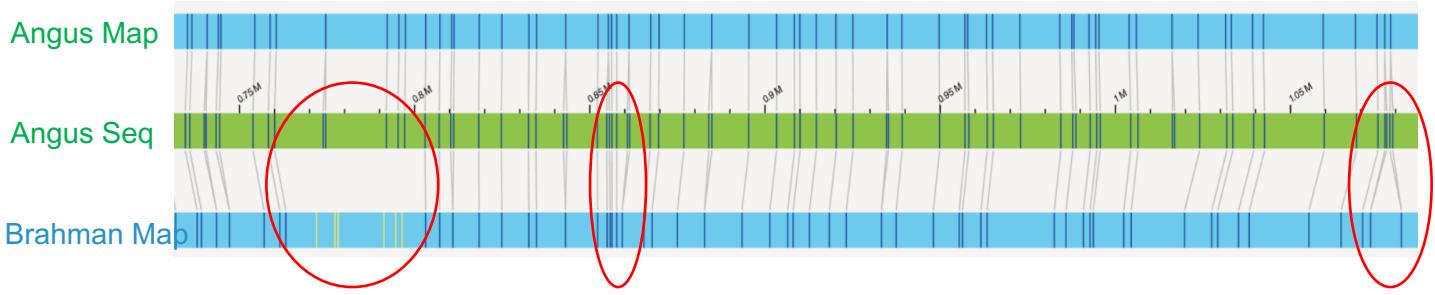
Trio binning



- Molecules were aligned to the Angus and Brahman sequences.
- Based on the alignment score of each molecule to each sequence, they were assigned to the pool (Angus/Brahman) that they have better alignment score with.
- To maintain equivalent coverage throughout each genome, molecules that aligned to both sequences with similar scores (difference <2) were split by half and assigned to each pool (Angus/Brahman).
- Molecules that did not align to either sequence were also split by half and assigned to each pool (Angus/Brahman).

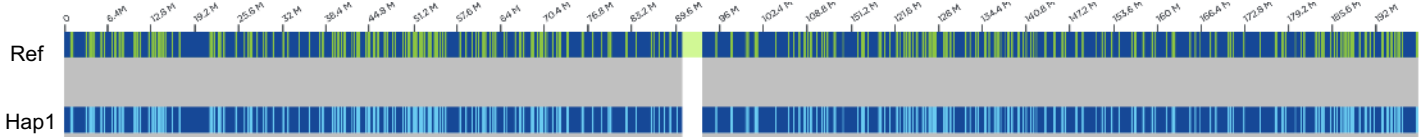
Trio binning Angus and Brahman cattle

	Expect	Trio binning	
		Angus	Brahman
Total length	~3 Gbp	2.79 Gbp	2.87 Gbp
Map N50		33.97 Mbp*	28.62 Mbp*

