OCTOBER 2019

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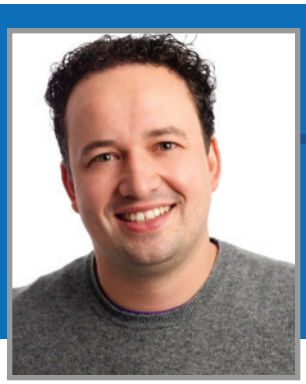
HIGHLY SENSITIVE STRUCTURAL VARIANT DETECTION FOR MEDICAL GENETICS: A COMPARISON TO THE STANDARD OF CARE

A **GENOMEWEB** WHITE PAPER

This white paper is based on a webinar presentation by Alexander Hoischen of Radboud University Medical Center, in which he discussed the promise of optical mapping technology for medical genetics.

Dr. Hoischen shared details of a proof-of-concept study his lab is conducting to evaluate the Saphyr whole-genome imaging technology from Bionano Genomics as a possible replacement for karyotyping, fluorescent in situ hybridization, and copy number variant microarrays.

Dr. Hoischen noted that optical mapping promises "next-generation cytogenetics" because it provides 10,000-fold improved sensitivity compared to karyotyping while maintaining many of the advantages of classical cytogenetics methods. "We still get a genome-wide analysis; we still can get positional information; and, as a best-case scenario, can also even reach single-molecule resolution," he said.



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The Bionano system uses microfluidic chips with nanochannels only the width of a molecule of DNA. The system images barcoded DNA molecules as they move through the nanochannels and then algorithmically converts them into megabase-length molecules, which are aligned to create a *de novo* whole-genome map that can be compared to a reference genome to identify structural variants.

Dr. Hoischen said that one reason his team decided to evaluate the Saphyr system now

