Evaluation of the Bionano Optical Mapping Technology as a Replacement of Conventional Cytogenetics in a Diagnostic Setting

Charlotte Keith[1], Eddy Maher[1], Jon Warner[1] and Alex Hastie[2]

[1]South East Scotland Genetic Laboratories, Western General Hospital, Edinburgh

[2] BioNano Genomics, Inc, San Diego, CA, USA .

Poster No.

1. Introduction

The diagnostic remit of a clinical cytogenomic laboratory is to identify DNA copy number variations (CNVs) and structural variations (SVs) that are likely to impact phenotype.

Current methodologies have significant limitations. Within the South East Scotland Genetic Service, chromosomal microarray (Affymetrix 750k) is used to diagnose CNVs greater than 10 kilobase (Kb) in size, but cannot detect "balanced" aberrations such as translocations or inversions. G-banded chromosome analysis detects most structural variation, but at a lower resolution of 3-5 Megabases (Mb) and Fluorescent In Situ Hybridisation (FISH) often requires prior knowledge of the variant.

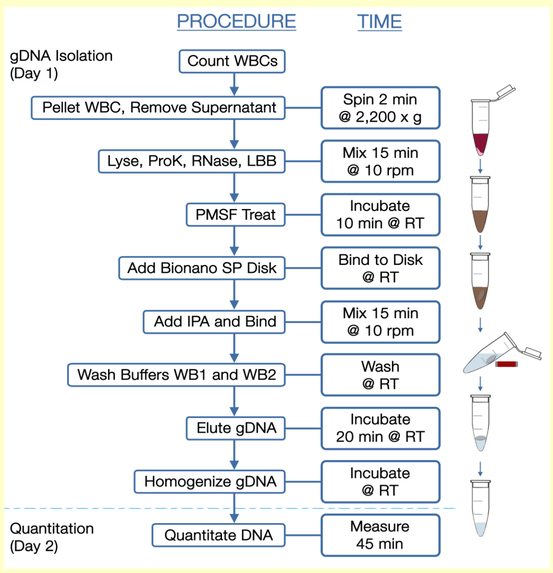
Sequencing technology has improved, with significant progress in the detection of single nucleotide changes and short indels, but most methods produce short-read sequences and therefore fail in GC rich or repetitive regions and cannot detect larger variants accurately.

