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# Optical genome mapping workflow for identification and annotation of variants in hematological diseases

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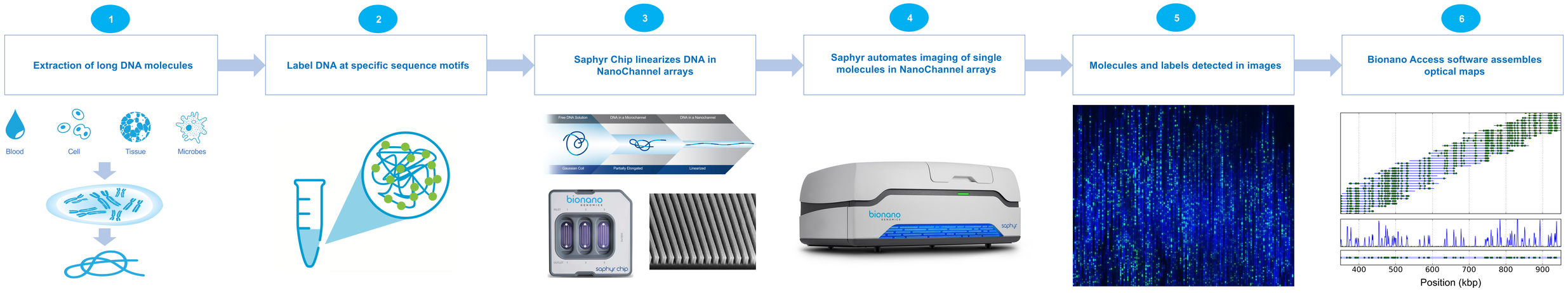
## Abstract

Guidelines from WHO, NCCN and others, for the genetic analysis of hematological malignancies included structural variation analysis. Traditionally, this has relied on a combination of three cytogenetic technologies for structural variation analysis: karyotyping, FISH, and microarray, are used to detect copy number variants, translocations, inversions. Next generation sequencing is also applied for mutation analysis but has not been successful for structural variation analysis. These traditional methods have many very manual aspects and require extensive expertise. Optical genome mapping (OGM) consolidates assays into a single laboratory assay in which the output provides the visualization of structural and copy number variants at one time.
OGM is able to comprehensively detect structural variations genome wide down to 5% variant allele fraction for CNVs, inversions, and translocations from blood and bone marrow aspirates making it an attractive choice for hematologic malignancy genomic analysis. Preanalytical and analytical steps require approximately 4-5 days from sample to processed data with structural variation calls. Dynamic filtering in the user interface can be configured to remove most polymorphic variants and prioritize relevant variants. In addition, the OGM graphical user interface software, Bionano Access 1.7, allows for the user to assign classification/relevance to the variants for each case. For example, an ALL sample with t(9;22), deletion of *CDKN2A*, and whole chromosome gains of 4,6, and 10 can be easily visualized with the Circos plot and, then, can be further examined and annotated as needed. A second analyst can repeat the process blind to the first analysis and a supervisor can adjudicate the classifications. A variety of cases with hallmark abnormalities from various leukemias will be presented with the filtering and prioritization workflow used to derive them. This comprehensive technology allows for a quicker, more reliable output than traditional cytogenetic approaches.

## Methods



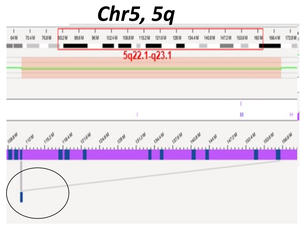

**(1)** Long molecules of DNA are labeled with Bionano reagents by **(2)** incorporation of fluorophores at a specific sequence motif throughout the genome. **(3)** The labeled genomic DNA is then linearized in the Saphyr chip using NanoChannel arrays **(4)** Single molecules are imaged by the Saphyr instrument 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.

## Virtual Panels

All classes of structural variations can be detected by OGM in a single assay. Above, deletions, amplifications, inversions, and translocations are shown to represent all variants recommended by the National Comprehensive Cancer Network (NCCN).

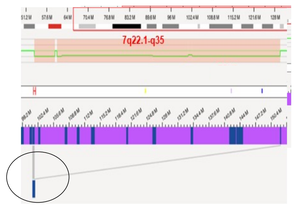
### Acute Myelogenous Leukemia

***Chr5, 5q***



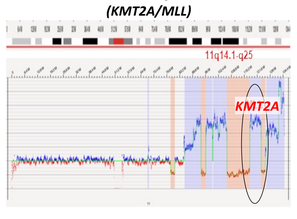

CSF1R deletion seen through copy number loss indicated by the red shaded box covering 5q14-q35.1. in addition, the fusion across the deletion is shown through alignments of the fusion map to 5q14 and 5q35 (circled).

***Chr7, 7q***



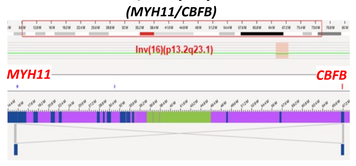

EZH2 deletion seen through copy number loss indicated by the red shaded box covering 7q22.1-q35. The fusion across the deletion is shown through alignments of the fusion map to 7q22.1 and 7q35 (circled).

***Chr11q23***
***(KMT2A/MLL)***




KMT2A/MLL is amplified in this case, the CNV profile shows a CN state of 5 (circled)

***t/inv(16)***
***(MYH11/CBFB)***

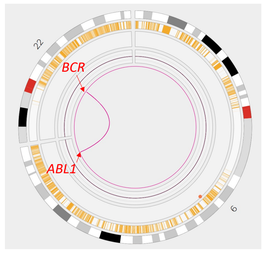



inv(16) shown by abnormal fusions between MYH11 and CBFB and its reciprocal fusion. These are shown in two maps whose alignments cross.