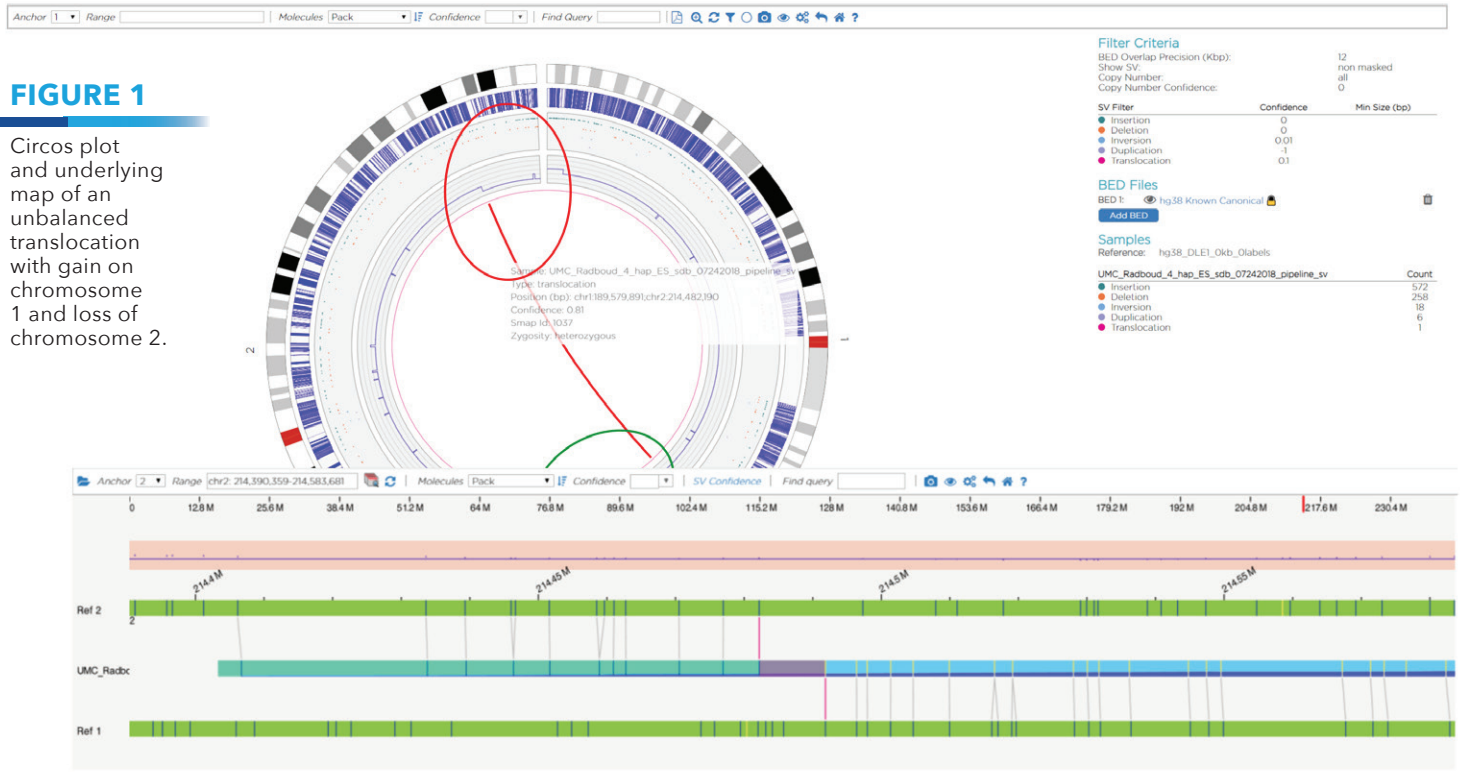


FIGURE 1

Circos plot and underlying map of an unbalanced translocation with gain on chromosome 1 and loss of chromosome 2.

is that "the pricing started to get competitive." Currently around $500 per sample, the technology is "comparable to standard CNV microarrays," he said, adding that "there may be a chance to bring down the price quite significantly in the next month and years."

The current version of the system provides up to 400-fold coverage of the genome, which was another factor in Dr. Hoischen's decision to bring it in house, he said.

The Radboud University team is testing a total of 150 samples with the system – 100 leukemia samples and 50 samples with constitutional cytogenetic aberrations. They have so far completed 26 of the leukemia samples and 17 of the samples with germline structural variants and expect to complete the project by the end of the year.

The aim of the study, Hoischen said, is to determine whether optical mapping can replace karyotyping, FISH, and microarrays – the current gold-standard cytogenetic methods in the diagnostic setting.

"The ultimate goal would be to implement [optical mapping] as part of our routine process and replace the classical methods, at least to a large degree," he said.

So far, the Bionano Saphyr system is generating average read lengths of around 270kbp – "much, much larger than we use for any sequencing" – and average coverage of up to 400-fold. The platform detects on average between 5,000 and 6,000 structural variants per sample.

Dr. Hoischen provided details on several of the leukemia samples where the Bionano system identified structural variants that were not detected on other platforms.

In one case, the Saphyr system was able to detect "in one go" an unbalanced translocation with a net gain of chromosome 1 and a loss of chromosome 2 material that otherwise would have required a combination of karyotyping and array comparative genomic hybridization. Dr. Hoischen explained that the system allows users to easily visualize the genome in a circos plot, and then click on any specific structural variant to zoom down into the underlying maps *(see figure 1)*.

In this case, the Bionano technology also detected a new translocation between chromosomes 5 and 14, as well as the breakpoint, which the team was able to confirm by FISH.

In another case of a chronic myeloproliferative neoplasm, the Radboud team used a new rare variant software tool from Bionano to detect a

FIGURE 2

Optical mapping detected a candidate *de novo* structural variant downstream of the 17q21.31 region in a patient with intellectual disability that appears to affect the NSF gene.

deletion on chromosome 3, "which initially was not spotted by our classical methods."

The deletion affects a gene called FOXP1 that is "not a known driver gene for leukemia, but it is known to be deleted in other cancers, so potentially, this would be interesting to follow up," Hoischen said.

"In other cases, we've spotted novel events that were not visible before, sometimes simply because they were not tested for, but now, with Bionano, we always get a genome-wide view instead of, for example, a limited set of FISH probes," he said.

He added that his team has also found a number of novel fusions "with a one-click analysis: clicking on the circos plot and directly seeing where those fusions are and how many molecules support those fusions."

Dr. Hoischen recalled that it used to take "months" to characterize a single breakpoint with M-FISH in combination with PCR. "Now, we all see it after a simple analysis. That's quite remarkable progress."

With the leukemia samples the team has studied so far, "we've identified all the previously reported, clinically relevant variations as long as they had a variant allele frequency of 10 percent or higher," he said. "Quite often, we had to conclude that the previously called rearrangement was actually more complex after we saw it by the Bionano analysis."

UNSOLVED RARE DISEASE CASES

Dr. Hoischen said that in addition to the proof-of-concept study in leukemia and germline variants, his team is also evaluating the Bionano platform to study unsolved rare disease cases – particularly in the area of severe intellectual disability.

He noted that his team has access to a cohort of around 1,500 patients with severe intellectual disability. "Whenever a new laboratory test came available, we tested this cohort or a representative fraction with the latest tools."