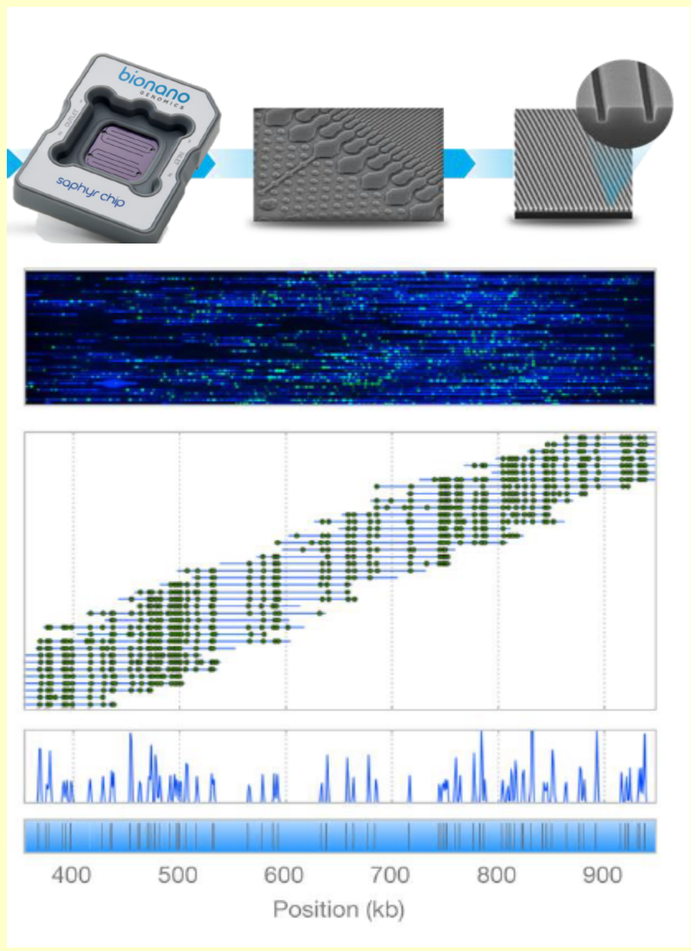
2. Optical Mapping

Bionano's Saphyr system is an optical mapping technology that utilises single, megabase-size native DNA molecules, identifying a custom recognition sequence to use fluorescent labelling for alignment. The automated calling algorithms aim to detect all copy number and major structural variation types (including balanced rearrangements), potentially replacing the need for multiple analytical techniques.

The Bionano Saphyr extracts intact single DNA molecules between 100 kb and 2.5 Mb in length.

Once DNA is extracted, it is fluorescently labelled, linearised in NanoChannels and imaged. (see three diagrams below)





There is no amplification, providing the long-range contiguity critical for de novo sequence scaffolding. Labels and molecules are detected, de novo assembled and aligned to another genome map or a sequence assembly that provide dense genome-wide anchor points for ordering and orienting sequencing contigs or scaffolds to greatly increase completion and accuracy of de novo assemblies.

By observing changes in label spacing and comparisons of order, position, and orientation of label patterns, Bionano's algorithms reportedly detect all major structural variation types with data generation of 320Gb.

This new system was used on four (anonymised) diagnostic samples from Edinburgh to see if copy structural variants as well as copy number variants could be detected. Results were compared to those obtained previously by multiple traditional cytogenomic techniques.

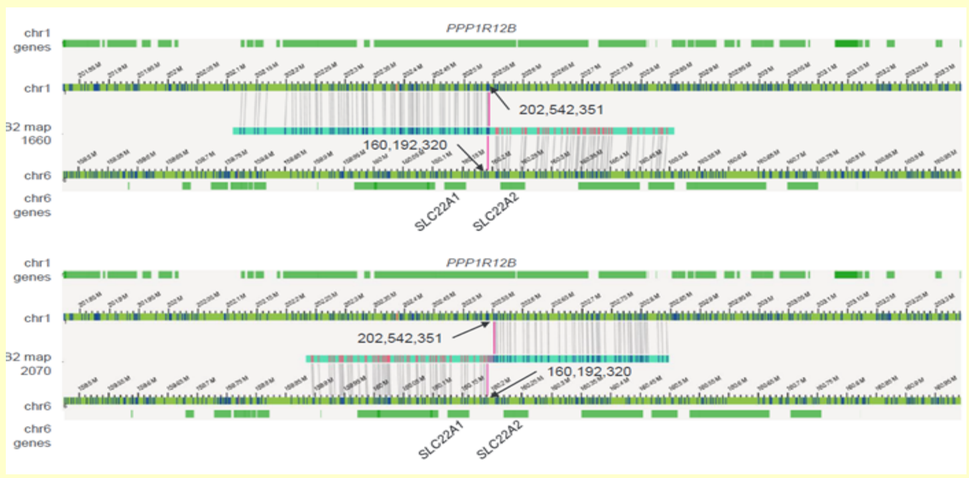
3. Results

SAMPLE 1: (A peripheral blood sample from a patient with a history of abnormal pregnancies.)

G-banded chromosome analysis showed an apparently balanced translocation between the long arms of chromosome 1 and chromosome 6.

46,XX,t(1;6)(q32;q25)

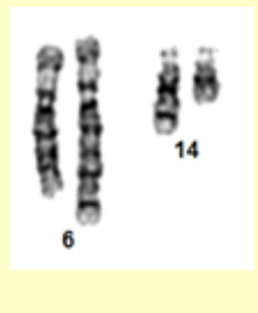
G-banded chromosomes, imaged by Leica hardware and Cytovision software



