Real-time metagenomic outbreak surveillance

Field-based analysis of human pathogens

The facility to take sequencing to the sample source is a significant advantage for outbreak surveillance, saving considerable time and supporting the rapid implementation of control measures. After successful deployment for genomic surveillance of recent Ebola[1], Zika[2], and Yellow fever[3] outbreaks, Kafetzopoulou *et al.*[4] utilised nanopore sequencing for on-site metagenomic sequencing and real-time analysis of a major Lassa fever virus (LASV) outbreak in Nigeria. Over seven weeks, the team sequenced 120 infected samples, revealing extensive diversity and phylogenetic intermingling with strains from previous years. The results suggested that rodent hosts were the main source of the upsurge in cases, allaying fears of human-to-human transmission. The unbiased metagenomic sequencing approach deployed by the team also allowed, in one sample, the identification of co-infection with Hepatitis A virus. The results from this study were immediately reported to the WHO and Nigerian authorities, supporting a rapid public health response to the outbreak.

Disease surveillance and control in livestock

As infectious diseases caused by diverse pathogens (e.g. viruses, bacteria, fungi, and parasites) can present with the same or similar symptoms, metagenomic approaches to pathogen identification —where no *a priori* knowledge of the infectious organism is required — hold much promise for timely detection and therapeutic intervention. Many researchers have demonstrated the suitability of portable, real-time nanopore sequencing for advancing veterinary medicine, through on-site identification of pathogenic infections.

> ***'Portable metagenomic sequencing of genetically diverse RNA viruses on the MinION...and with no pathogen-specific enrichment, is shown to be a feasible methodology enabling a real-time characterization of potential outbreaks in the field'***[4]

In one recent example, researchers from the U.S. Department of Agriculture (USDA) used metagenomic nanopore sequencing to identify African swine fever virus (ASFV) in infected pigs[5]. ASFV is a large double-stranded DNA virus (170-193 kb), with high mortality rate and rapid spread. The last decade has seen significant geographical spread of the virus, presenting a severe threat to global pig farming. According to O'Donnell *et al.*[5], early detection of infection is essential to controlling outbreaks and preventing further infection, but current sequencing-based techniques are limited by time- and resource-intensive protocols, significant capital investment, and lengthy sequencing runs. To overcome these challenges, the USDA utilised the MinION to perform real-time sequencing of samples from infected pigs.

Methylated host DNA was depleted using a commercially available microbiome enrichment kit, increasing the proportion of unmethylated viral DNA. The team reported that, for enriched samples, sufficient data was available for complete ASFV genome resolution within 10 minutes of initiating the sequencing run. This compares favourably with unenriched samples that required 64 minutes of sequencing for complete genome coverage. Rather than deploy taxonomic classification of all sequence data, the team developed ASF-FAST, which maps the sequence data in real time against an ASFV reference genome, resulting in rapid generation of a reference-guided genome assembly. Together with their ASF-FAST data analysis pipeline, nanopore sequencing dramatically reduced the time required for pathogen identification and characterisation (**Figure 1**).

Rapid metagenomic analysis of porcine pathogens has also been reported by both Theuns *et al.*[6] and Sutton *et al.*[7] who identified the causative agents in enteric disease and congenital tremor samples, respectively.

> ***'This work demonstrates the utility of this technology for sequence-based diagnostics, supporting effective emergency management in the event of an outbreak of the [ASF] disease'***[5]

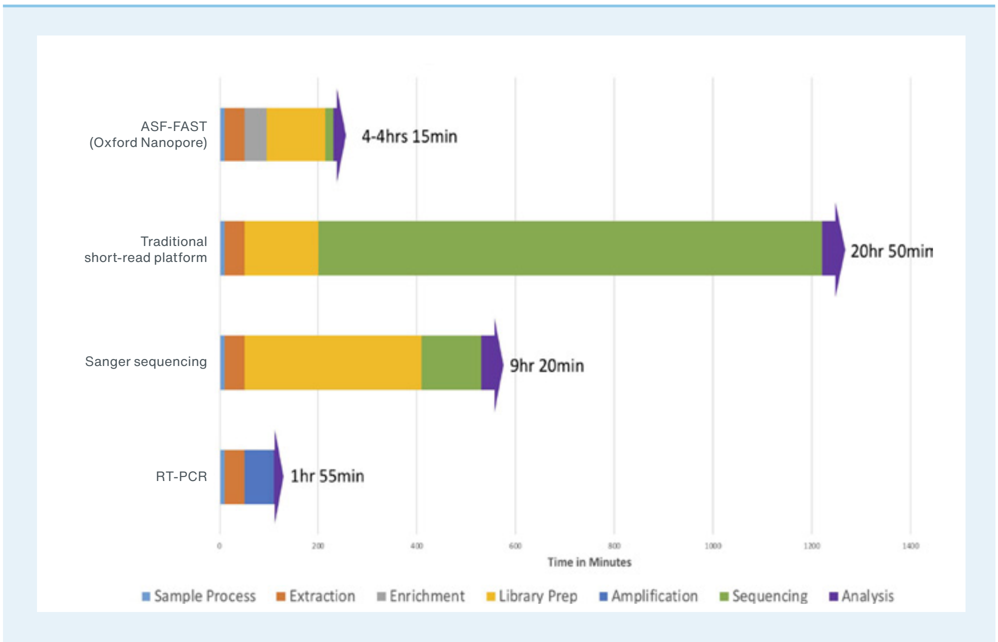

Figure 1
Comparison of the timelines for different ASFV detection methods. Metagenomic sequence-based analysis, as provided by nanopore and short-read sequencing technologies, delivers more comprehensive and informative genomic information than that offered by Sanger sequencing and RT-PCR. Figure adapted from O'Donnell *et al.*[5]

Find out more about metagenomic sequencing at www.nanoporetech.com/applications

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