

Validating a molecular cloning experiment using *de novo* assembly of nanopore reads

wf-clone-validation – for quickly verifying artificial constructs such as plasmids, bacterial artificial constructs (BACs) and fosmids

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More information can be found at: labs.epi2me.io/workflows/wf-clone-validation/

Data used in this analysis is available to download from labs.epi2me.io/lc2024-datasets

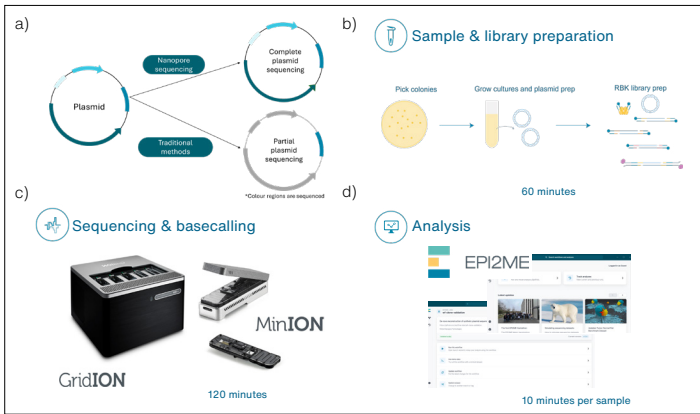


Fig. 1 a) Traditional methods vs clone validation end-to-end workflow b) Sample and library preparation diagram c) sequencing and basecalling devices d) EPI2ME™ application for analysis

End-to-end solution

Traditional methods for validation of cloned vectors usually only consider a small section of the construct and ignore the rest. Therefore, they can miss changes in the vector backbone, such as recombination and mutations, or insertion failure (Fig. 1a). The end-to-end clone-validation workflow overcomes these issues by assembling whole constructs, ranging in size from 1,500 to 300,000 bp (Fig. 1b,c,d). To demonstrate its utility, a plasmid containing non-coding DNA as an insert was prepared with the Rapid Barcoding Kit (RBK) and sequenced on a GridION™. Basecalling was performed using the Dorado dna_r10.4.1_e8.2_400bps_hac@v4.2.0 HAC model and the FASTQ files were analysed with the EPI2ME™ wf-clone-validation workflow on a standard laptop (16 GB RAM; 8 CPUs).

