Next-generation cytogenetics: high-resolution optical mapping to replace FISH, karyotyping and CNV-microarrays

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Introduction

Structural variants (SVs) are an important source of genetic variation in the human genome and they are involved in multiple diseases, including cancer and developmental disorders. In a diagnostic set-up, a comprehensive analysis of all molecular cytogenetic aberrations still requires a combination of different techniques, such as CNV-microarrays, karyotyping and fluorescence *in situ* hybridization.

Hypothesis

A combination of classical approaches could be largely replaced by the novel optical mapping technology.

Material and Methods

In total we have now successfully run 36 samples (DNA derived from blood or bone marrow) using the Saphyr system (Bionano genomics). All the samples were first analyzed using standard of care workflow and the residual material was processed for Bionano optical mapping to detect chromosomal aberrations (**Fig. 1**). Briefly, the samples were run on the Saphyr to generate a 100-300-fold genome coverage by imaging long chromosomal fragments. Chromosomal aberrations were detected by comparing optical maps to a reference and control dataset, and a coverage-based CNV calling was performed. Currently, a detailed analysis of 40 samples has been finished (see Table 2 for examples).

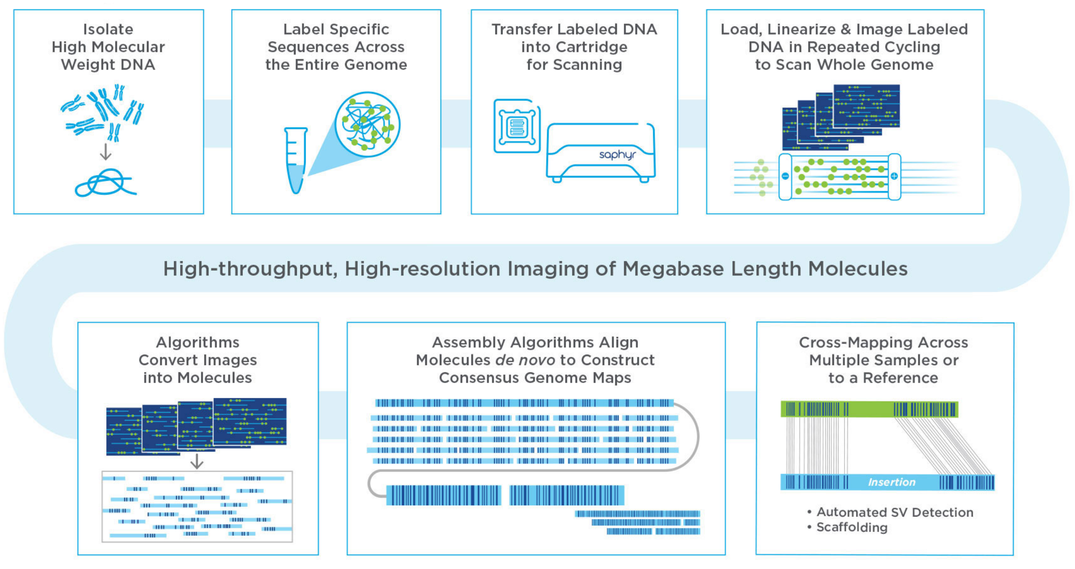

Figure 1. *The Saphyr system worklow to image and analyze extremely long, high-molecular-weight DNA for SVs (https://bionanogenomics.com/technology/platform-technology/).*

Results

Optical mapping identified all the previously known clinically relevant aberrations from the analyzed samples (**Table 1**). This held true for deletions, insertions, inversions and translocations, including a 3-way Philadelphia chromosome (**Figs. 2A** and **2C**). Importantly, we were able to identify aberrations in leukemia samples with a cancer cell content under 40% by using Bionano's latest single-molecule SV detection tool, which also enabled to resolve chromothripsis events. In addition, optical mapping identified multiple novel events in leukemia, such as an inversion in chromosome 11 (chr11:24,875,044-26,299,641) and a translocation t(5;14)(q35.2;q32.2), both validated afterwards. Overall, thousands of SVs (without filtering for SV size or population frequency) were identified in each leukemia sample (**Table 2**).

Table 1. Detection of clinically relevant aberrations with optical mapping.

Sample	Previous diagnostic test result	Optical mapping
CML	t(9;14;22)(q34;q11.2;q11)	(**Fig. 2A**)
ALL-1	der(2)t(1;2)(q31;q35)	(**Fig. 2B**)
MPN	45,XX,-6,-9,-11,-22,+3mar / (6)cth	
ALL-2	Multiple CNVs	
CLL	Multiple CNVs	
Congenital-1	8p22 loss	(**Fig. 2D**)
Congenital-2	22q11.2 loss	

ALL: Acute Lymphocytic Leukemia, CML: Chronic Myeloid Leukemia, MPN: Chronic Myeloproliferative Neoplasms, CLL: Chronic lymphocytic leukemia

Table 2. High-confidence SV calls in leukemia samples.