In "the dark ages of human genetics," clinicians would request a single-gene test, which only delivers a diagnostic result in 1 percent to 5 percent of cases, he said. "Then, in the mid-2000s, genomic microarrays for CNV profiling came around and they usually solve anything between 10 percent and 15 percent of the cases." While exome sequencing and whole-genome sequencing have increased the diagnostic yield, Dr. Hoischen noted that around 40 percent of all cases still do not have a definitive diagnosis.

"The big question for that is, what have we missed so far?" Maybe, he said, "we simply haven't sequenced the mutation itself or, maybe equally likely, there was a structural variant that remained undetected with the current genome sequencing or exome sequencing."

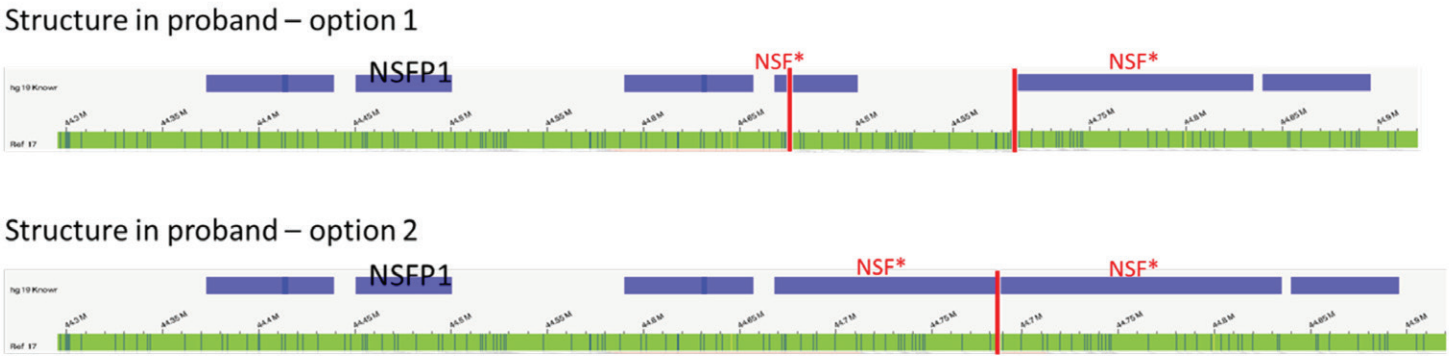
With this working hypothesis, Dr. Hoischen and colleagues set out to use long-read sequencing to find "hidden" *de novo* structural variants in unsolved intellectual disability trios. They used Pacific Biosciences SMRT sequencing on five patient-parent trios in which the disease cause had not yet been found.

While PacBio sequencing identified around 25,000 structural variants and up to 40,000 indels per genome, "to our disappointment, we were not able to find a single *de novo* structural variant in any of those five patient parent trios and we also did not find a likely pathogenic mutation in any of those cases so far," Dr. Hoischen said.

Dr. Hoischen and his team are currently analyzing the same five trios with the Bionano system and are seeing very high concordance between the two platforms.

FIGURE 3

The optical mapping data suggests two possible breakpoint scenarios, either of which would disrupt both copies of the NSF gene.



In one case, the Bionano technology detected a candidate *de novo* structural variant "in the most complicated region of the human genome" – just downstream of the Koolen-de Vries microdeletion syndrome region (17q21.31). *(see figure 2)*

Bionano flagged this as a deletion affecting the NSF (N-Ethylmaleimide Sensitive Factor) gene, but the PacBio coverage was very low in this region. "One reason may be that the PacBio data should have been mapped back to an alternative reference haplotype," he said.

"If we then look in greater detail into the Bionano call, then there's two options for this event," he said. "It could be one or two breakpoints … In either of those options, it is most likely that a partial duplication of this locus is disrupting both NSF copies on this allele. We also don't see any support in the parental assemblies for this event, so it makes it very likely that this is a true *de novo* event." *(see figure 3)*

Whether this variant is related to the disease "is very difficult to judge at this moment," Dr. Hoischen said, but "it's important to know that NSF actually shows multiple copies in the general population, so it's a copy number variable gene. To my understanding, there was never an individual reported with just one allele, which this individual may carry. So, that's very interesting to follow up."

Dr. Hoischen said his team is launching another research program using optical mapping in cases where the linkage region for a disease has been known for years "and none of the sequencing approaches have yet identified the [causative] mutations."

The researchers are also using the Bionano technology in cases where they suspect very strongly recessive disease. "So far, we've identified the pathogenic mutation on one of the alleles but the second mutation remains undiscovered. We think Bionano may discover those."

So far, the early data they've seen "encourages us to believe that this may replace a lot of the classical cytogenetic technologies in the next one to two years," Dr. Hoischen said.

Bionano technology is for research use only and not for diagnostic procedures.