Detection of Genomic Structural Variation in Primary and Metastatic Ovarian Cancer Using a Novel Genome Wide High-Resolution Optical Mapping Approach

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Abstract

Introduction: Next generation sequencing (NGS) is a powerful method to detect small genomic alterations such as single nucleotide variations and small deletions, however, it is limited in its ability to detect large scale genomic structural variations that could play significant roles in cancer. The Bionano Genomics Saphyr® single molecule optical mapping system is a novel approach that allows rapid and efficient large scale structural evaluation of the genome which could complement NGS analysis. We report utilization of this novel genomic analysis approach to evaluate structural changes in ovarian carcinoma specimens, including 2 cases with matched primary tumor and metastatic cells in associated malignant ascites.

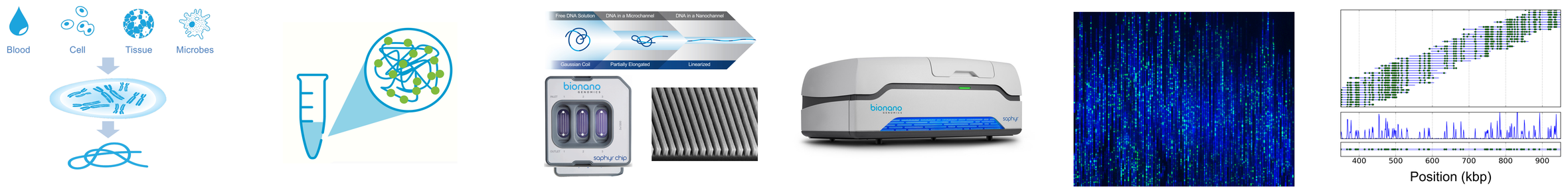
Methods: High molecular weight DNA was extracted from fresh frozen samples using the Bionano Prep kit and recommended protocol. DNA molecules were labeled at specific recurrent nucleotide sequences using Direct Label and Stain chemistry, electrophoresed into nanochannels, and imaged at >300x coverage of human genome per flow cell per run. Digital representation of the imaged DNA molecules were de novo assembled, and genome maps were aligned to the reference to identify and classify structural variants. Subsequently, the Bionano variant annotation pipeline was run to annotate variants with overlapping gene info, frequency of variants and the presence of the variants in the matched control samples, when available. A new single molecule analysis pipeline, the Rare Variant Pipeline, was used to call structural variants present at low allele frequency.

Background

Whole genome structural variation analysis has been intractable by DNA sequencing and extremely low resolution by cytogenetic methods. The Saphyr whole genome imaging instrument provides direct visualization of ultra-long DNA molecules in their native state, which accurately represent the structure of the genome. Consensus assemblies are used to accurately call structural variation between samples and the human reference genome on a genome-wide scale at resolution 1,000x higher than chromosomal banding from karyotype analysis. Leveraging the high throughput of the Saphyr system, variants are also detected in heterogeneous cancer tissues with 5% allele fraction in a sample allowing analysis of very complex and heterogeneous samples.

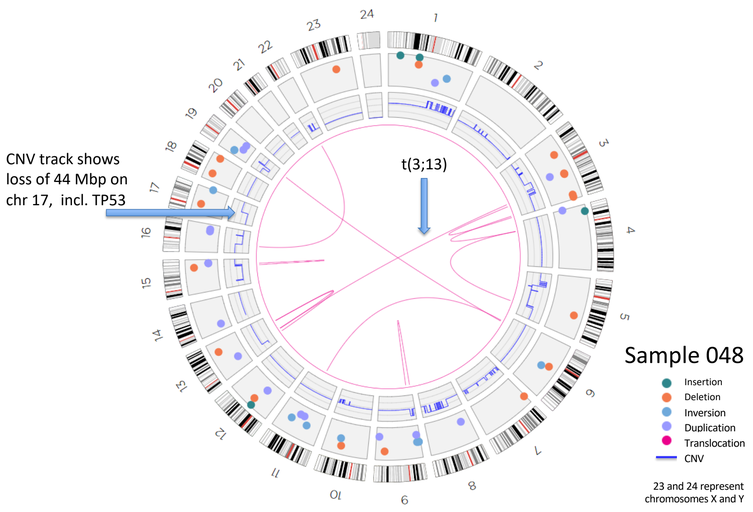
Methods

1. Extraction of long DNA molecules
2. Label DNA at specific sequence motifs
3. Saphyr Chip linearizes DNA in NanoChannel arrays
4. Saphyr automates imaging of single molecules in NanoChannel arrays
5. Molecules and labels detected in images
6. Bionano Access software assembles optical maps