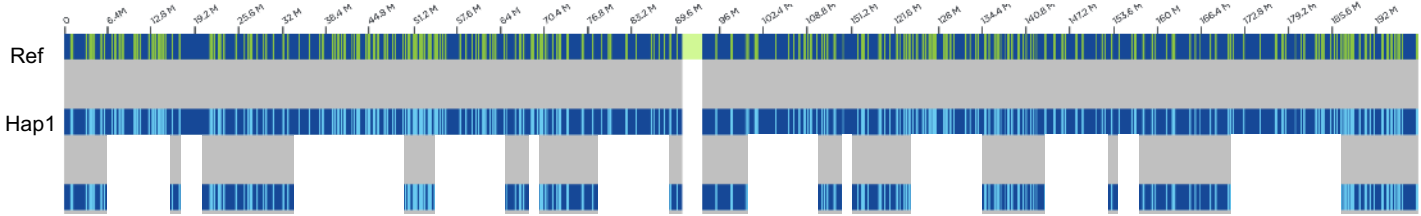


Resolving heterozygosity without requiring a trio

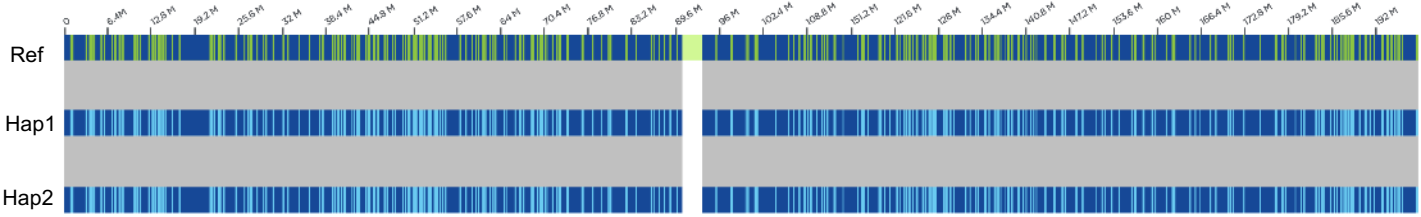
No haploid assembly (recommended for scaffolding) -> 1N



Extend and split – resolving large (>30kbp) het. sites -> 1-2N



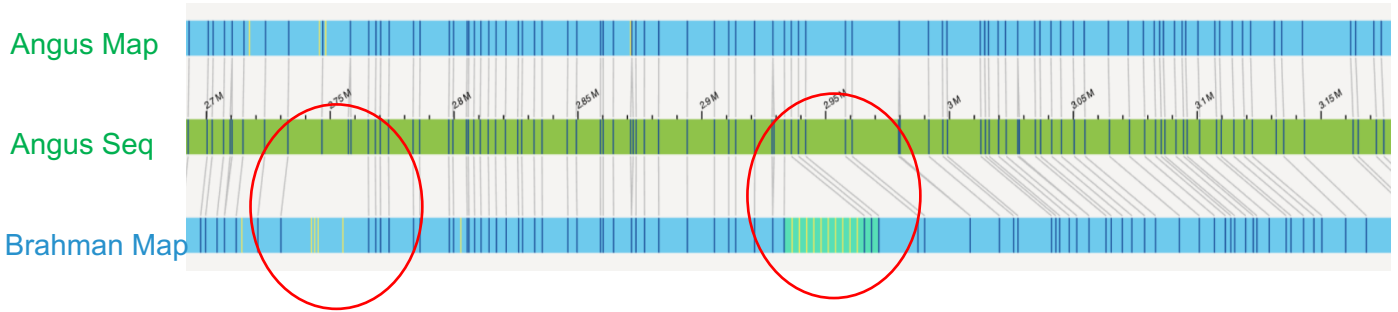
Extend and split + haplotype refinement - resolves >500bp het. sites (for human SV studies) -> 2N



Phasing is based on SVs at molecule ranges

Assembly of Angus and Brahman cattle

	Expect	Trio binning Angus	Trio binning Brahman	ES	ES + Hap refinement
Total length	~3 Gbp	2.79 Gbp	2.87 Gbp	3.37 Gbp	5.01 Gbp
Map N50		33.97 Mbp*	28.62 Mbp*	71 Mbp	69 Mbp
Ins-Del vs Angus		587	NA	3727	5101



Caution: Haplotype refinement is only designed for human SV studies, we have seen over splitting with some non-human genomes. Optimization of parameters may be needed.

De novo haplotype map resolution is possible – how about scaffolding

- Sequence assembly should have both alleles represented
- Map and sequence assembly should be put into phase, can be partially handled by Bionano hybrid scaffolder
- Bionano hybrid scaffold can perform diploid hybrid scaffold only with high heterozygosity
  - **It may be adaptable for diploid scaffold**
- Diploid aware scaffolder may be needed to optimize scaffolding

In Conclusion….

- Many improvements in the development pipeline
  - Iterative products
  - Workflow improvements
  - Technology advancements

- To learn more
  - Stop by the Bionano Genomics Booth #225
  - Visit our website at www.bionanogenomics.com
  - Follow us on social media

Thank you for your attention!

