

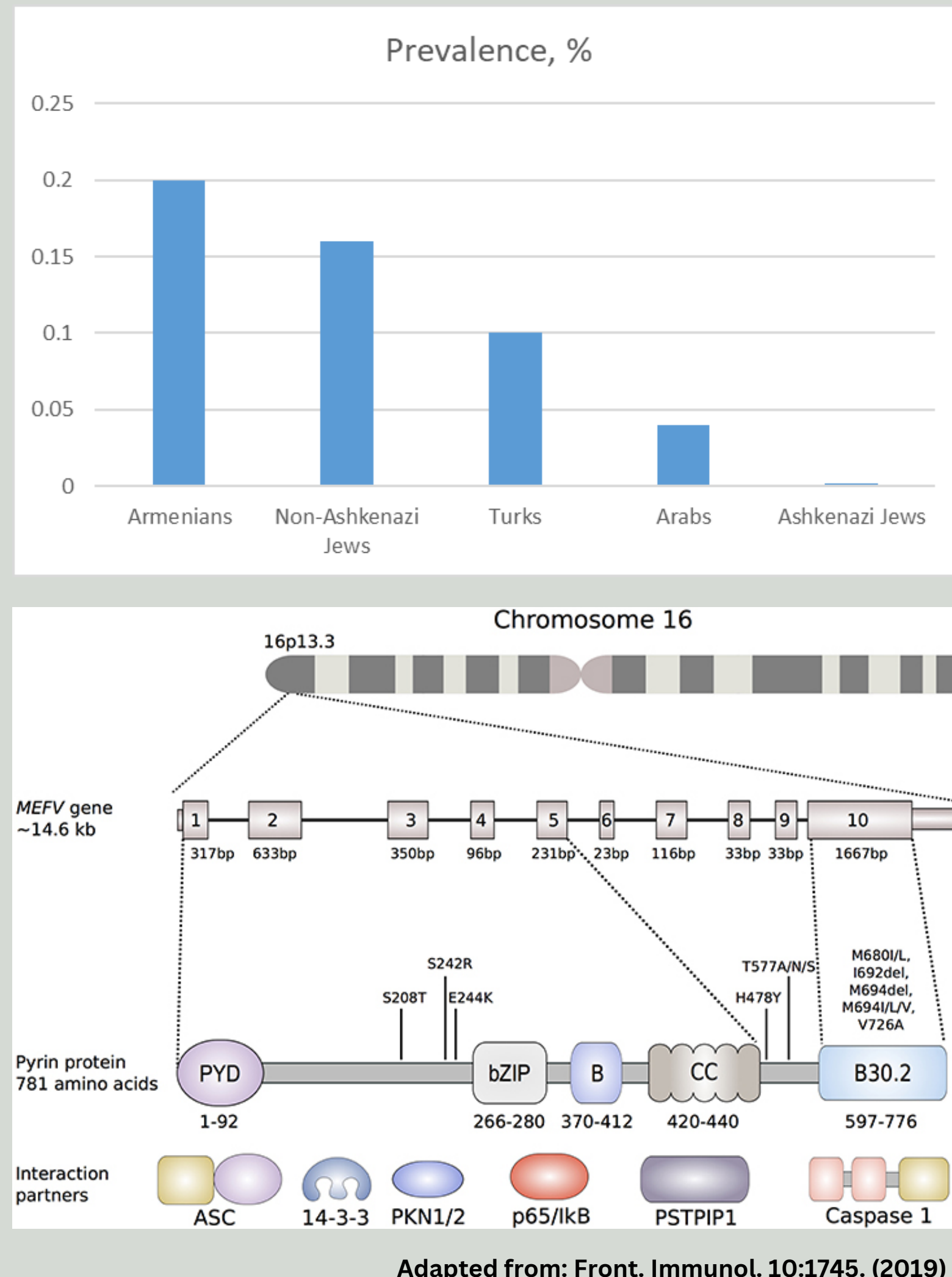
Introduction

Familial Mediterranean Fever (FMF), is a genetic disorder, which primarily affects populations originating in the Mediterranean region, particularly people of Armenian, Arab, Turkish, or Jewish ancestry.

The *MEFV* gene encodes pyrin, which functions as an innate immune sensor that can trigger the formation of an inflammasome, allowing the production of inflammatory mediators during infection. Around 50 mutations in the *MEFV* gene are associated with FMF.

Aim:

Our main aim was to investigate if *MEFV* mutations can be identified with nanopore sequencing.