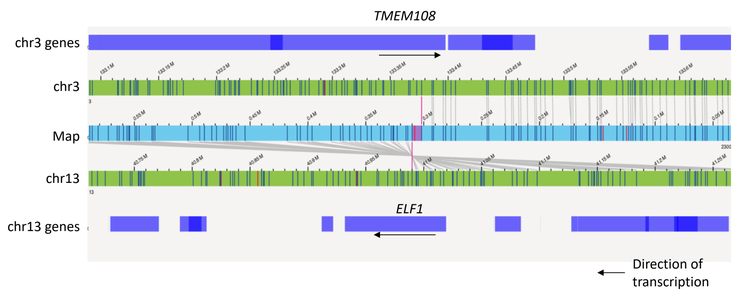


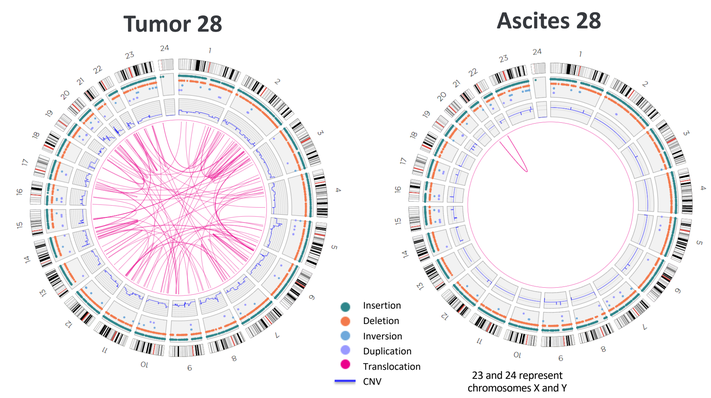
(1) Long molecules of DNA are labeled with Bionano reagents by (2) incorporation of fluorophores at a specific sequence motif throughout the genome. (3) The labeled genomic DNA is then linearized in the Saphyr Chip using NanoChannel arrays (4) Single molecules are imaged by Saphyr and then digitized. (5) Molecules are uniquely identifiable by distinct distribution of sequence motif labels (6) and then assembled by pairwise alignment into *de novo* genome maps.

Results



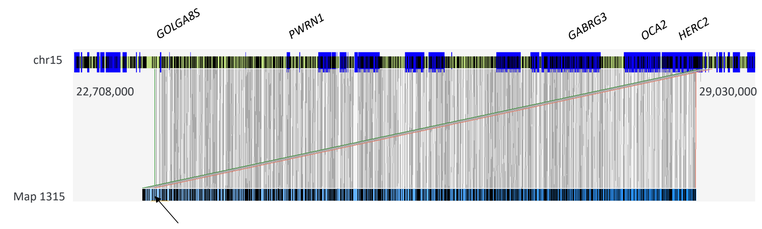
HPV+ ovarian tumor sample 048 shows the loss of 44 Mbp on chr17, encompassing the gene TP53 (above); a ELF1-TMEM108 potential fusion gene created by a t(3;13) (below).



