Oxford Nanopore Technologies

End-to-end workflow for haplotype-resolved genetic and epigenetic variant calling using Oxford Nanopore sequencing

Comprehensive identification and interpretation of single nucleotide variants (SNVs), structural variants (SVs), copy number variants (CNVs), and epigenetic modifications from a single PromethION™ Flow Cell

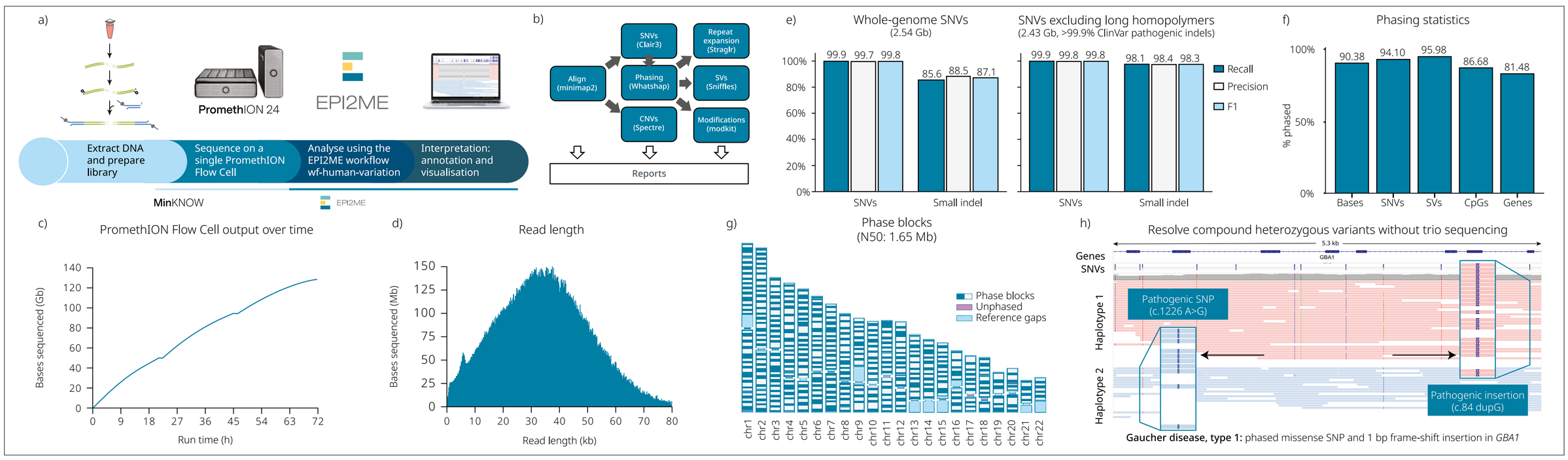

Fig. 1 a) Overall workflow b) analysis pipeline c) output d) read length distribution e) Genome in a Bottle (GIAB) SNV evaluation f) phasing performance g) phase block length h) *GBA1* region.

Oxford Nanopore sequencing enables calling and phasing of SNVs with high accuracy, even in difficult genomic regions

Oxford Nanopore provides end-to-end workflows for variant detection across the human genome* (**Fig. 1a**), from extraction from cell lines, blood, saliva, or buccal swab research samples, through to analysis using the EPI2ME™ platform (**Fig. 1b**). Briefly, for blood research samples, gDNA is extracted using the QIAGEN Puregene Blood Kit, size selected using the Oxford Nanopore Short Fragment Eliminator Kit and fragmented using Megaruptor 3. Libraries are prepared using the Ligation Sequencing Kit and sequenced on a PromethION Flow Cell. Flow cells are washed and re-loaded with library after ~24 and 48 hours (**Fig. 1c**). Size selection ensures the generation of long reads, with a read length N50 of ~30 kb (**Fig. 1d**). To validate our 30 kb end-to-end workflow, we sequenced the HG002 cell line on a PromethION device, basecalled with HAC v5.2.0 and resampled the reads to match the depth and read length of a blood research sample prepared as described. Results show high accuracy for SNVs across the whole genome (**Fig. 1e, left**) as well as for small indels (**Fig. 1e, right**) when excluding long homopolymers (≥10 bp; <1% of the genome and <0.1% of pathogenic ClinVar variants). The read lengths obtained enable phasing, with a phase block N50 of 1.65 Mb and most genes phased end to end (**Fig. 1f,g**). Phasing enables the detection of compound heterozygous variants without having to resort to trio sequencing. **Fig. 1h** shows an example of a pathogenic SNV), as well as a pathogenic 1 bp frame-shift insertion in *GBA1*, correctly called and fully phased.