VERTEBRATE GENOMES PROJECT

DIGITAL NOAH'S ARK GENOME LIBRARY

Bionano Advances for Chromosome-level Haplotype-resolved *de novo* Genome Map Assemblies

Alex Hastie, PhD Director, Customer Solutions | Bionano Genomics
ahastie@bionanogenomics.com

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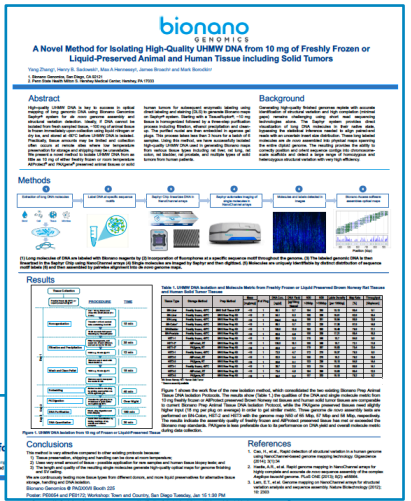
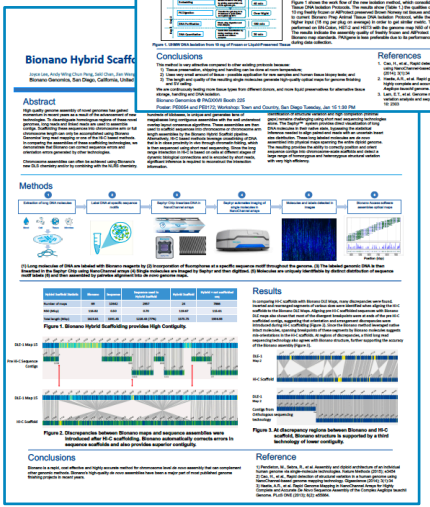
Bionano Posters Presentations @PAGXXVII

A Novel Method for Isolating High-Quality UHMW DNA from 10 mg of Freshly Frozen or Liquid-Preserved Animal and Human Tissue including Solid Tumors (**#PE0172**)

Bionano Hybrid Scaffold Assemblies Provide High Contiguity and Accuracy (**#PE0054**)

