Rapid identification of variants associated with cystic fibrosis using a long-read multiplexed amplicon panel

Simultaneous amplification of twenty four ~1.5 kb regions covering all major cystic fibrosis mutations allows rapid detection of variants and unambiguous identification of paralogous genes

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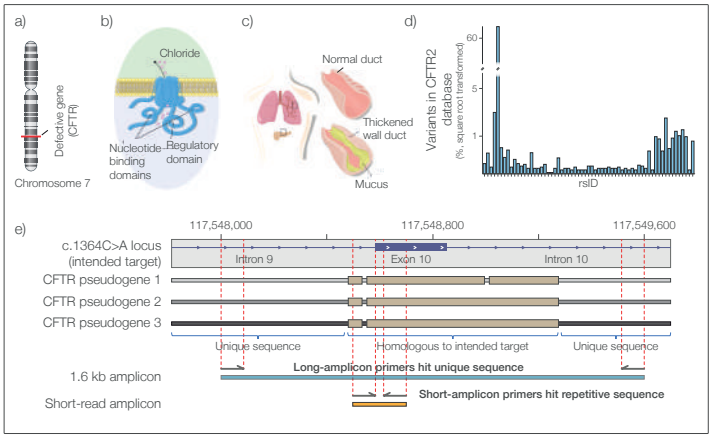

Fig. 1 Cystic fibrosis a) locus b-c) condition d) mutations e) avoiding pseudogenes

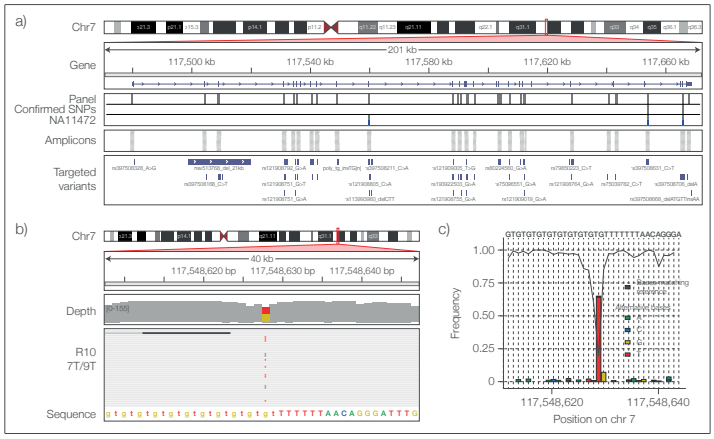

Fig. 2 24-plex long-amplicon panel a) variant-calling b) and c) resolving homopolymers with R10

Cystic fibrosis is a hereditary disease affecting the lungs and digestive systems

Cystic fibrosis is a recessive genetic condition caused by mutations to the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein (Fig. 1a), which controls movement of salt and water into and out of cells (Fig. 1b). Patients are prone to breathing difficulties and infections (Fig. 1c) and have reduced life expectancy. There are many disease-related CFTR mutations (Fig. 1d). Detecting these variants provides a way to screen populations, confirm clinical diagnosis, determine treatment options and to perform prenatal diagnosis. Sequencing long amplicons allows unambiguous identification of paralogous genes (Fig. 1e).

Resolving the intron 9 poly-T homopolymer tract variants using the R10 pore

We constructed a multiplexed PCR panel consisting of twenty four ~1.5 kb amplicons covering all major CFTR variants. We called single nucleotide variants and small indels from human cell line NA11472 using *Clair* and correctly identified all confirmed heterozygous SNPs as well as an unreported SNP (Fig. 2a). The intron 8 poly-T tract represents a more challenging variant to detect, consisting of 5, 7 or 9 consecutive Ts followed by a dinucleotide repeat. Our new nanopore, R10 enables resolution of longer homopolymers, such as the (T)9 heterozygous homopolymer, which can be seen in approximately half of the aligned sequences (Figs. 2b and

