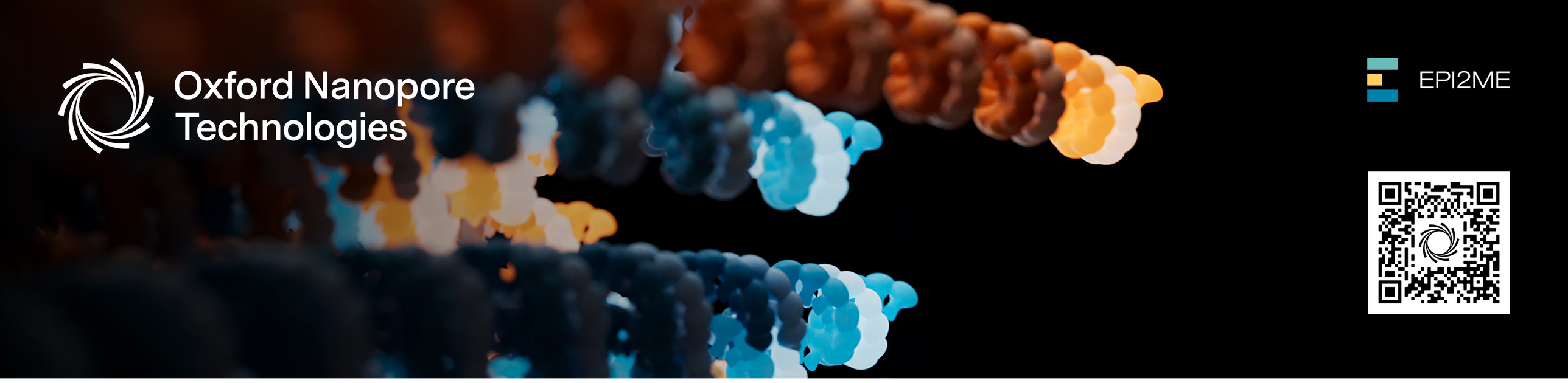

Quick and simple analysis of PCR products with Oxford Nanopore reads

wf-amplicon — use Oxford Nanopore sequencing to generate a *de novo* consensus of your amplicons or call variants against a known reference

Contact: **support@nanoporetech.com**
More information at: **epi2me.nanoporetech.com/epi2me-docs/workflows/wf-amplicon**
Data used in this analysis is available to download from **epi2me.nanoporetech.com/lc2024-datasets**

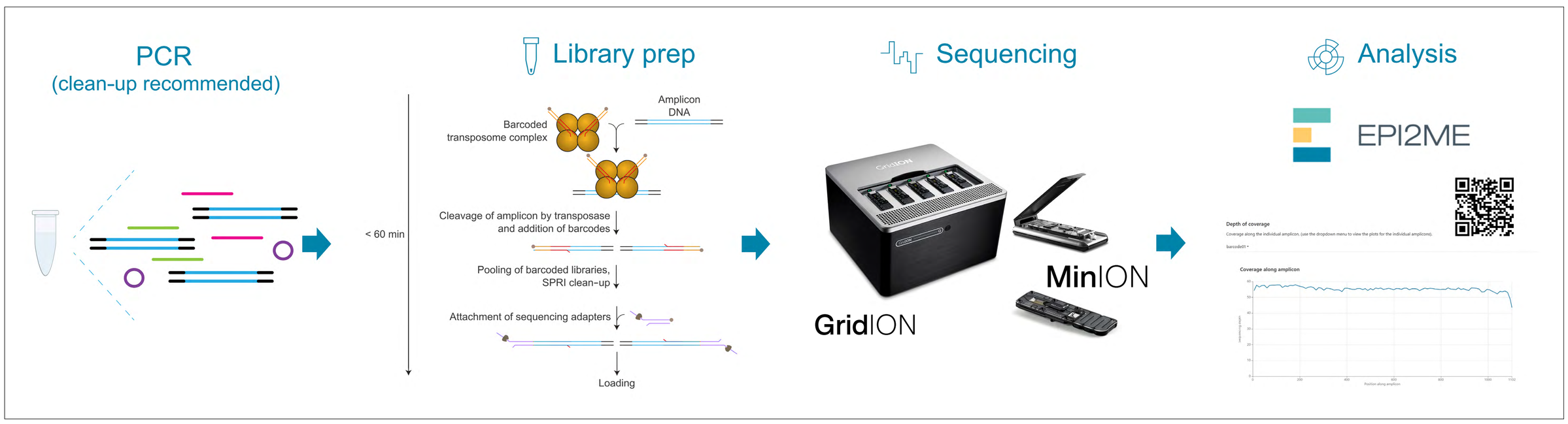

Fig. 1 End-to-end workflow for Oxford Nanopore sequencing of amplicons.

