CASE STUDY

Rapid, low-cost detection of genomic aberrations with Flongle

The extensive role of gene fusions in malignancies is well recognised, ranging from the characteristic *BCR-ABL1* gene fusion found in breast tumours to the diverse range of *FGFR2* fusions seen in certain liver cancers[1]. Consequently, gene fusion detection is central to the diagnosis and treatment of many tumours. Jeck and colleagues at Duke University Medical Center, US, have been evaluating ways to improve how fusions can be detected and assessed[2].

A commonly used method for fusion detection employs fluorescence *in situ* hybridisation (FISH), which provides relatively quick results, but is limited to the detection of a single gene target[2]. Similarly, quantitative RT-PCR requires prior knowledge of fusion partners[2]. Short-read sequencing technology is capable of simultaneously surveying numerous targets, whilst often providing the resolution to determine fusion breakpoints and partners. However, doubts over the economic viability of such short-read sequencing tests exist; Jeck *et al.* note that *'a full sequencing run on most devices is excessive for the testing of a single patient'*. Although multiplexing reduces costs, it may greatly extend turnaround times and, in many cases, time to action is critical for successful outcomes. To that end, the researchers evaluated the potential utility of the Oxford Nanopore Flongle device for fusion detection; at only $90 per flow cell, costs were greatly reduced compared to commonly used methods*.

Moreover, in terms of detecting fusions, results obtained via the Flongle sequencing pipeline implemented by the team showed *'excellent concordance'* with those obtained using a short-read-based approach. Flongle also outshone the selected short-read sequencing technology for detecting an aberration within a 3.3 kb tandem repeat, highlighting the benefits of long nanopore sequencing reads. The team stated that Flongle was *'particularly strong in identifying notoriously difficult to detect* CIC-DUX4 *translocations'*.

> ***"Flongle is a promising platform for single specimen fusion identification[2]"***

* Oxford Nanopore Technologies products are not intended for use for health assessment or to diagnose, treat, mitigate, cure, or prevent any disease or condition

Products used

Kit	Ligation Sequencing Kit
Device	Flongle
Tools	Custom pipeline

Find out more: nanoporetech.com/products

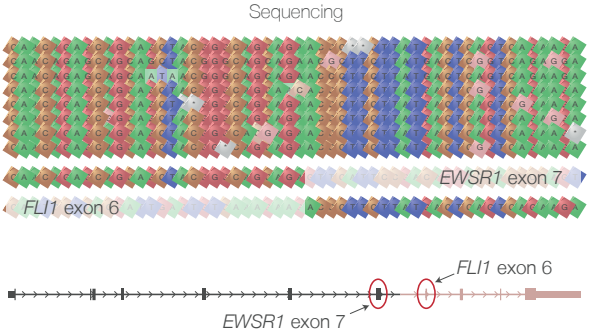

Figure 1
A demonstration of fusion detection using long nanopore sequencing reads. Taken from the Oxford Nanopore Technologies cDNA applications poster, this image shows a sequence alignment of long nanopore reads to the *EWSR1* gene, associated with various forms of cancer, which enabled both the breakpoint junction and the fusion partner to be identified. Read poster: nanoporetech.com/poster-cdna-biology

Also leveraging the Flongle for its *'low per-run unit cost'*, Watson *et al.* based at St James's University Hospital in Leeds, UK, assessed the performance of nanopore sequencing to identify a *PMS2* insertion-deletion mutation associated with Lynch syndrome[3], a genetic predisposition to different cancer types. The variant under study proved difficult to validate using Sanger sequencing due to the complexity of the surrounding genomic sequence. Watson and colleagues demonstrated 100% sequence identity following pairwise comparison between a verified benchmark sequence and consensus assembly of nanopore reads. Furthermore, results from the bioinformatic analysis were much *'simpler to interpret'* than the chromatograms generated using orthogonal techniques. Taken together, Watson *et al.* concluded that long-read capable devices, such as the Flongle are, in the future, *'likely to become essential tools in diagnostic genetics'**.

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Products used

Kit	Ligation Sequencing Kit
Device	Flongle
Tools	Minimap2 | Nanopolish

Find out more: nanoporetech.com/products

Figure 2
Flongle is an adapter for MinION and GridION devices that enables cost-effective, real-time sequencing on smaller, single-use flow cells.

NANOPORE SEQUENCING

- Enabled rapid detection of large genomic rearrangements using Flongle
- Provided rapid low cost assays with user friendly bioinformatic pipelines

> ***"In two cases, a CIC-DUX4 translocation that was not initially identified by the [short-read] sequencing pipeline were identified by Flongle[3]"***

Find out more about clinical research using nanopore sequencing: nanoporetech.com/applications/clinical-research

References

1. Arai, Y. et al. *Hepatology.* 59, 1427-1434 (2014).
2. Jeck, W., Iafrate, A. and Nardi, V. *The Journal of Molecular Diagnostics.* 23(5):630-636 (2021).
3. Watson, C. et al. *Cancer Genetics.* 256-257:122-126 (2021).

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