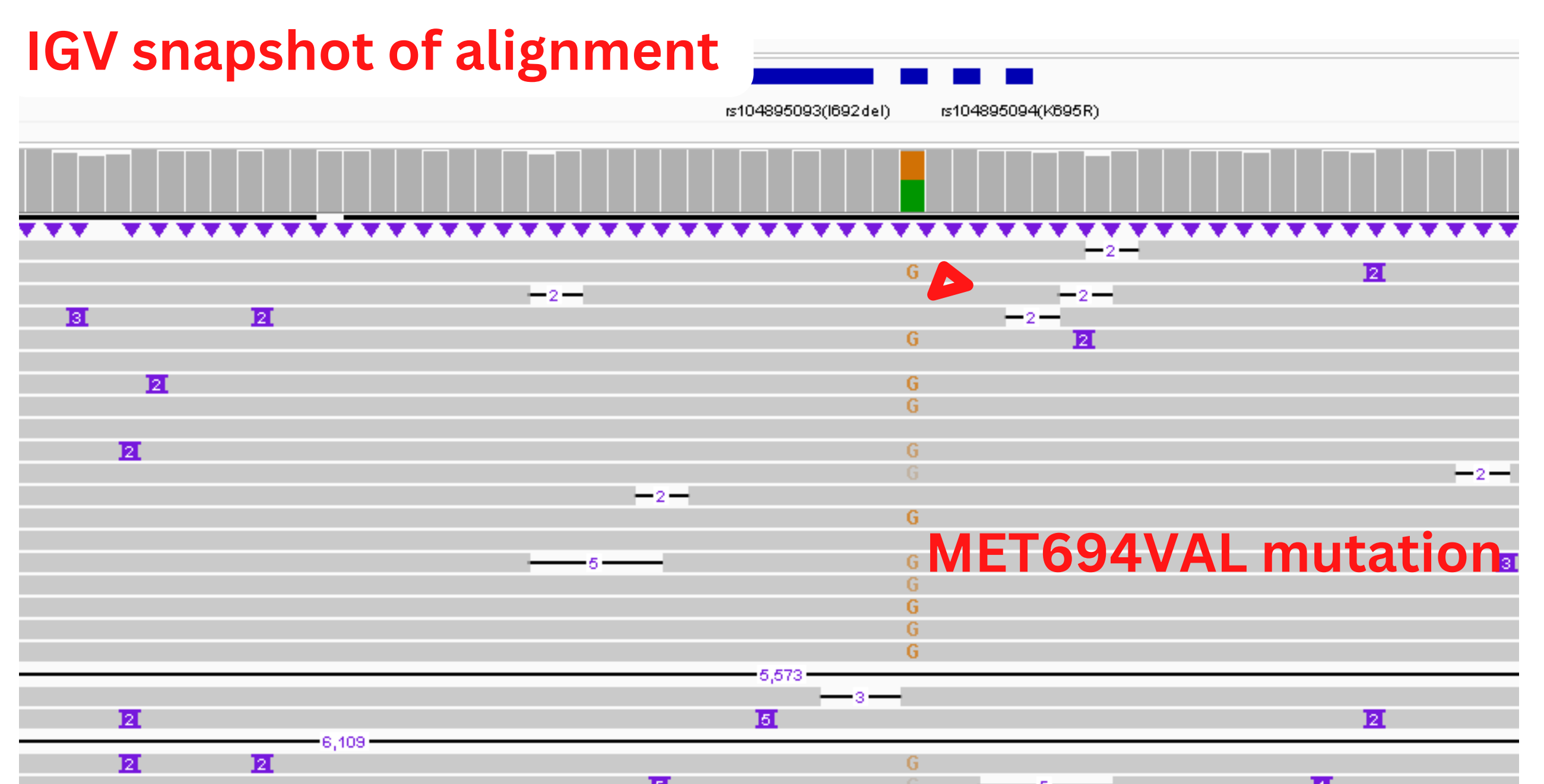


Results and Discussion

DNA samples from two FMF patients (F5 and F11) were used.

PATIENT	INITIAL CONC. (ng/ul)	LIBRARY CONC. (ng/ul)	SEQUENCING SUCCESS
F5	30.8	22.8	YES
F11	4.14	0.430	NO

Patient F5 was found to have 4 mutations in the *MEFV* gene, 3 of which are not known to cause FMF, and the mutation at (MET694VAL) had been proven to cause FMF.



Patient F11 was also found to have the symptoms of FMF, thus the whole process of sequencing was also performed on this sample, however because of low concentration of DNA library, no precise results of mutations were found.