Our end-to-end workflow: a simple, cheap, and quick (<60 minutes library prep time) way to sequence amplicons from 500 to 5,000 bp

Using Sanger sequencing to analyse amplicons leaves much to be desired. Most importantly, it has a maximum read length of about 800 bp. It also struggles with certain types of repeats and sequencing several amplicons at the same time is not possible. The EPI2ME™ wf-amplicon workflow can be used to analyse nanopore sequencing data created from amplicons, for example with the Rapid Barcoding Kit (RBK) end-to-end workflow (Fig. 1). wf-amplicon can be run in two different modes (variant calling mode and *de novo* mode), depending on whether reference sequences of the target amplicons are available. In variant calling mode, reads are trimmed before alignment against the reference and variant calling. The variants are then incorporated into the reference to create a consensus sequence. In *de novo* mode, the consensus sequence is assembled from the trimmed reads and then polished. For extra multiplexing, several different amplicons can be pooled using the same barcode in variant calling mode (if they are dissimilar). This is not possible in *de novo* mode.

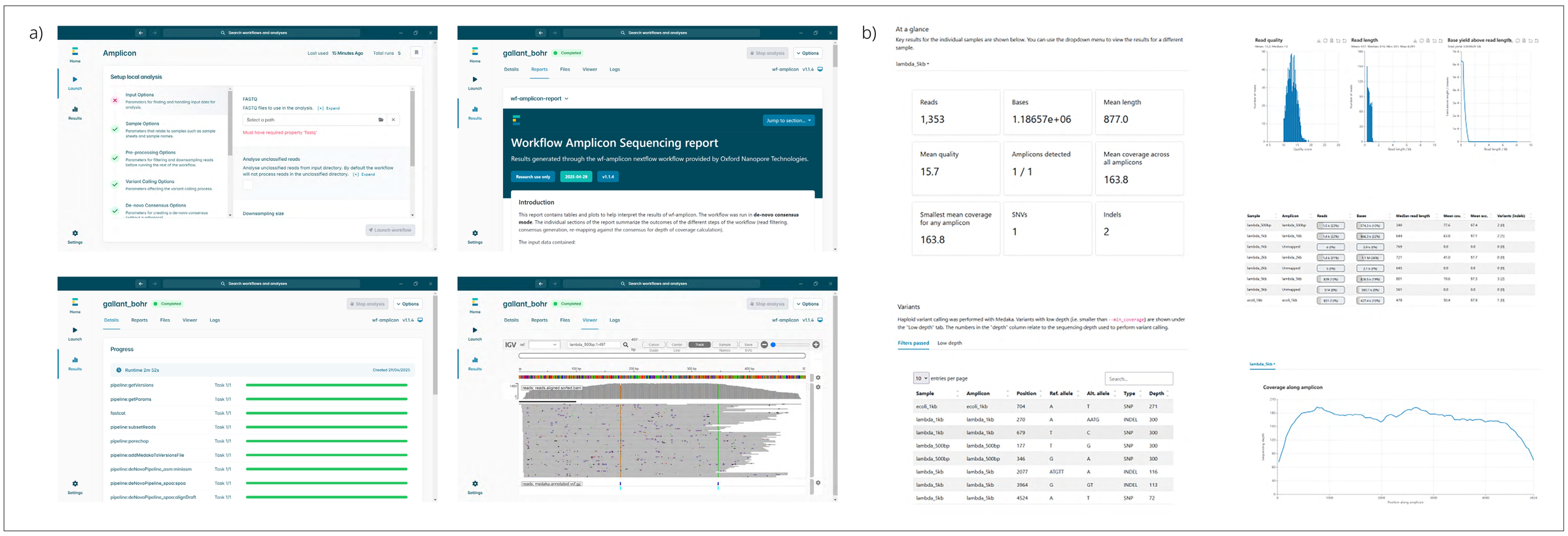

Fig. 2 Use the EPI2ME Desktop Application to analyse your data (on a local computer or in the cloud) and view the results in an interactive report and embedded Integrative Genomics Viewer (IGV) panel.

Not just VCF files; wf-amplicon creates a diverse set of outputs

wf-amplicon can be run via either the cross-platform EPI2ME Desktop Application (Fig. 2a) or using the command line. In both cases, after selecting the input data (basecalled reads of one or more samples in FASTQ or BAM format), the progress of the analysis is displayed before it produces an interactive HTML report upon completion. The report illustrates the analysis results and can be viewed directly in the desktop application or any modern browser. The application also features an embedded IGV panel, which can be used to visualise the aligned reads and any called variants. The outputs shown in Fig. 2b were produced from a selection of five amplicons with lengths between 500 and 5,000 bp (0.5, 1, 2, and 5 kb from lambda phage; 1 kb from *E. coli*). They include summary statistics, histograms of read length and quality, as well as tables of alignment statistics and the called variants. In *de novo* mode, the elements of the report related to variant calling are replaced with information about the assembled consensus. The outputs also include the BAM and VCF files generated by the workflow.