Comprehensive and routine analysis of leukaemia samples

Compared to current methods for detecting gene fusions, such as real-time PCR and fluorescence *in situ* hybridisation (FISH), which take days or even weeks to perform, nanopore technology offers the advantages of rapid sequencing with real-time data generation.

With the aim of rapidly detecting and characterising a variety of oncogenic fusion events, including the *BCR-ABL1* fusion (which is present in nearly all patients with chronic myeloid leukaemia [CML]), Jeck *et al.* developed a workflow based on a modified Anchored Multiplex PCR (AMP) method for library construction. Initial proof of concept experiments in the K562 cell line using gene-specific primers against *BCR* exons 1 and 2, along with sequencing on the MinION, demonstrated the ability of this technique to precisely delineate the *BCR-ABL1* fusion junction (**Figure 1**)[1]. Furthermore, real-time analysis enabled confident detection of the fusion within just 5 minutes, with the first fusion read being generated within five seconds.

Based on these promising results, Jeck *et al.* applied an expanded AMP-based assay, targeting an array of oncogenic fusions, to a number of haematological malignancy specimens. Various fusion events were detected including *PML-RARA* — the hallmark of acute promyelocytic leukaemia.

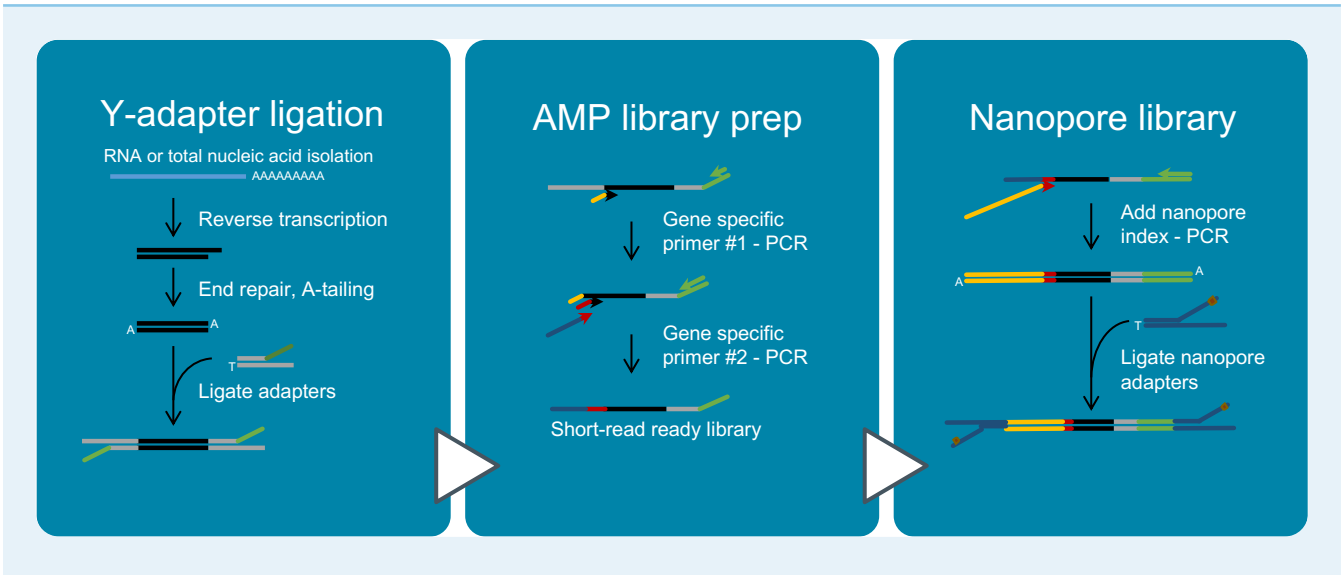

Figure 1
Method of library construction for MinION sequencing using Anchored Multiplex PCR (AMP). Adapted from Jeck *et al* [1].

Sensitivity to this critical fusion was 100% in clinical research samples, even with a 1:10 dilution specimen. When multiplexing four samples on a fresh flow cell, all fusions could be detected within 6 hours of sequencing.

Application of this assay to previously characterised libraries from formalin-fixed, paraffin-embedded (FFPE) sarcoma specimens detected a range of gene fusions with high specificity, demonstrating that the problem of fragmentation and lower DNA quality in FFPE specimens did not impede accurate fusion detection using nanopore sequencing. Furthermore, the fraction of MinION reads mapping to a given fusion was higher than that observed with traditional short-read sequencing technology in all but one case, which the authors suggest is likely due to longer read length. The authors concluded that *'…nanopore sequencing has great promise as a broad fusion detection platform…'*[1].

In a slightly different approach, Cumbo *et al.* compared FISH followed by Sanger sequencing to long-range template multiplex PCR and nanopore sequencing, to analyse *BCR-ABL1* DNA fusions[2]. With a sequencing depth of 400x over the *BCR* region, the team observed concordance between the nanopore and Sanger sequencing results in all CML samples studied, stating that *'…the very low costs, the ease of use, and the length of the reads (hundreds of kilobases), make MinION an ideal tool for target sequencing'* [2].

Researchers from the Fred Hutchinson Cancer Research Centre in Seattle, aimed to develop a single method allowing the detection and analysis of mutations in *FMS-like tyrosine kinase 3 (FLT3)*, a tyrosine kinase receptor involved in haematopoietic cell proliferation, differentiation, and apoptosis[3]. Duplications in *FLT3* are associated with aggressive acute myeloid leukaemia (AML). The quick workflows and real-time data acquisition afforded by nanopore sequencing offers a distinct advantage over traditional sequencing technologies. The researchers designed an RNA amplicon sequencing assay producing a 2,400 bp product covering well-defined hotspot regions in *FLT3*. MinION sequencing provided rapid acquisition of full-length reads, which allowed the reliable detection of internal tandem duplication mutations[3].

Find out more about sequencing for clinical research at www.nanoporetech.com/applications

References

1. Jeck, W.R. et al. A nanopore sequencing–based assay for rapid detection of gene fusions. J Mol Diagn. S1525-1578(17)30630-X (2018).
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3. Yeung, C. and Sala-Torra, O. Clinical applications for real-time sequencing in leukaemia. Presentation. Available at: https://nanoporetech.com/resource-centre/direct-rna-cdna-sequencing-human-transcriptome. [Accessed: 07 February 2019]