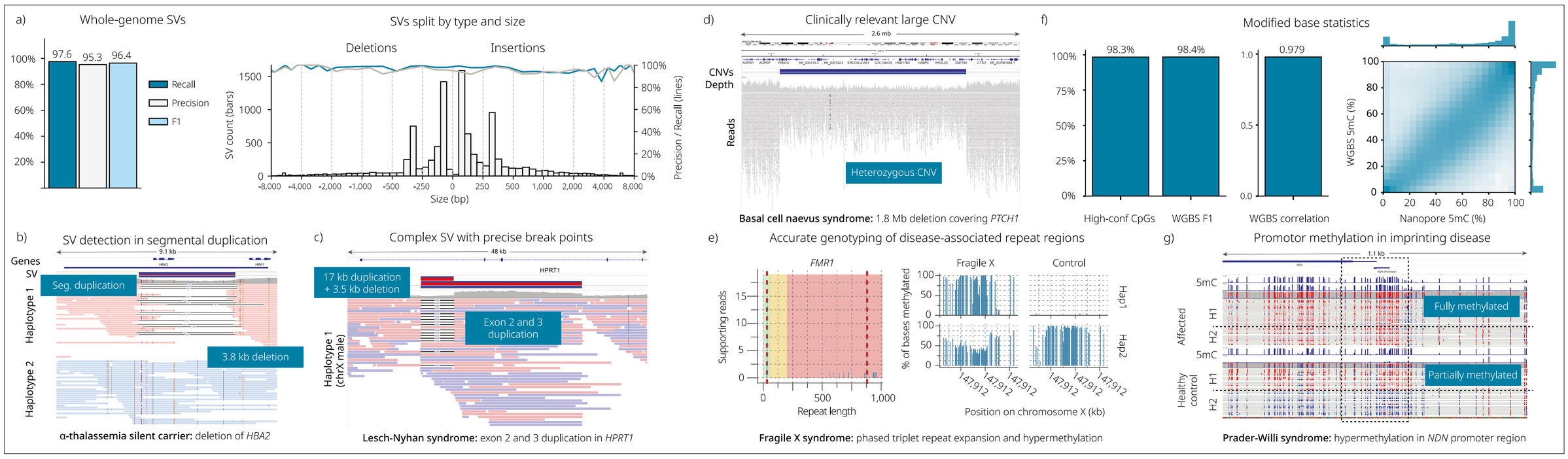

Fig. 2 a) GIAB SV evaluation b) SV in segmental duplication c) complex SV d) large CNV e) repeat expansion calling in *FMR1* f) 5mC calling performance g) promoter methylation.

Long, native Oxford Nanopore reads enable precise calling of phased SVs, CNVs, repeat expansions, 5mC, and 5hmC in a single assay

Next, we evaluated SV calling performance and observed high accuracy, largely independent of SV size or class (**Fig. 2a**). The size distribution of called SVs shows the expected *ALU* and *LINE1* peaks around 300 bp and 6,000 bp, respectively. Most importantly, high SV calling performance enables the detection of SVs in highly repetitive regions, such as segmental duplications (**Fig. 2b**). Furthermore, long reads can span and thus resolve complex SVs and detect exact break points. **Fig. 2c** shows an example of a complex SV, including a 17 kb duplication flanked by a 3.5 kb deletion. Accurate calling of the event by Sniffles shows that exons 2 and 3 of *HPRT1* are affected by the duplication, while the deletion only spans intronic sequence. Large CNVs (>100 kb) are facilitated by segmental duplications or large low-complexity repeats. Many of these regions are not resolved in the current human reference genome, making the detection of break points impossible. Thus, we use CNV calling (Spectre) to accurately detect these events (**Fig. 2d**). Specialised tools like Straglr enable accurate calling of tandem repeat expansions (**Fig. 2e**). Finally, nanopore sequencing yields exceptional coverage of CpGs, with 98.3% of hg38 CpGs covered by >10 observations (**Fig. 2f**) enabling high-quality calling of 5mC frequencies. At sites mappable by whole-genome bisulfite sequencing (WGBS), we observe high correlation between Oxford Nanopore 5mC and short-read WGBS calls. **Fig. 2g** shows an example of a hypermethylated promoter, which is characteristic for Prader-Willi syndrome.

*Oxford Nanopore Technologies products are currently for research use only (RUO).

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