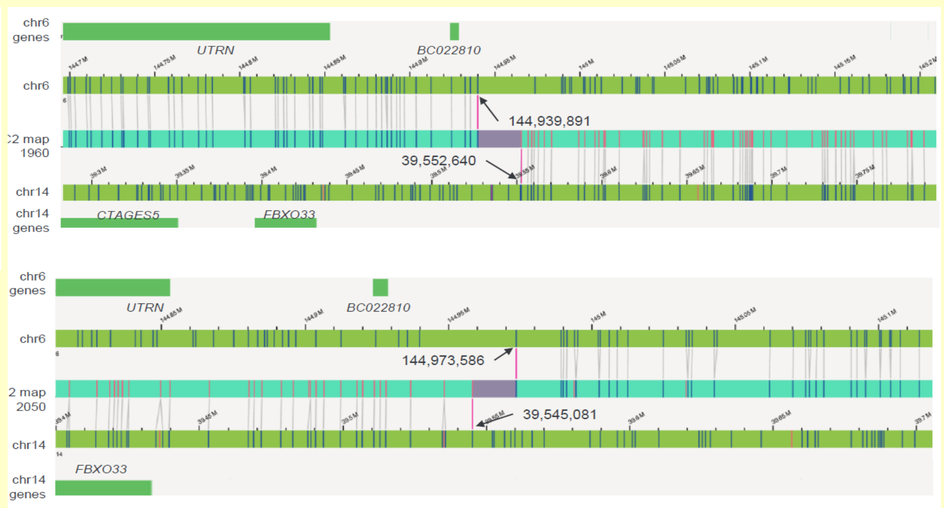
Bionano Solve v3.2 presentation of reciprocal translocation, showing assembled maps versus expected maps with basepair coordinates indicating where assembled map changes from aligning with chromosome one, to chromosome six and vice versa.

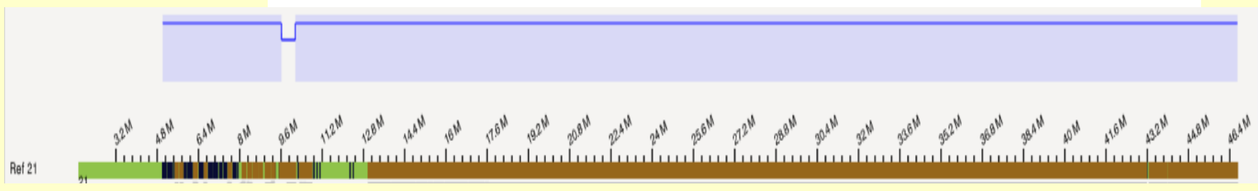
SAMPLE 2: (A chorionic villus sample from a high risk pregnancy serum screening).

The additional chromosome 21 had already been detected in rapid quantitative fluorescent PCR investigations (standard in prenatal cases) and G-banded chromosome analysis followed for any additional variants.
It showed an apparently balanced translocation between the long arms of chromosome 6 and chromosome 14.



