Assessing the readiness of Oxford Nanopore sequencing for clinical genomics applications

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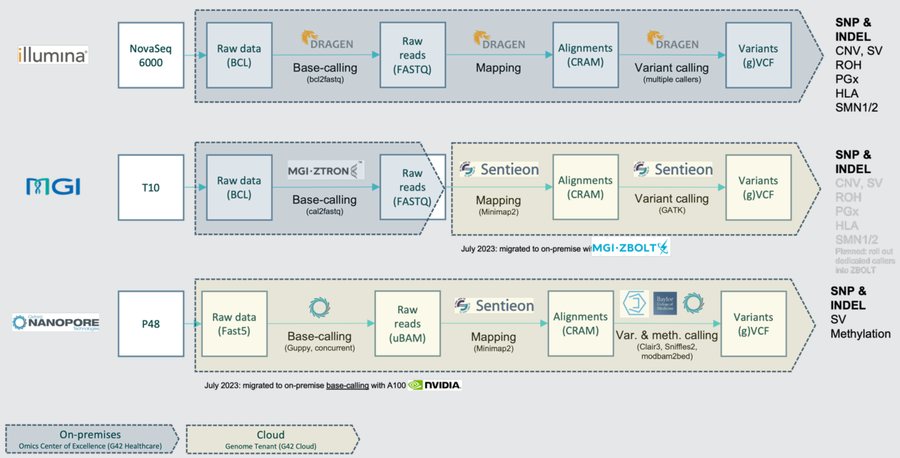
Introduction

Long-read sequencing (LRS), particularly Oxford Nanopore Technologies (ONT), has opened new avenues in genomics by allowing the detection of complex genomic variations, such as structural variations (SV) and repetitive regions, which are often missed by short-read sequencing (SRS). This technology has demonstrated potential for clinical genomics, offering improvements in variant detection and phasing information critical for understanding genetic inheritance. In this study, we assessed the readiness of ONT for clinical applications by benchmarking its performance against traditional SRS platforms, focusing on key factors such as error rates, variant calling accuracy, and the ability to detect clinically relevant mutations.

Methods

17 Coriell samples were sequenced at 30X coverage using whole genome sequencing (WGS) on three high-throughput sequencing (HTS) platforms **(Figure 1),** generating CRAM and VCF outputs for variant analysis.

Figure 1. Analysis workflows across sequencing platforms.



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Results & Discussion

Table 1. Summary of (a) genome-wide SNV/INDEL calling performance in the Coriell trio and (b) detection (✓) or not (✗) of the expected genotype for each of the 14 positive control Coriell reference samples.

Sample	Description	Variant Type	Illumina	MGI	ONT (R9)	ONT (R10)
a			F-score			
GM24143	Trio (Mother)	SNV	0.980	0.975	0.981	0.978
		INDEL	0.936	0.928	0.678	0.744
GM24149	Trio (Father)	SNV	0.981	0.975	0.978	0.980
		INDEL	0.936	0.929	0.659	0.758
GM24385	Trio (Child)	SNV	0.982	0.977	0.983	0.981
		INDEL	0.940	0.932	0.685	0.746
b						
GM13708	BRCA1	SNV	✓	✓	✓	✓
GM27630	MRD40	SNV	✓	✓	✓	✓
GM27631*	MRD40	SNV	✓	✓	✓	✓
GM27632*	MRD40	SNV	✓	✓	✓	✓
GM10354	USH1C	SNV	✓	✓	✓	✓
GM07828	CF	INDEL	✓	✓	✓	✓
GM07829	CF	INDEL	✓	✓	✓	✓
GM07830	CF	INDEL	✓	✓	✓	✓
GM08211	CF	INDEL	✓	✓	✓	✓
GM04099	DMD	CNV (Deletion)	✓	✓	✓	✗
GM10684	SMA1	CNV (Deletion)	✓	✓	✓	✓
GM03990	DM	STR	✓	✓	✓	✓
GM09145	FXS	STR	✗	✗	✓	✓
GM03620	HD	STR	✓	✓	✓	✓

Robust genome-wide SNV detection across platforms, with INDELs challenges in ONT

SNV and INDEL detection was assessed across all platforms using a parent-offspring trio **(Table 2 & Figure 2).**

Overall, ONT achieved similar SNV detection performance to SRS, with notable improvements in INDEL detection using R10, particularly in exonic regions.

Strong Detection of Disease-Causing Mutations

11 pathogenic mutations linked to clinically relevant conditions were examined in the remaining 14 samples **(Table 2)**:

- SRS and LRS accurately identified all SNVs, including the *de novo* MRD40 mutation.
- INDEL detection was consistent across platforms, with an unreported 1-bp annotation error in GM07829 confirmed via Sanger validation.
- SRS and ONT R9 detected the DMD CNV, while ONT R10 missed it due to variant-calling limitations.
- ONT outperformed SRS in detecting large STR expansions, underscoring its clinical potential **(Figure 3)**.

Figure 2. SNV and INDEL calling performance across sequencing platforms.

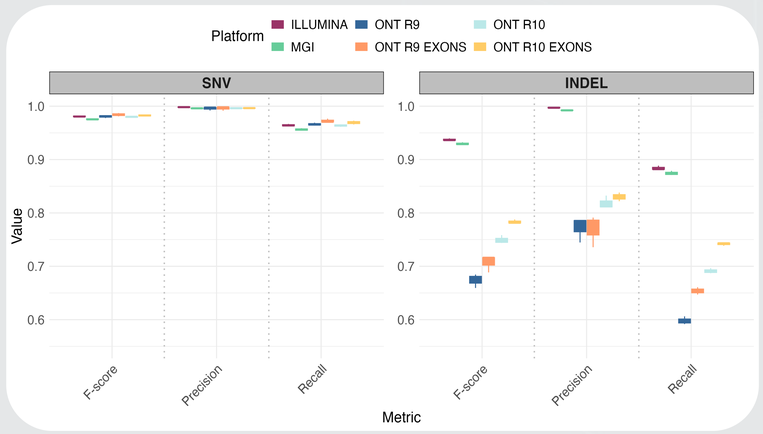

Figure 3. STR detection for genes FMR1, HTT and DMPK in the 14 Coriell.