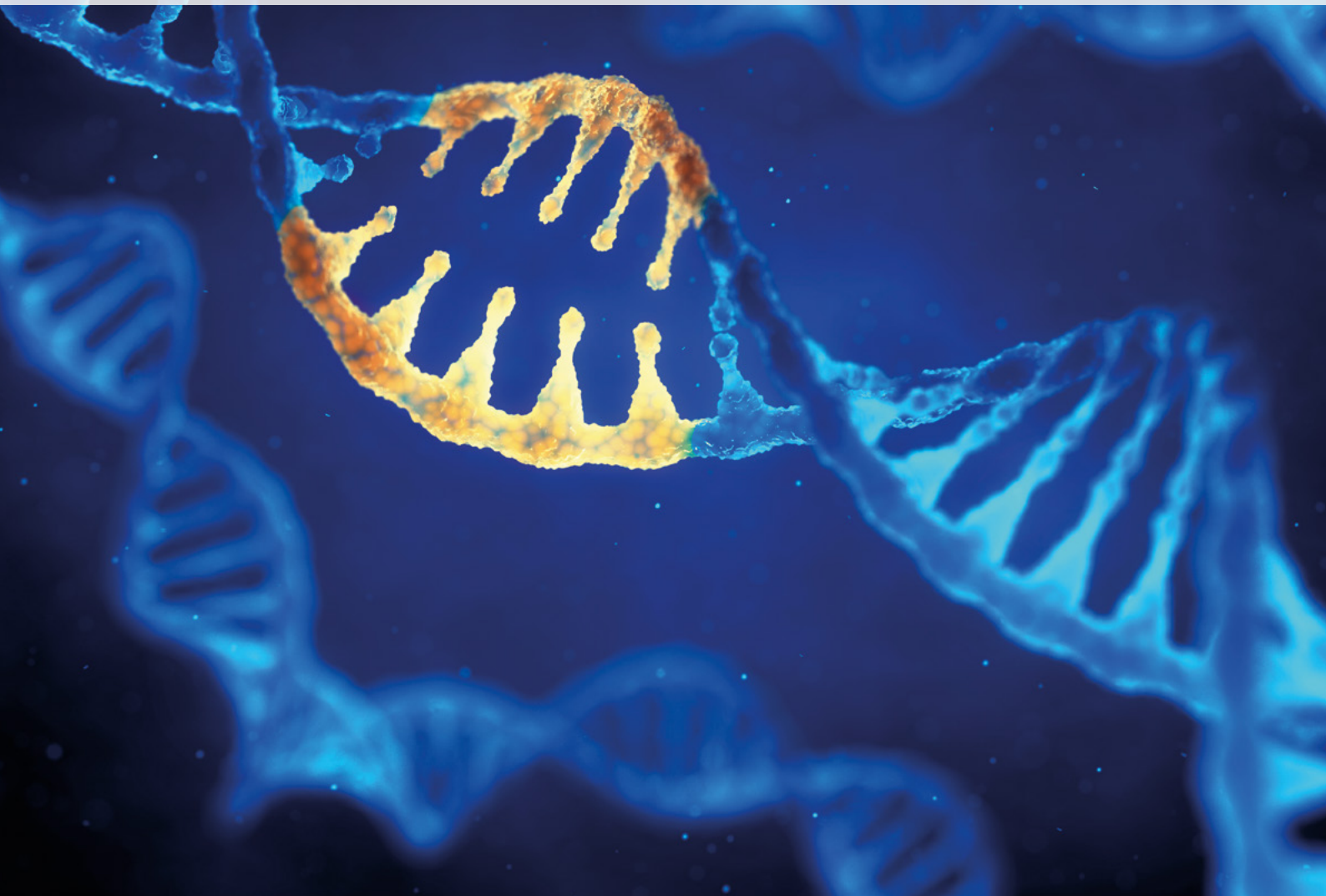


Targeted, PCR-free analysis of cancer-associated genes with Cas9

Cas9 enrichment with Oxford Nanopore enables the capture and sequencing of large regions of native DNA, allowing simultaneous evaluation of structural variation, SNPs and methylation patterns



Timothy Gilpatrick and his team utilised Cas9 to enrich for and comprehensively analyse multiple cancer-associated loci in breast cell lines

Impact

This study demonstrated the capacity of Cas9 enrichment and long-read nanopore sequencing to thoroughly characterise cancer driver genes. Providing both sequence and modification data in a single experiment, this rapid, low-cost method can help further our understanding of complex diseases.

Results

Several hundred-fold enrichment of target loci enabled identification of known large structural variants, SNPs, and differential methylation patterns in genes with prognostic implications in breast cancer.

10

cancer-associated loci enriched in multiplex

680x

median coverage per locus from one MinION™ Flow Cell

155 kb

deletion detected in GM12878

KRT19

gene hypomethylation in two breast cancer cell lines

TP53

variants phased in GM12878

HOW?

Ten cancer-associated loci were enriched in DNA from 3 human breast cell lines and the well-studied GM12878 cell line, then sequenced in multiplex. Structural variants and SNPs were identified, and CpG methylation evaluated.

Kit	Oxford Nanopore Ligation Sequencing Kit + third party reagents Cas9 Sequencing Kit now available here: https://store.nanoporetech.com/us/catalog/product/view/id/461/s/cas9-sequencing-kit/category/28/
Device	GridION™ with MinION and Flongle™ Flow Cells
Tools	Custom analysis pipeline, including: <ul style="list-style-type: none">- Minimap2 (alignment)- Nanopolish (CpG methylation calling)- Sniffles (deletion calling)- Samtools, Clair, Medaka or Nanopolish (new variant calling)- WhatsHap (phasing) Find out more about analysis pipelines available here: community.nanoporetech.com/knowledge/bioinformatics

Find out more at: nanoporetech.com/applications/targeted-sequencing

Publication

T. Gilpatrick *et al.* Targeted nanopore sequencing with Cas9-guided adapter ligation. *Nature Biotechnology* (2020). DOI: <https://doi.org/10.1038/s41587-020-0407-5>

Presentation

Timothy Gilpatrick – Targeted nanopore sequencing with Cas9 for studies of methylation, structural variants and mutations. Available at: <https://nanoporetech.com/timothy-gilpatrick-1c19> [Accessed 11 September 2020]



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