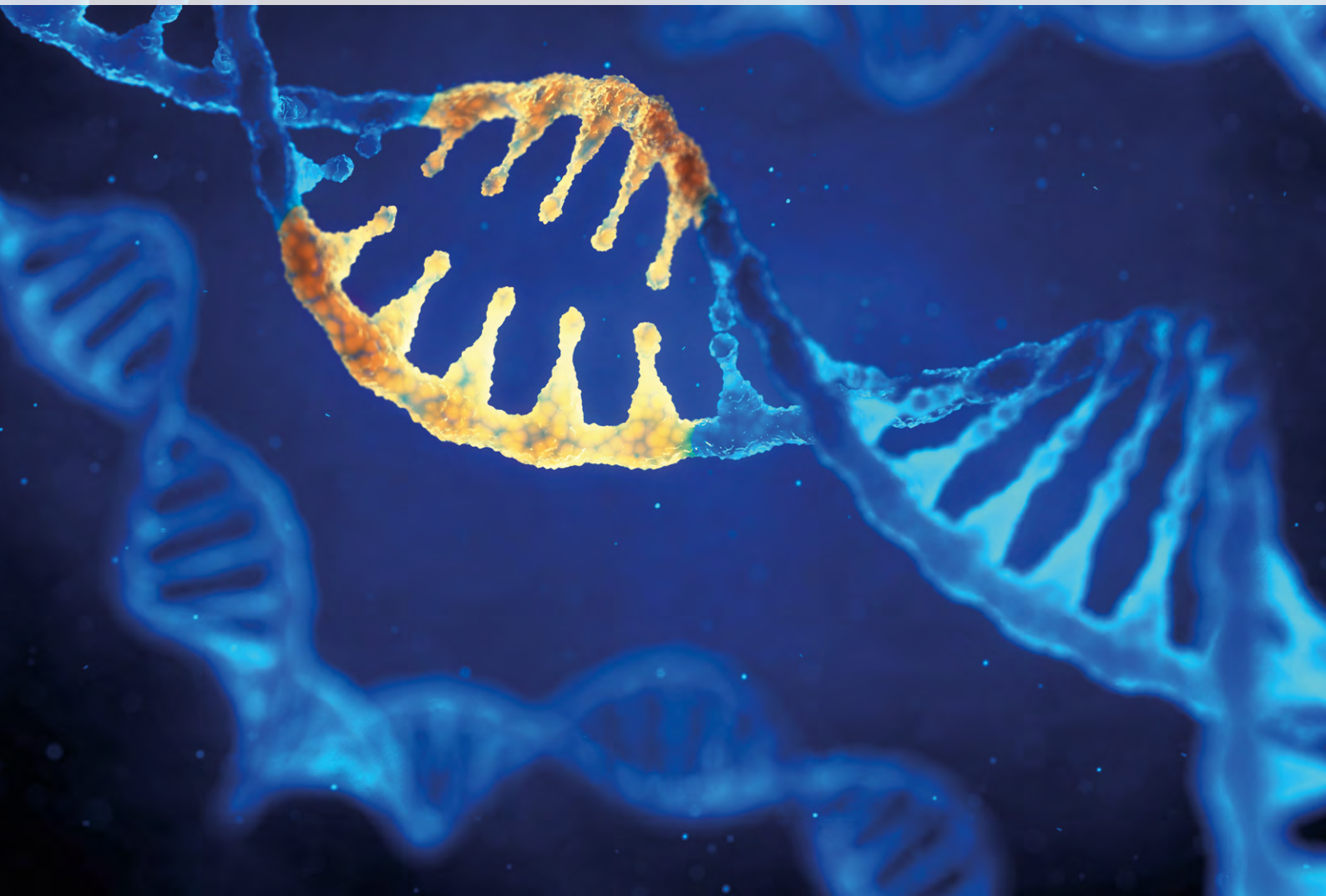


Targeted, PCR-free analysis of cancer-associated genes with Cas9

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

165x

median coverage per locus from one MinION™ Flow Cell

150 kb

deletion detected in GM12878

KRT19

gene hypomethylation in two breast cancer cell lines

GSTP1

gene promoter hypermethylation in ER(+) breast cancer cell line

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
Available here: store.nanoporetech.com/ligation-sequencing-kit.html

Device GridION™ with MinION and Flongle Flow Cells

Tools Custom analysis pipeline, including:
– Minimap2 or NGMLR (alignment)
– Nanopolish (CpG methylation calling)
– Sniffles (structural variant calling)
– Nanopolish or samtools (*de novo* variant calling)
– HapCUT2 (haplotype assembly)

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 for studies of methylation, structural variants, and mutations. BioRxiv (2019). DOI: <https://doi.org/10.1101/604173>

Presentation

Timothy Gilpatrick – Targeted nanopore sequencing with Cas9 for studies of methylation, structural variants and mutations. Available at: <https://nanoporetech.com/resource-centre/targeted-nanopore-sequencing-cas9-studies-methylation-structural-variants-and-0> [Accessed 25 June 2019]



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