47,XX,t(6;14)(q25;q22),+21



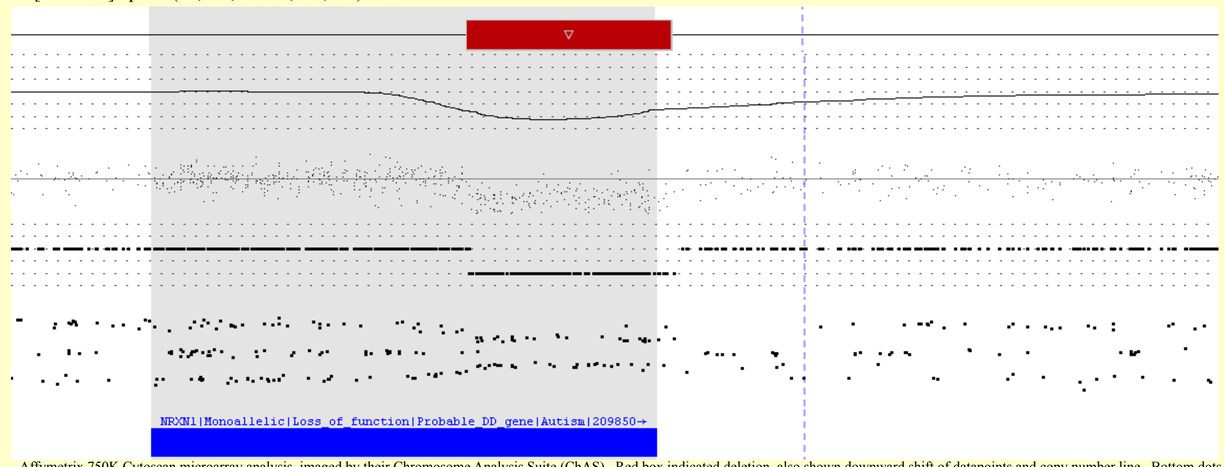


Raised blue line indicating copy number 3

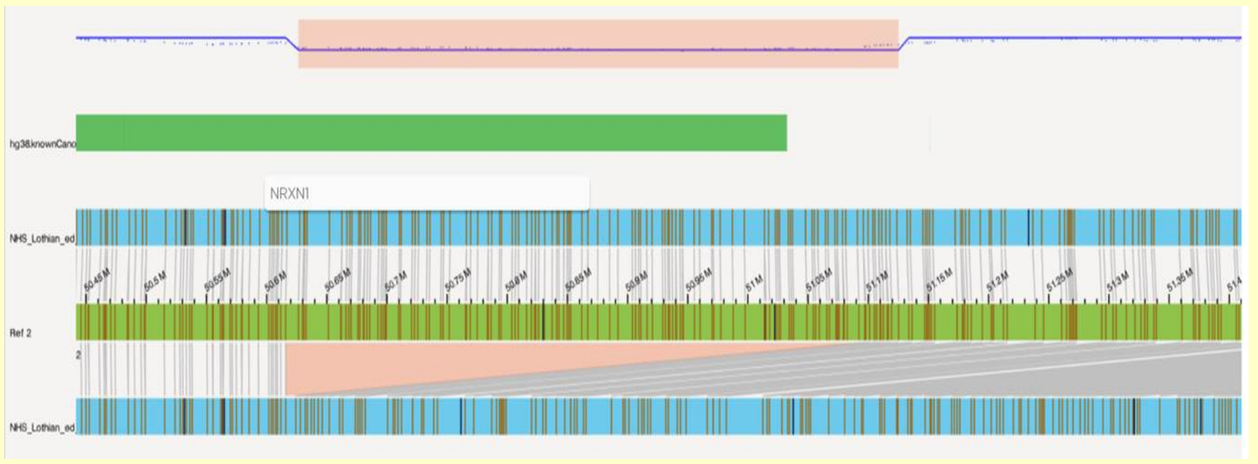
SAMPLE 3: (DNA extracted from an amniotic fluid sample from a high risk pregnancy serum screening)

The rapid QFPCR showed no evidence of trisomy 13, 18 or 21 so the sample was run on the Affymetrix 750K Cytoscan microarray platform. Five calls greater than 200Kb in size were found, with a 450Kb deletion within NRXN1 being clinically significant.

arr[GRCh37] 2p16.3(50,844,816-51,294,688)x1 dn



Affymetrix 750K Cytoscan microarray analysis, imaged by their Chromosome Analysis Suite (ChAS). Red box indicated deletion, also shown downward shift of datapoints and copy number line. Bottom dots represent SNPs and grey box/blue line is the NRXN1 gene region



SAMPLE 4: (A peripheral blood sample with no clinically significant variants.)

This sample was from the parent of a child with an unbalanced translocation. G-band chromosome analysis showed no evidence of any translocation.
The Bionano platform detected no translocations, but showed 16 insertions, 9 deletions, 6 inversions and 4 duplications. These variants were all found in the Bionano control sample database so were not clinically significant.

4. Conclusions

The Bionano optical mapping system not only detected all copy number variants with a higher resolution than microarray, it also detected all balanced structural variants. Although this cohort was a very small proof of principle study, the results are extremely encouraging and a large scale validation will be arranged with the hope of implementing this technology as a diagnostic service.

5. Acknowledgments

Thank you to all my colleagues in South East Scotland Genetics Service, and to Joe Butler, Andy Pang, Joyce Lee, Sven Bocklandt and the team at Bionano Genomics Inc.