Gaucher disease (GD), the most common lysosomal storage disorder, is caused by biallelic mutations in the GBA gene. Heterozygous mutations in this gene are also a significant risk factor for Parkinson’s disease and other disorders. The complex structure of the genomic region incorporating GBA, which includes multiple pseudogenes, complicates analysis using PCR and traditional short-read DNA sequencing techniques. Leija-Salazar et al.¹ assessed the utility of long-read nanopore sequencing to overcome these challenges. The MinION™ provided rapid and comprehensive analysis of the entire ~8 kb GBA gene, allowing the detection and phasing of single nucleotide variants (SNVs) and deletions.

**Sample preparation**

DNA was purified from brain tissues using phenol-chloroform² and from saliva using the Oragene-DNA Kit according to manufacturer’s instructions.

**Library preparation**

As per Ligation Sequencing 1D Kit* and PCR Barcoding Kit I (EXP-PBC001).

0.5 nM amplicon DNA.

**AMPLIFICATION**

PCR amplification of 8.9 kb gene sequence using Kapa Hi-Fi polymerase. Primers incorporated barcoding adapters.

**CLEAN-UP**

Amplicons purified using QIAquick PCR Purification Kit as per manufacturer’s instructions and quantified on Qubit.

**BARCODING**

Barcoding using PCR Barcoding Kit I (EXP-PBC001) as per instructions.

**CLEAN-UP**

Clean-up using AMPure XP beads according to standard protocol.

**ADAPTER LIGATION**

Barcoding adapter ligation and clean-up according to standard protocol.

**SEQUENCING**

Higher throughput and lower cost sample analysis can be achieved using the PCR Barcoding Kit 96 (EXP-PBC096), which enables 96 samples to be run on a single flow cell.

*Laboratory Note: Ligation Sequencing Kit 1D SQK-LSK108 has since been superseded by kit SQK-LSK109.
Data analysis

Only downstream analysis tools recommended by the authors are presented; however, other tools were assessed. More information can be found in the full publication.

References


Find out more about real-time, long-read amplicon sequencing at www.nanoporetech.com.

*Metrichor is no longer available for basecalling; the local basecaller Guppy is now recommended.