Targeted sequencing of long custom fragments to study exonic, intronic and promoter regions, to determine the phase of two variants and to discriminate the gene from possible pseudogenes.

This technique is highly suitable in cases of:

- strong clinical suspicion involving a single gene
- detection of a single exonic variant (recessive diseases) to search for a second variant,
- prior identification of variant(s) that can only be confirmed and/or phased by sequencing long fragments (unavailable parents, pseudogenes, poorly covered regions, structural variants, etc.)

The Nanopore technique is used routinely in the Eurofins Biomnis laboratory for the study of *MEFV* or *TTR* genes, or for other genetic targets on request. It is now also being used in oncogenetics to study the *PMS2* gene.

Constitutional oncogenetics

Using Nanopore long-read sequencing: the example of the *PMS2* gene

More information

Eurofins Biomnis Ireland - Blackthorn Road

Unit 3, Sandyford Business Centre
Sandyford Business Park - D18 E528
E-mail: sales@ctie.eurofinseu.com
Phone: 1800 303 349
www.eurofins-biomnis.ie

Eurofins Biomnis International

17/19 avenue Tony Garnier - BP 7322 - 69357 LYON Cedex 07 - FRANCE

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Long-read sequencing (or third-generation sequencing) makes it possible to produce significantly longer reads than second-generation sequencing (short-read), i.e. several thousand (or even millions) of base pairs compared with a few hundred *(Figs. 1 and 2)*.

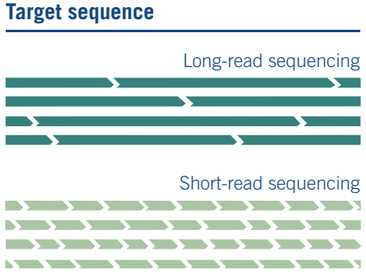

Figure 1 : Genomescan

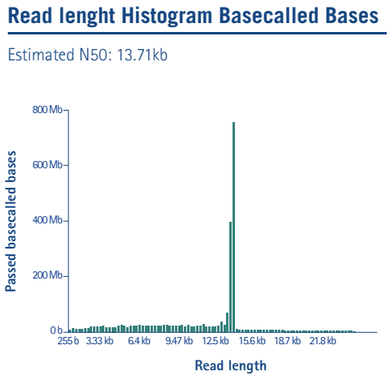

Figure 2: Read size: fragments long fragments >10 kb

Oxford Nanopore technology, which enables very long fragments to be sequenced, is based on the detection of an ionic current passing through biological nanopores inserted into a synthetic membrane. When a DNA molecule passes through the pore, a change in current is detected, enabling the nucleic acid sequence to be identified *(Fig.3)*.

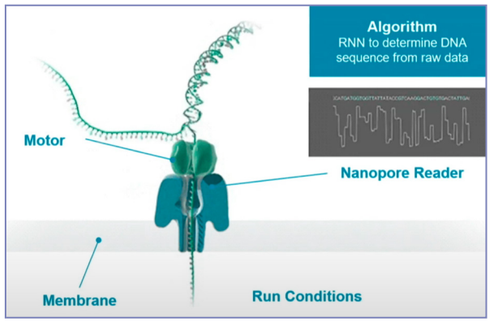

Figure 3: The nanopore sequencing process

The short-read approach has its limitations when it comes to studying genes that share too much sequence homology with other regions of the genome (notably certain pseudogenes).

Several studies have demonstrated the relevance of long-read approaches, which make it possible to circumvent the difficulty posed by sequence homologies[1-2].

In oncogenetics, this is the case for the *PMS2* gene, for example, which has homologies of over 99% with the *PMS2CL* pseudogene for certain exons

Clinical case

12-year-old girl, T lymphoblastic leukaemia.
Haematological malignancy panel negative.

Exome detection of two variants in the gene. *PMS2* gene in non-specific exons:
NM_000535.7:c.1831dup (exon 11)
NM_000535.7:c.137G>T (exon 2)

Variations already reported as pathogenic by the Nanopore technique

- Specific alignment on *PMS2 (fig 4 and 5)*
- Confirmation of the presence of the two variants on the *PMS2* gene and parallel confirmation at the reference centre
- Diagnosis of MMRd syndrome confirmed

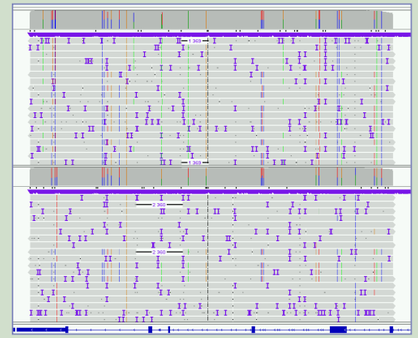

Figure 4: Alignment with *PMS2*

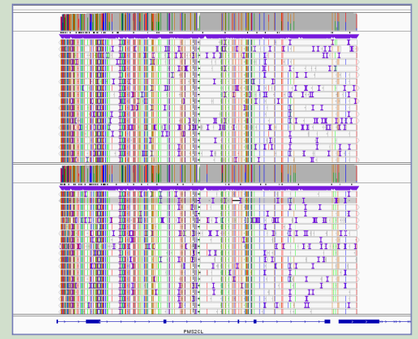

Figure 5: Alignment with *PMS2CL*

1 Watson CM, Crinnion LA, Simmonds J, Camm N, Adlard J, Bonthron DT. Long-read nanopore sequencing enables accurate confirmation of a recurrent PMS2 insertion-deletion variant located in a region of complex genomic architecture. Cancer Genet. 2021;256-257:122-126. doi:10.1016/j.cancergen.2021.05.012

2 The Value of Long Read Amplicon Sequencing for Clinical Applications. Kornelia Neveling et al.

Figure 1 : https://www.genomescan.nl/long-read-sequencing-2/

Figure 2: Molecular study of the MEFV gene using Nanopore sequencing. Eurofins Biomnis.

Figure 3 : Lin B, Hui J, Mao H. Nanopore Technology and Its Applications in Gene Sequencing. Biosensors. 2021; 11(7):214. https://doi.org/10.3390/bios11070214

Figures 4-5: Screenshots of Integrative Genomics Viewer. Eurofins Biomnis.