Detection of *GBA* missense mutations and other variants using the Oxford Nanopore MinION

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-chloroform[2] and from saliva using the Oragene-DNA Kit according to manufacturer's instructions.

Library preparation
Brain: ~ 3 days
Saliva: ~ 2–3 hours

DNA (100 ng).

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.

Library preparation

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

Library preparation
~ 2.5 hours

0.5 nM amplicon DNA.

BARCODING →

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

END-REPAIR, dA-TAILING →

Pool up to 12 barcoded samples and perform standard end-repair and dA-tailing reaction.

CLEAN-UP

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

ADAPTER LIGATION →

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

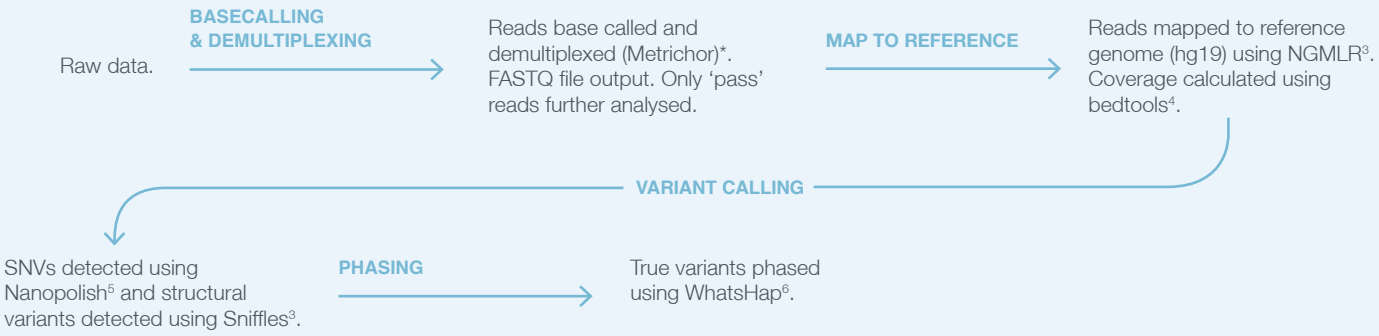
SEQUENCING →

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.

*Ligation Sequencing Kit 1D SQK-LSK108 has since been superseded by kit SQK-LSK109.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

1. Leija-Salazar, M. et al (2019) Evaluation of the detection of *GBA* missense mutations and other variants using the Oxford Nanopore MinION. Molecular Genetics and Genomic Medicine DOI: 10.1002/mgg3.564
2. Nacheva, E. et al (2017) DNA isolation protocol effects on nuclear DNA analysis by microarrays, droplet digital PCR, and whole genome sequencing, and on mitochondrial DNA copy number estimation. PLoS One12:e0180467.
3. Sedlazeck, F. J. et al (2018) Accurate detection of complex structural variations using single-molecule sequencing. Nature Methods 15(6):461-468
4. Quinlan, A.R. and Hall, I.M. (2010) BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26(6):841-2.
5. Loman, N.J., Quick, J. and Simpson, J.T. (2015) A complete bacterial genome assembled *de novo* using only nanopore sequencing data. Nature Methods 12(8):733-735.
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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

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