***t(8;21)***
***(RUNX1/RUNX1T1)***

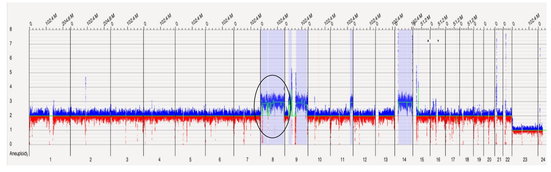

t(8;21) fusion (circled) shown by the sample map (light blue bar) which aligns to *RUNXT1* on the right/above and to *RUNX1* on the left/below.

### Chronic Myelogenous Leukemia




***BCR/ABL***

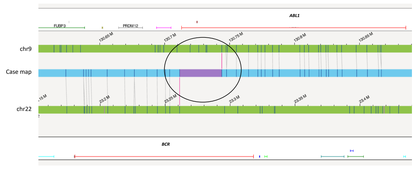
Fusion between *BCR* and *ABL1* is seen by the blue case's map aligned to *ABL1* on the right/top and *BCR* on the bottom/left.



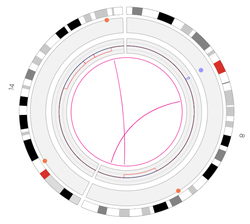
***Trisomy 8***

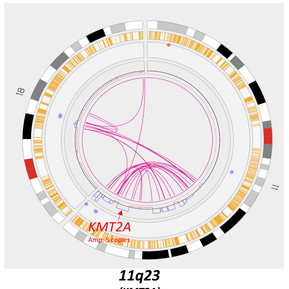
Trisomy 8 is called with whole genome CNV profile based on molecule counts across the genome. This case also has +9, +14.

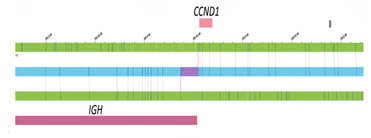
### Acute Lymphocytic Leukemia



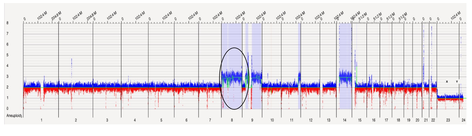

***BCR - ABL1***








Not shown: CEP4, 10, 17, 9p21/9q21

## Publications

| Reference | Cohort size | Clinical referral | Samples type | Variant types | Number of abnormalities included | Concordance with cytogenetics results | OGM additional findings |
|---|---|---|---|---|---|---|---|
| **Radboud University** Neveling et al., 2020 | 48 | Myeloid and lymphoid neoplasms (AML, MDS, CML, CLL, ALL, MM, MPN, T-PLL, LYBM) | Peripheral blood or bone marrow | Copy number gains/losses, inversions, translocations, aneuploidies, LOH | 112 | 100% | 18 potential gene fusions absent from COSMIC database.<br>26 insertions/deletions overlapping with well-established cancer genes |
| **Cancer Genomics Consortium** Levy et al., 2020 | 100 | Acute myeloid leukemia | Peripheral blood or bone marrow | Copy number gains/losses, inversions, translocations, aneuploidies | NA | 100% | 3 translocations, 1 inversion, 2 deletions and 1 derivative chromosome |
| **CHU Amiens** Lestringant et al., 2021 | 10 | B and T Acute lymphoblastic leukemia | Peripheral blood or bone marrow | Copy number gains/losses, inversions, translocations, aneuploidies, LOH | 78 | 76 out of 78* | 4 fusions, 6 deletions, 2 gains, 1 duplication, 3 complex chromosomal rearrangements |
| **M.D. Anderson** Yang et al., 2020 | 12 | Myelodysplastic Syndromes | Fresh/ frozen bone marrow aspirate | Copy number gains/losses, inversion, aneuploidies, translocations/ derivative chromosomes and 1 isodicentric chromosome | 28 | 26 out of 28**<br>93% with conventional karyotyping; 100% with chromosomal microarray | 6 cryptic aberrations (in 33% patients): 4 deletions, 1 duplication and 1 translocation |
| **Johns Hopkins University** Stinnett et al. 2021 | 5 | Leukemia/Lymphoma and Solid Tumors | Peripheral blood, bone marrow, or fresh kidney tissue | Copy number gains/losses, inversions, translocations, aneuploidies | 30 | 100% KT/FISH<br>100% CMA >10% VAF *** | 71 additional calls (7.7% involving cancer genes) |
| **University Hospital Olomouc** Kriegova et al. 2021 | 11 | Multiple myeloma | Bone marrow | Copy number gains/losses, inversions, translocations, aneuploidies | NA | 98% | |

## Conclusions

- ✓OGM allows for the detection of all classes of structural variations occurring in the human genome
- ✓Removes the need for tiered testing as part of a clinical work-up
- ✓OGM can pick up all actionable SVs as per NCCN/WHO guidelines
- ✓Bionano's Access software solution - ease of use
- ✓Graphical user interface allows filtering and manual evaluation on a per SV basis including access to raw data support
- ✓Fast TAT (sample to answer in 4 days)
- ✓Multiple studies have demonstrated 100% concordance with cytogenetic methods (karyotype, microarray, FISH) for constitutional and hematologic malignancy
- ✓Multiple studies showing increase in diagnostic yield against the standard of care in pediatric and heme