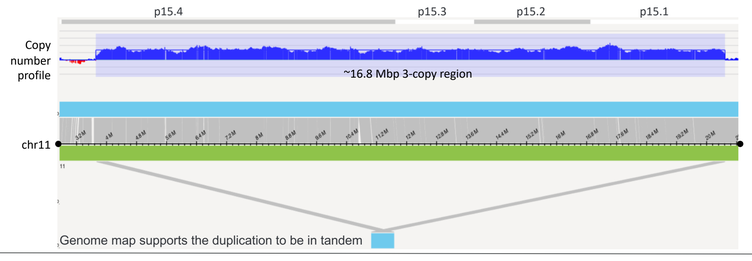


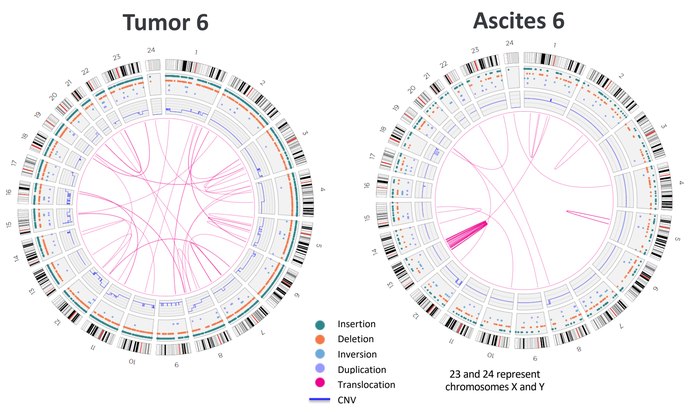
SVs called by molecule aligning to hg38	Shared	T28-unique	Asc28-unique
Insertions	4	15	15
Deletions	14	145	12
Inversion breakpoints	3	8	2
Duplications	3	26	5
Interchromosomal translocations	0	88	0
Intrachromosomal translocations	0	45	1

Primary ovarian tumor sample T28 shows many more rearrangements than the matching ascites sample Asc28, with very few shared variants (above).

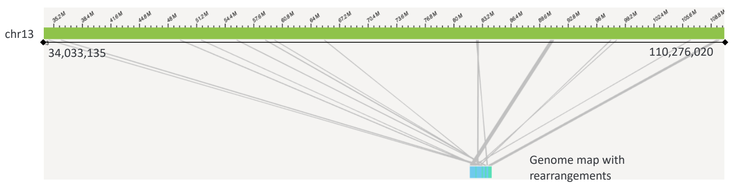


The Rare Variant Pipeline and CNV tool detected a 5.3 Mbp tandem duplication overlapping with HERC2 unique to T28, at low allele fraction, supported by just 7 molecules (above). A 16.8 Mbp duplication was detected in T28 by the CNV tool, and a *de novo* genome map confirmed the duplication to be in tandem (below).

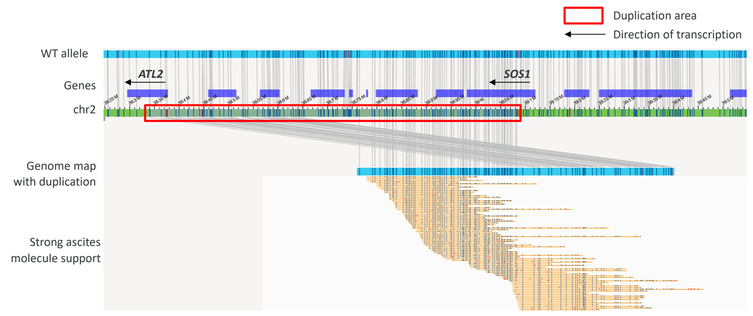




Primary ovarian tumor sample T6 shows many more rearrangements and an unstable copy number profile. The matching ascites sample Asc6 has a stable copy number, and rearrangements clustered on chr 13 (above). One Asc6 genome map capturing multiple chr 13 junctions is shown below.



Asc6-unique heterozygous 741 kbp tandem duplication was detected, creating a SOS1-ATL2 fusion (below).
SOS1: guanine nucleotide exchange factor for RAS proteins, regulates RAS proteins by facilitating exchange of GTP for GDP
ATL2: atlastin GTPase2.



Conclusions

Matched primary ovarian tumor-ascites samples showed extreme genetic divergence between primary tumor and malignant ascites, considered the primary route of metastasis and origin of recurrent disease. The optical mapping approach to structural genomic analysis is highly effective in detecting large scale deletions, duplications, insertions, and translocation all in one assay. Bionano offers a rapid informative method to study structural variation in ovarian tumors and is an important new tool to understand the molecular basis of ovarian cancer during tumor progression. The method is complementary to NGS and could help identify novel biological mechanisms of cancer progression as well as new actionable therapeutic targets.