Materials and Methods

The study was approved by the IRB of the Institute of Molecular Biology NAS RA.

Methods:

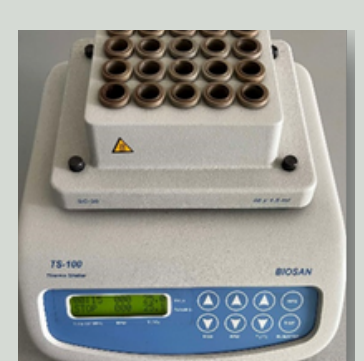
- Oxford Nanopore sequencing
- Electrophoresis
- PCR/ RT-PCR

Materials:

- Oxford Nanopore MinION sequencer
- Electrophoresis machine
- PCR amplifiers
- Qubit fluorometer
- Centrifuge/Microfuge
- Vortex mixer
- Thermocyclers
- Magnetic beads and racks
- Pipettes

Bioinformatics programs:

- Open CRAVAT
- Infevers
- IGV, USCS
- Command line
- VCF and BAM files



Thermostat



Qubit fluorometer



Vortex Mixer



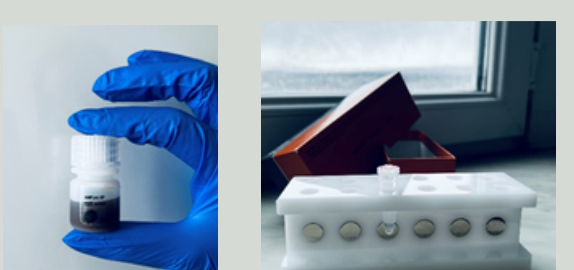
Electrophoresis machine



MinION



