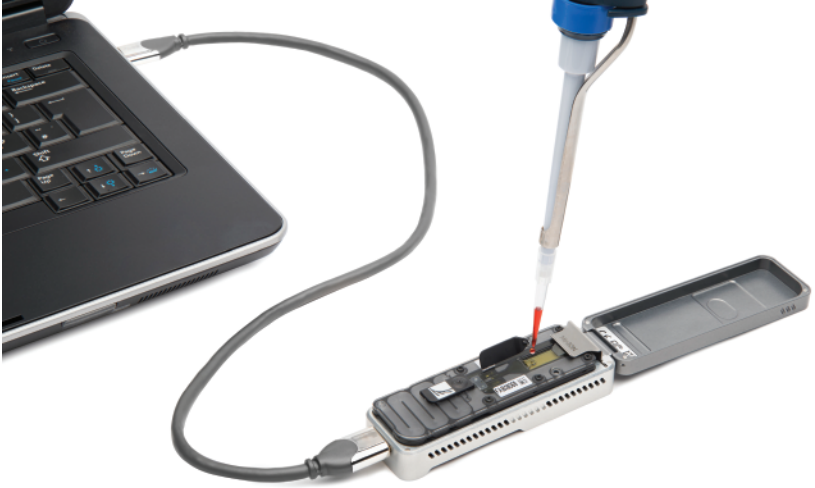

Potential for self-monitoring of chronic myelogenous leukaemia gene fusion using VolTRAX™ and MinION™

Quantitative home-monitoring of the BCR-ABL1-expressing Philadelphia chromosome by automated library preparation on VolTRAX, sequencing on MinION and analysis

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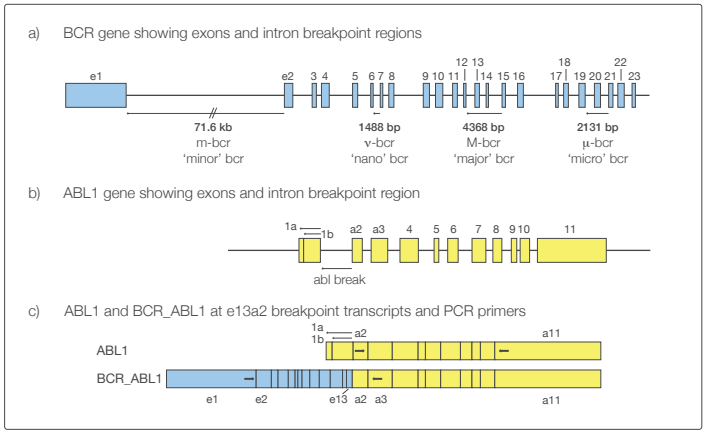


Fig. 1 Schematic representation of the BCR and ABL1 genes that break and fuse

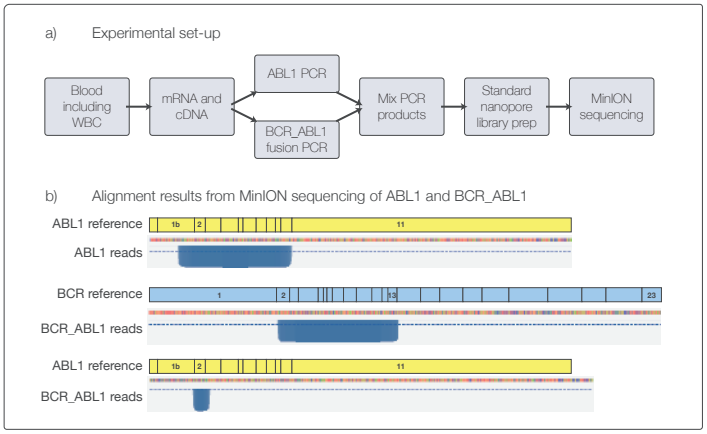
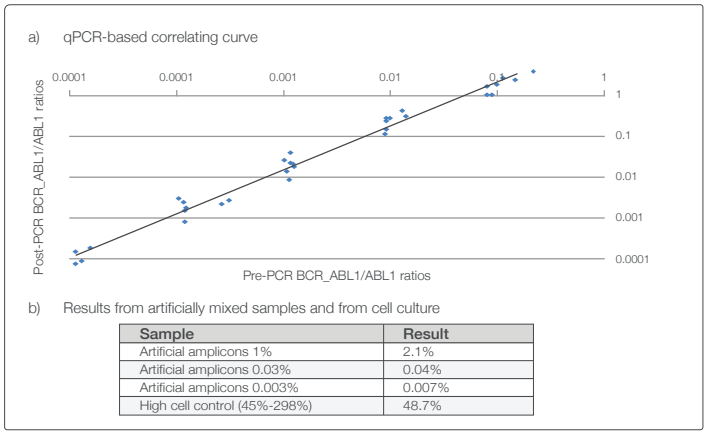