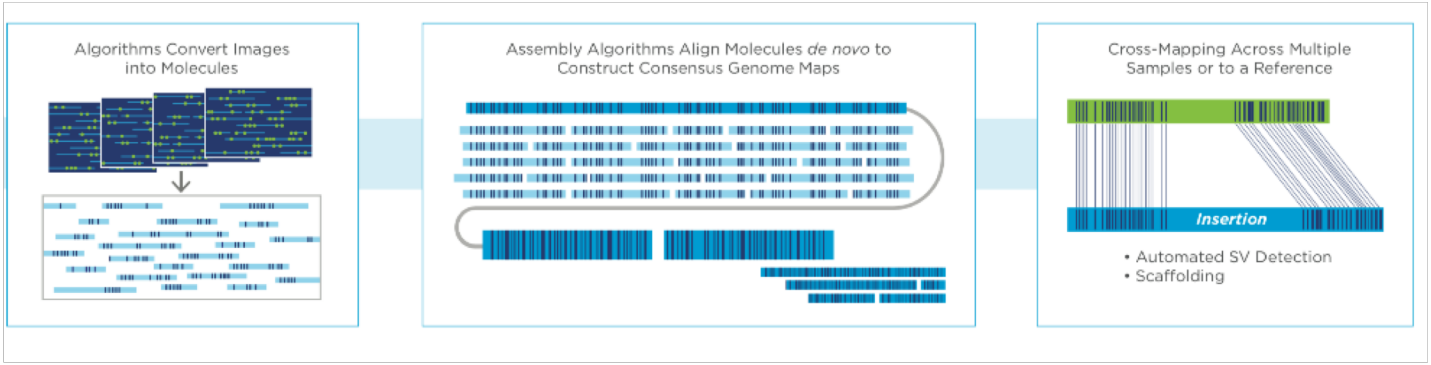




Print copies available in Booth #225

Electronic copies available via survey form request

Bionano Advancements Coming Soon



Bionano Compute On-Demand

- cost-effective; secure; easy

SVs from low allelic fractions

PAG variant analysis workflows

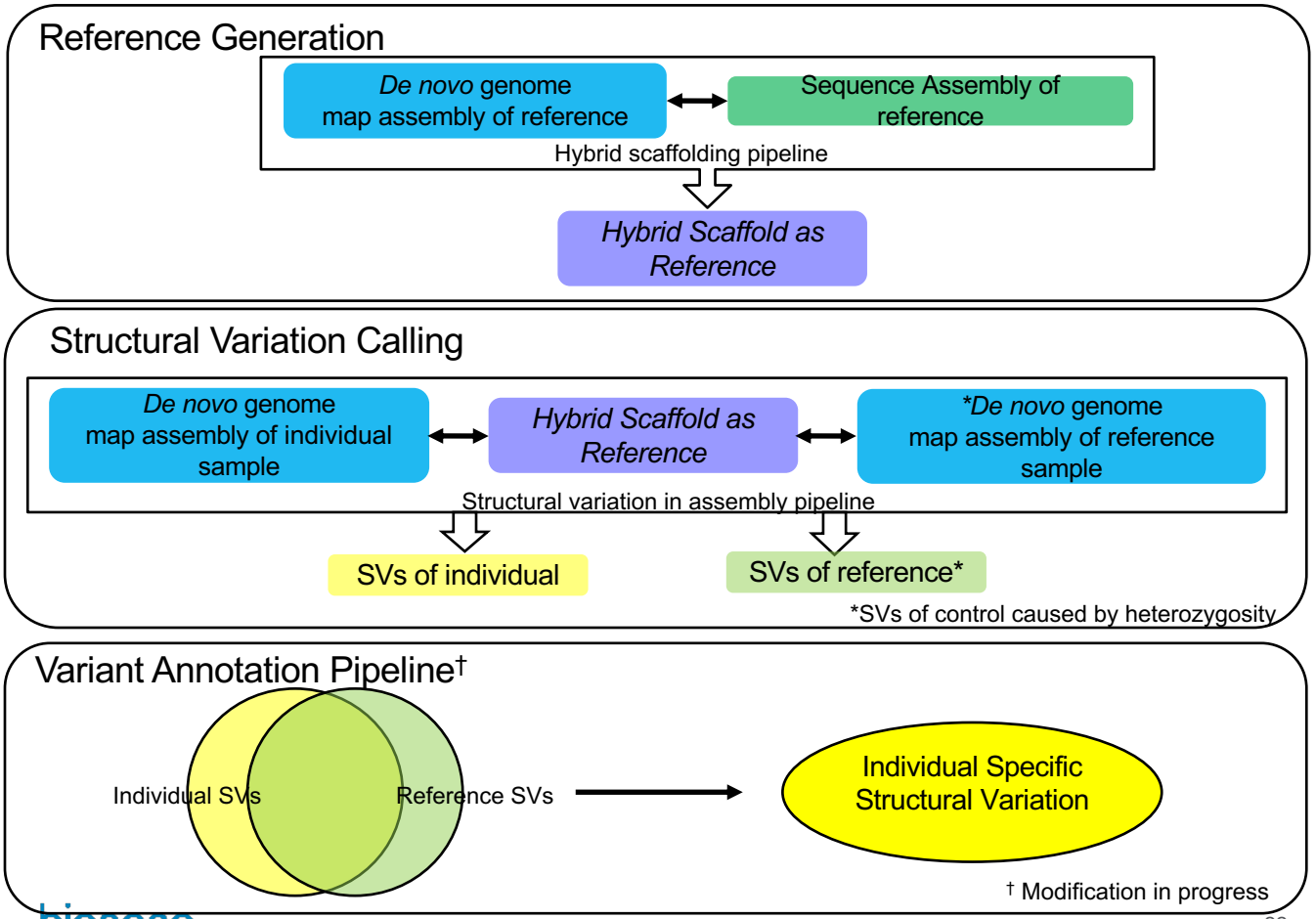