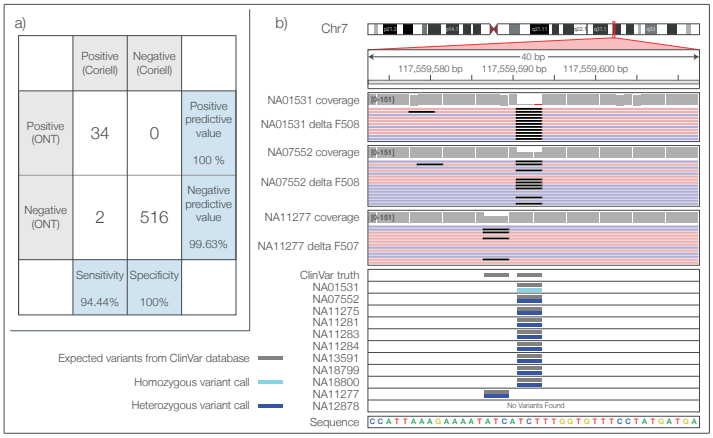

Fig. 3 Variant-calling using the MUTCF-2 panel a) overall results b) delta F507 and F508 variants

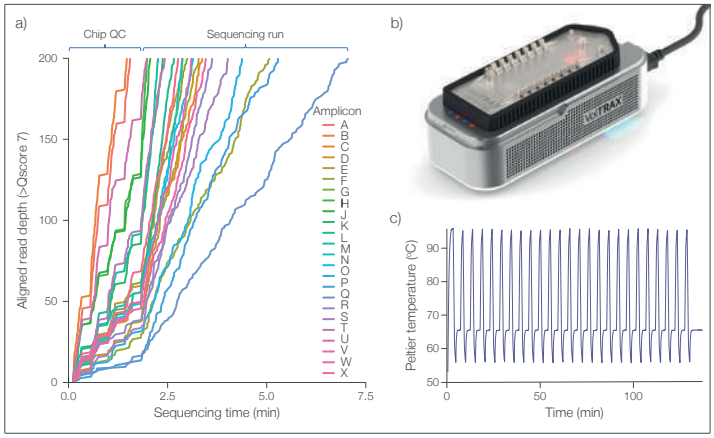

Fig. 4 a) real-time data generationon, b-c) the VolTRAX device and PCR temperature profile

Calling variants in the MUTCF-2 sample panel with high specificity and sensitivity

The MUTCF-2 panel consists of 23 samples, each with a unique CF-related variant. We used the panel to determine variant-level specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) for the multiplexed PCR assay (Fig. 3a). The multiplexed PCR was performed on each sample individually along with a negative sample (NA12878) and libraries were barcoded, pooled and run on a MinION flow cell. Variant calling was performed with *Clair* after down-sampling to a maximum read depth of 150 per amplicon. We achieved a sensitivity of 94.44% and specificity of 100%, with a PPV of 100% and NPV of 99.63%. Figure 3b shows the most common pathogenic variant (delta F508) in homozygous and heterozygous form.

PCR of the 24-plex CFTR panel on VolTRAX, a portable library preparation device

Because sequencing on nanopore devices takes place in real time, sufficient coverage for variant-calling can be obtained from all amplicons in around five minutes of sequencing (Fig. 4a). VolTRAX is a portable, USB-powered library preparation device which can perform a wide range of molecular biological manipulations without the need for human intervention (Fig. 4b). VolTRAX utilises an array of pixels and by applying a charge, reagent and sample droplets are moved in a path programmed by software, which allows library preparation processes to be performed sequentially. The device is also capable of heating and cooling droplets, enabling PCR (Fig. 4c). We successfully performed the 24-plex CFTR PCR on VolTRAX in ~3 hours in a volume of 10 µl.

NA11742 genomic DNA and the MUTCF-2 panel were obtained from the Coriell Institute for Medical Research