Fig. 2 Alignment of MinION reads to ABL1, BCR and BCR_ABL1 reference sequences

Overview of CML: BCR-ABL1 fusion gene arises from Philadelphia translocation

The Philadelphia chromosome is a rearrangement that is present in the white blood cells of 90% of people with chronic myelogenous leukaemia (CML). It arises from a chromosome 9 and chromosome 22 translocation, generating a fusion gene from the breakpoint cluster region (BCR) and the Abelson leukaemia (ABL1) gene. Several breakpoints have been identified in BCR, and the fusion of these different breakpoints to ABL1 results in the production of a non-regulated tyrosine kinase, transforming normal cells to neoplastic CML cells, and leading to unlimited propagation. BCR exon 13-ABL1 exon 2 (e13a2, p210) and BCR exon 14-ABL1 exon 2 (e14a2, p210) have been found in more than 95% of CML patients (Fig. 1).



b) Results from artificially mixed samples and from cell culture

Sample	Result
Artificial amplicons 1%	2.1%
Artificial amplicons 0.03%	0.04%
Artificial amplicons 0.003%	0.007%
High cell control (45%-298%)	48.7%

Fig. 3 Using MinION sequencing reads as a quantitative assay for BCR_ABL1

Detecting BCR_ABL1 fusion and ABL1 cDNA amplicons on MinION

MinION sequencing can be used to detect the presence of the BCR_ABL1 fusion transcript, and by using the normal ABL1 transcript as an endogenous control (EC) we have developed a quantitative assay. After mRNA extraction and cDNA synthesis, we run two gene-specific PCR reactions, each for a limited number of cycles, one amplifying ABL1, and the other amplifying BCR_ABL1. The BCR_ABL1 primers are positioned in such a way that they amplify all fusion breakpoints, making use of the long-read capability of the MinION. Fig. 2 shows alignments of the amplicon sequence data back to the ABL1 and the BCR_ABL1 fusion gene reference sequences.

Fig. 4 Monitoring in a non-laboratory environment

Quantitative monitoring of BCR_ABL1/ABL1 ratios by MinION sequencing

The expression level of the BCR_ABL1 fusion transcript is reduced by successful treatment, but relapses do occur, and sensitive and quantitative methods are required for their detection. RT-qPCR-based methods are commonly used to detect both the BCR_ABL1 fusion and ABL1 transcript, which is used as an endogenous control. In our sequencing-based workflow, the original BCR_ABL1/ABL1 ratio changes after the two gene-specific PCRs, due to different PCR protocols and efficiencies. Therefore, a correlating curve is built from a series of qPCR measurements, allowing conversion from the post-PCR ratio of BCR_ABL1/ABL1 to the pre-PCR ratio (Fig. 3).

MinION and VolTRAX allow testing in non-laboratory environments

The portability of the MinION sequencer, coupled with VolTRAX, our automated sample and library-preparation device, means that it is possible to perform sequencing-based assays outside of a laboratory environment. Together with user-friendly analyses, this could potentially allow testing for chronic conditions like CML to be performed in a doctor's surgery. As well as giving greater control to the patient, and making the testing process more convenient, this approach could minimise the amount of time taken to return results, meaning that any necessary action could be taken within hours of taking the sample, in contrast to the current waiting time of several weeks.