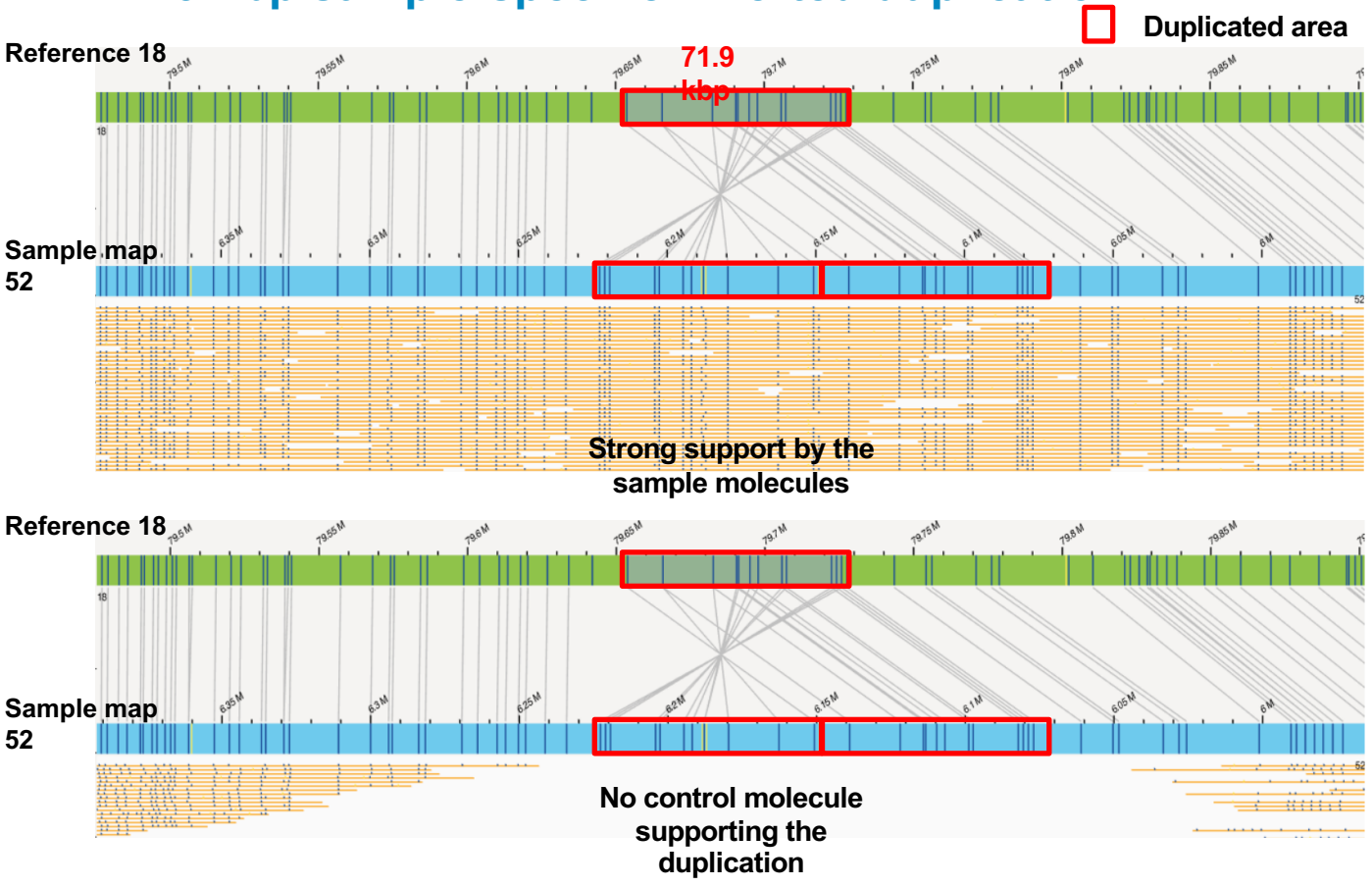
- traits in plants
- therapeutic response in model organisms

Lower $ per genome

Higher throughput/24hrs

Rarer all types of SVs

Analysis Workflow: Reference Generation and Sample Specific Structural Variation



A 71.9 kbp sample-specific inverted duplication