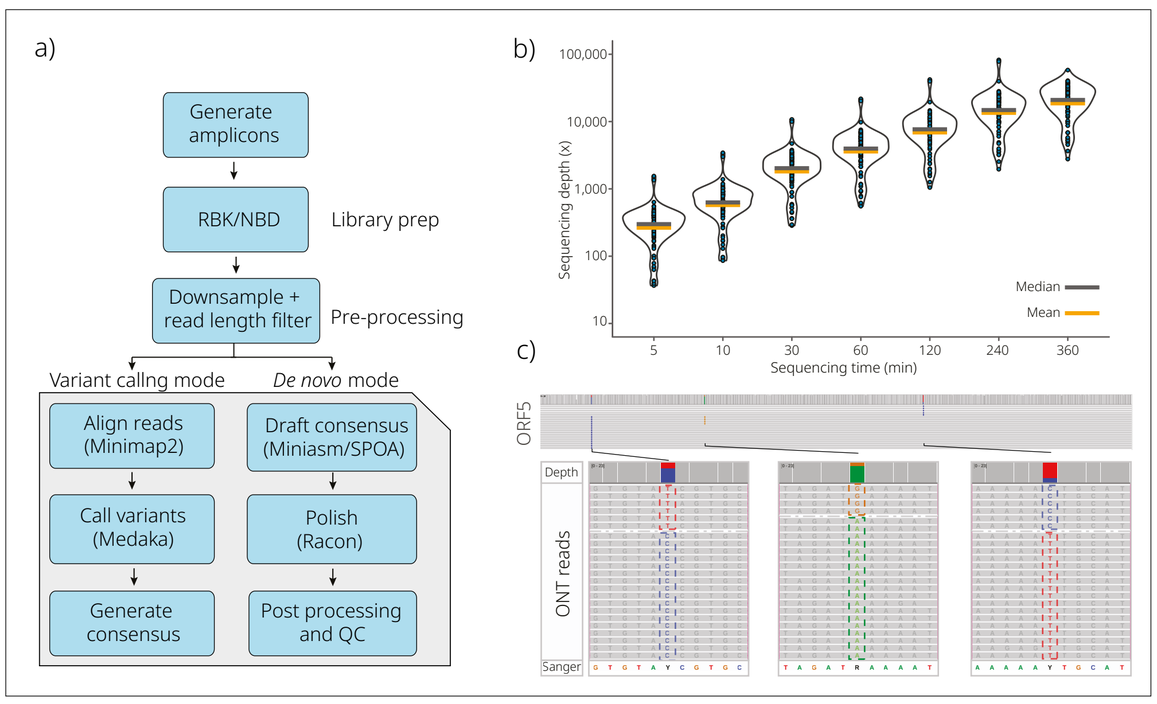
Oxford Nanopore Technologies

# Easy transfer of routine, day-to-day sequencing applications to the Oxford Nanopore platform

Sequencing applications traditionally performed on capillary-based systems can be easily transferred to the Oxford Nanopore platform and supported by end-to-end workflows with downstream data analysis solutions