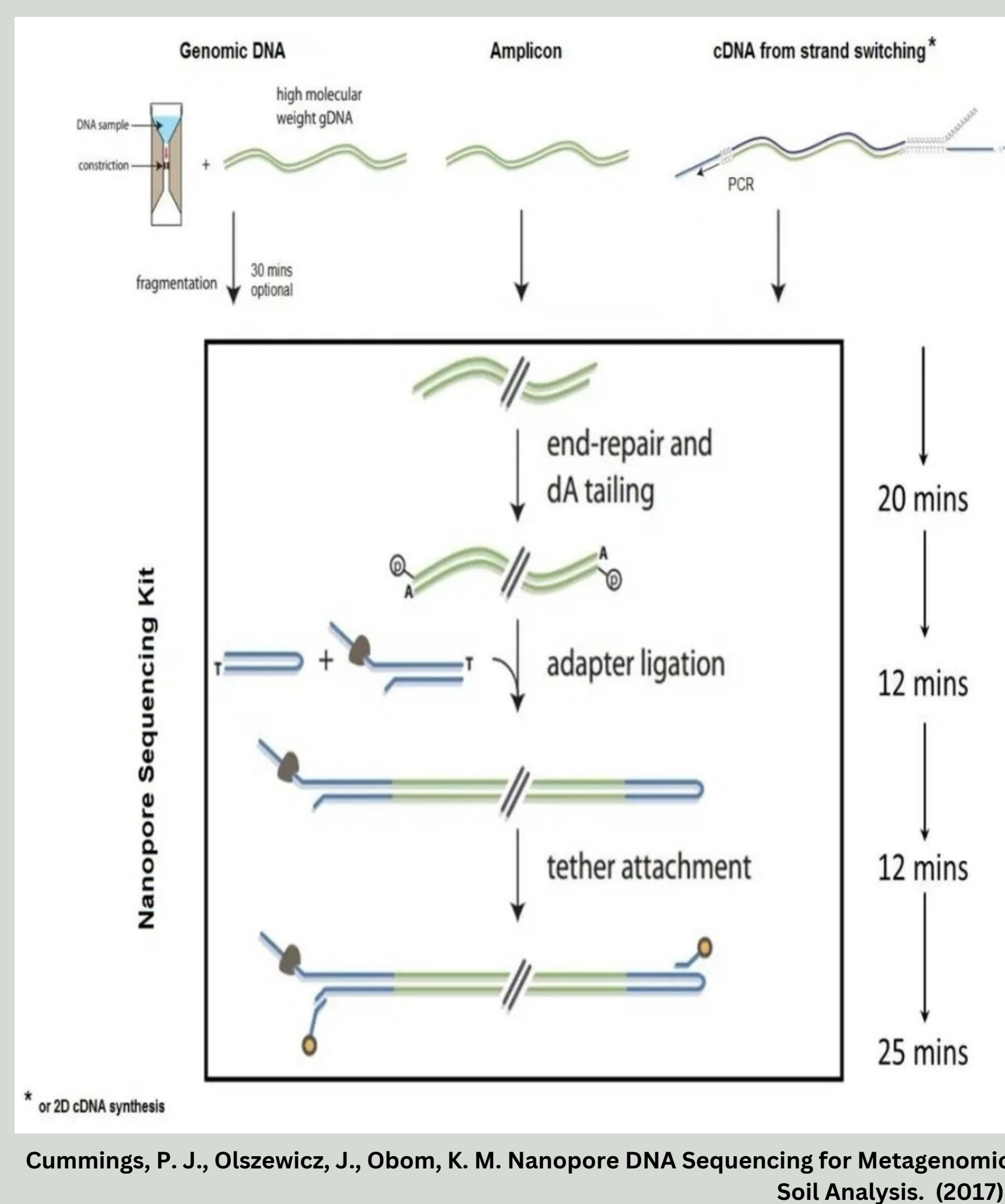
Centrifuge



Magnetic beads and separation rack



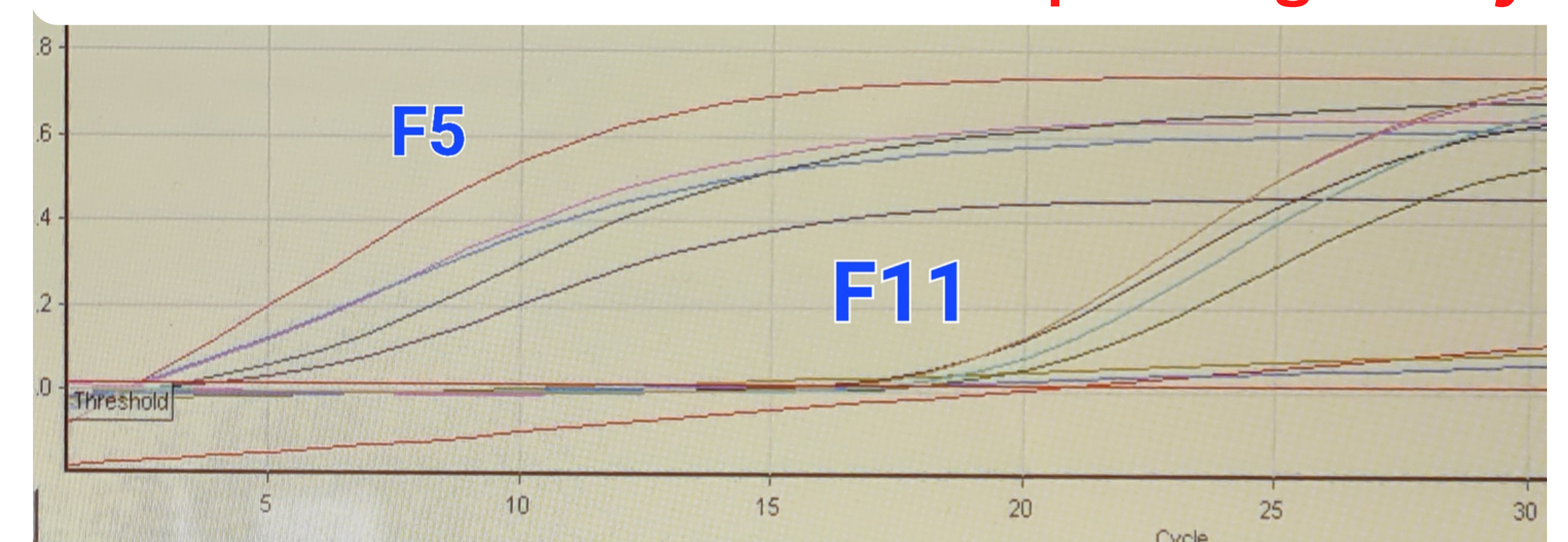
PCR Amplificator



IGV snapshot of alignment



Real-time PCR evaluation of sequencing library



Conclusions

- According to our research, **patient 1(F5)** had 4 mutations, 1 out of which was the causative for FMF.
- The second **patient 2(F11)** also had the FMF, however, as the library concentration was too low, no relevant results about mutations were obtained.
- Nanopore Sequencing is a great and cutting-edge technology for sequencing long DNA fragments, in the presence of a NORMAL DNA library concentration.