SV type	CML	ALL-1	MPN	ALL-2	CLL	Average
Insertions	3864	3804	3951	4043	4018	3936
Deletions	1482	1516	1511	1647	1790	1589
Inversions	84	86	95	81	181	105
Duplications	56	50	61	62	75	61
Translocations	3	2	1	6	3	3

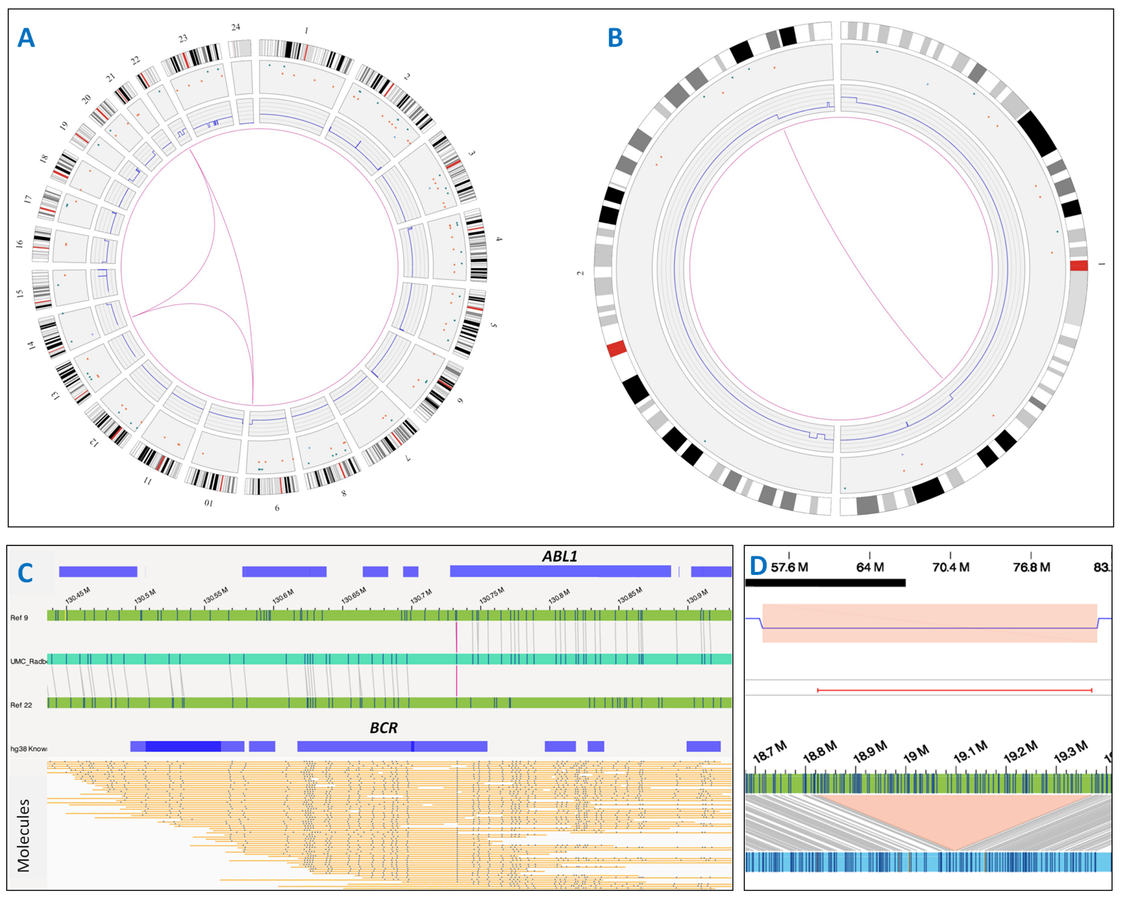

Figure 2. *Visualization of Bionano optical mapping data. **A**) Circos-plot view of the data from a CML sample showing a 3-way Philadelphia chromosome translocation t(9;14;22)(q34;q11.2;q11), **B)** Unbalanced translocation t(1;2)(q31;q35) resulting in a derivative chromosome 2 with gain of chromosome 1 and loss of chromosome 2, **C)** BCR-ABL1 gene fusion and the aligned DNA-molecules spanning the breakpoint, **D)** a congenital 8p22 deletion detected both with assembly- and coverage-based methods.*

Conclusions

We are currently conducting a study to systematically compare the sensitivity and specificity of optical mapping in 100 leukemia samples and 50 samples with known germline cytogenetic aberrations against the standard of care workflow. Of these, 17 with constitutional aberration and 19 leukemia samples have been now processed, so far all clinically relevant, previously reported, aberrations were identified by optical mapping.

We see potential that optical mapping may replace most classical cytogenetic tests.

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