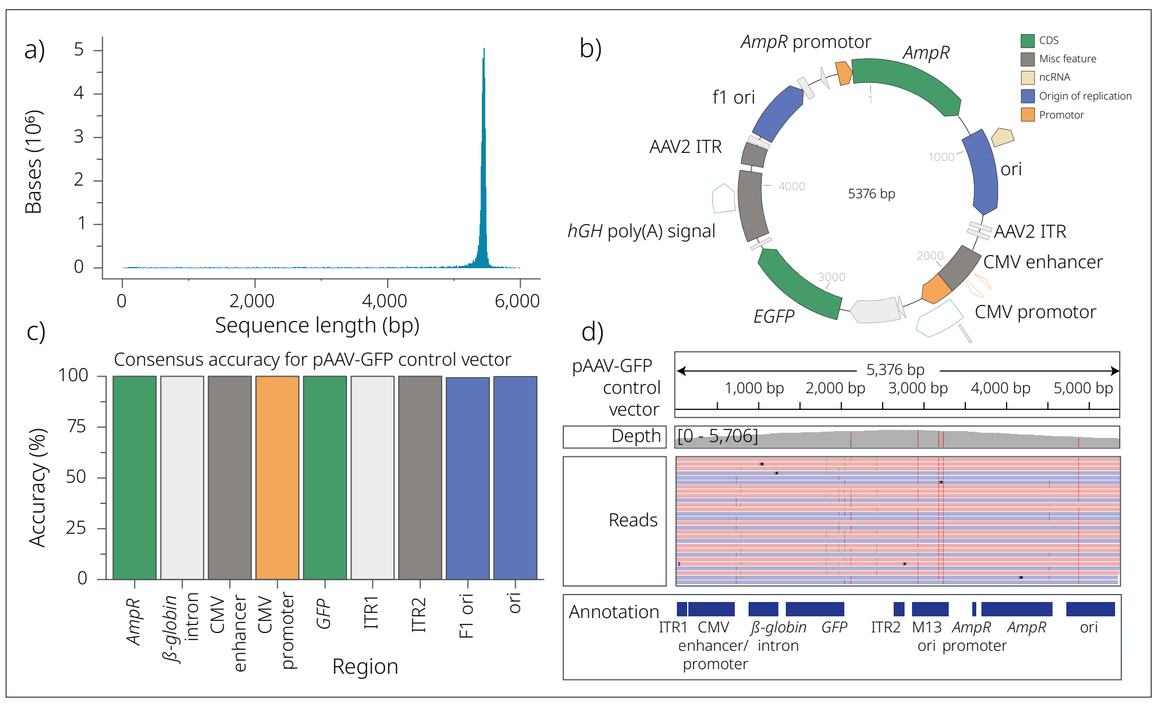




**Fig. 1** a) Consensus workflow b) sequencing time and depth c) resolving ambiguity in Sanger data.

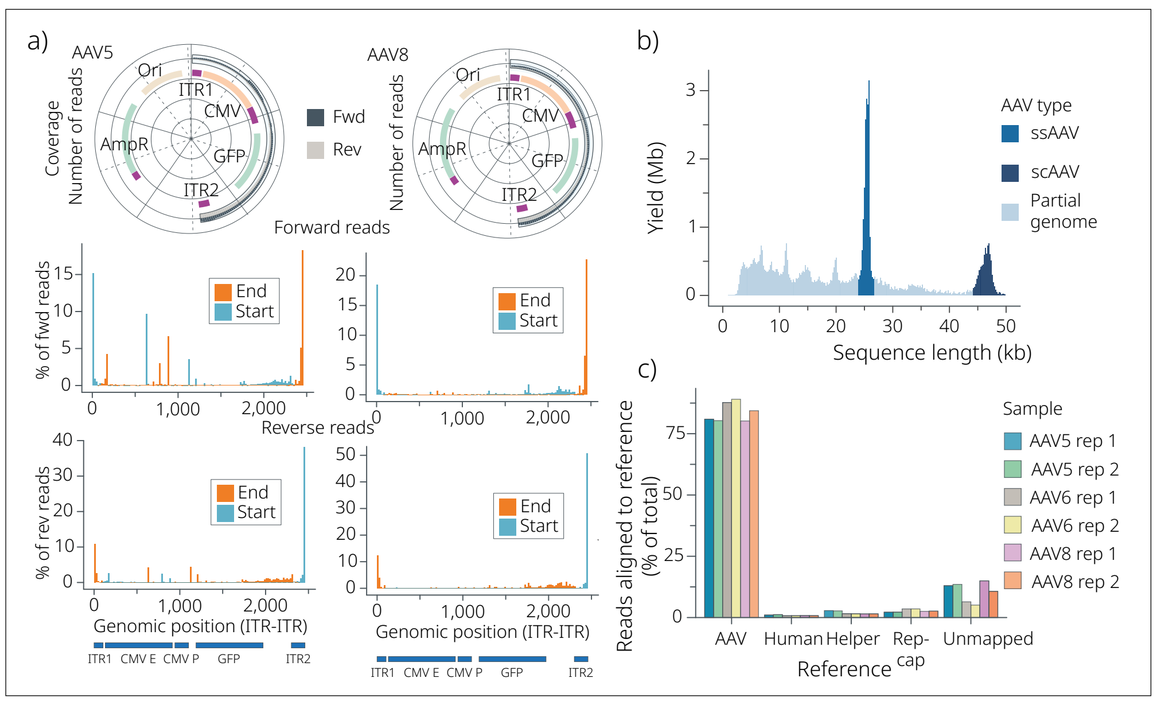
**Fig. 2** a) Read length b) plasmid map c) accuracy d) coverage.

## Scalable analysis of amplicons using EPI2ME™ wf-amplicon

The use of PCR to amplify targeted regions of a genome is a common procedure carried out across a wide range of molecular biology applications. Barcoding by either rapid or native approaches allows for scalable sequencing on the Oxford Nanopore platform. Using EPI2ME wf-amplicon (**Fig. 1a**), the *de novo* consensus sequence of an amplicon targeting the ORF5 region of the porcine reproductive and respiratory syndrome virus (PRRSV) was generated. Downsampling shows that even sequencing for 2 hours is sufficient to generate 1,000x coverage for 94 amplicons (**Fig. 1b**). Investigating a sample with ambiguous bases in the Sanger consensus, nanopore reads revealed the presence of three populations with distinguishable SNVs (**Fig. 1c**).

## Validation of plasmid constructs using EPI2ME wf-clone-validation

We sequenced a typical control AAV transgene plasmid (pAAV-GFP) following the end-to-end protocol, preparing the sample using the Rapid Barcoding Kit and performing data analysis using EPI2ME wf-clone-validation. Read length distributions show a peak at ~ 5.3 kb indicating a single-cut, full-length pAAV-GFP plasmid (**Fig. 2a**). We used the EPI2ME workflow to create a plasmid map identifying the presence of the transgene and ITR sequences (**Fig. 2b**), in addition to identifying all key plasmid components with high accuracy when compared to the manufacturer's reference (**Fig. 2c**). Alignments show even coverage indicating that circular plasmids were cut once with the rapid barcoding transposase (**Fig. 2d**).



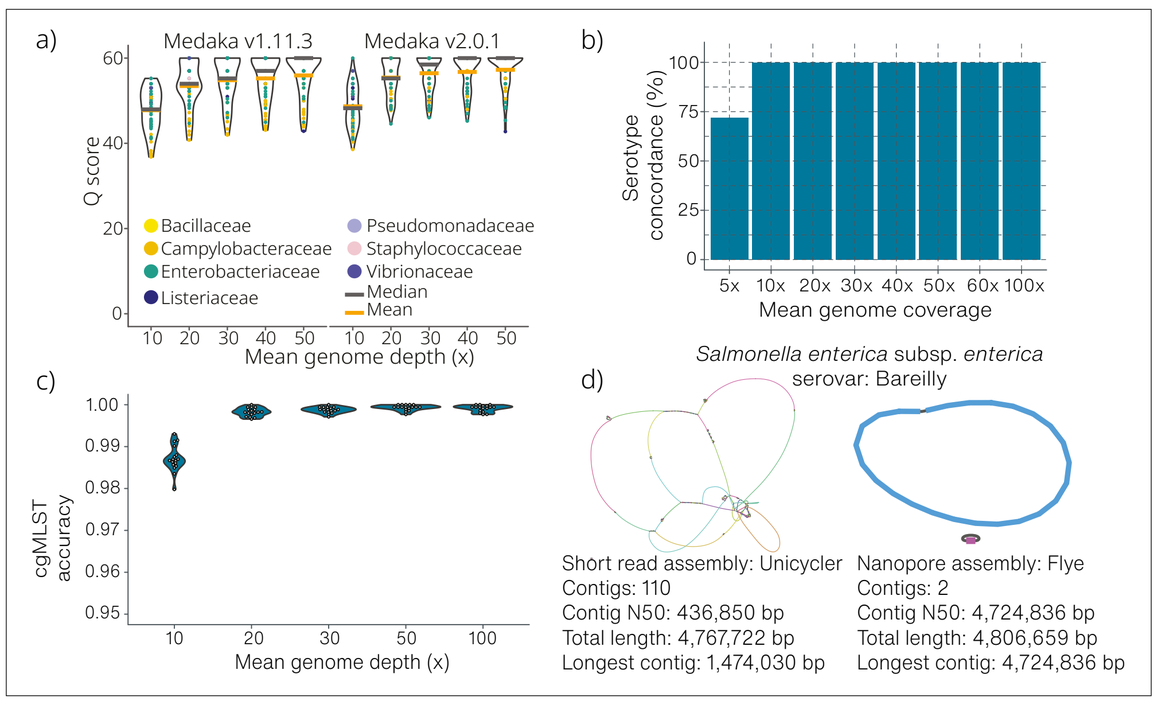




**Fig. 3** a) Coverage and truncation events b) read lengths c) contaminants by read alignments.

**Fig. 4** a) Accuracy b) *Salmonella* serotype c) cgMLST d) short- and long-read assembly.

## Characterisation of AAV genomes using EPI2ME wf-AAV-QC

Identifying truncation hotspots and generating consensus sequence of the adeno-associated virus (AAV) genome are common requirements for quality control of AAV preparations. Calculating the sum of the start and stop positions of primary read alignments can identify truncated genomes and their locations. Here the AAV5 sample shows truncations in the CMV promoter, which are absent in the AAV8 sample (**Fig. 3a**). Read length distributions for AAV5 show a mixed population of ssAAV and scAAV along with potential truncation events (**Fig. 3b**). To identify potential sources of vector contamination, we aligned reads to the host, helper, and rep-cap plasmids, identifying potential carryover of the material during manufacturing (**Fig. 3c**).

## Bacterial isolate analysis using EPI2ME wf-bacterial-genomes

Nanopore-only bacterial isolate genome assembly was benchmarked with Medaka v2.0.1 against hybrid nanopore-short read genome assemblies (n=61). Nanopore-only assemblies generated with Medaka v2.0.1 were improved compared with the previous version, achieving Q56/Q58.5 at 30x, and Q56/Q60 at 50x (**Fig. 4a**). *Salmonella* serotyping of 32 isolates spanning a range of closely related taxa showed complete concordance with known serotypes at 10x (**Fig. 4b**). Core genome MLST profiles exceeded 99.5% concordance at 20x coverage (**Fig. 4c**). Comparing short-read and Oxford Nanopore-only assemblies on the same isolate showed complete circular contigs were generated using the nanopore platform only (**Fig. 4d**).