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Abstract #2221

An accelerated HiFi whole genome workflow for comprehensive, rapid long-read sequencing

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Introduction

Rapid whole genome sequencing (rWSG) can help accelerate genomic insights in time-critical settings. While current rWGS workflows are typically built on short-read sequencing, they may miss or incompletely resolve complex variant classes such as structural variants (SVs), repeat expansions, phased haplotypes, and difficult-to-call genomic regions. PacBio HiFi sequencing offers long, highly accurate reads with the potential to capture these variant types in a single genome-wide assay.

Here, we developed and assessed an accelerated HiFi WGS workflow on the Revio system with SPRQ-Nx chemistry, designed to reduce turnaround time while preserving accuracy for comprehensive variant detection across diverse specimen types (Figures 1 and 2).

Methods

A total of 24 samples with previously characterized pathogenic variants were processed using the rapid workflow, including 23 buccal/saliva specimens and 1 blood specimen. Buccal/saliva samples were collected with DNA Genotek OCD100 collection devices and extracted with Thermo Fisher MagMAX kits. The whole blood specimen was extracted with Revvity chemagic.

Rapid whole genome libraries were prepared using an accelerated HiFi prep kit 96 workflow with automated pipette shearing targeting 10 –15 kb fragments, followed by repair and A-tailing, adapter ligation, nuclease treatment, and polymerase binding (Figure 3). The workflow omitted Short Read Eliminator (SRE) size selection. Libraries were sequenced on the PacBio Revio system using SPRQ-Nx chemistry with 15-hour movie times (Figure 4). GPU-accelerated secondary analysis enables alignment and variant calling in under 4 hours*.

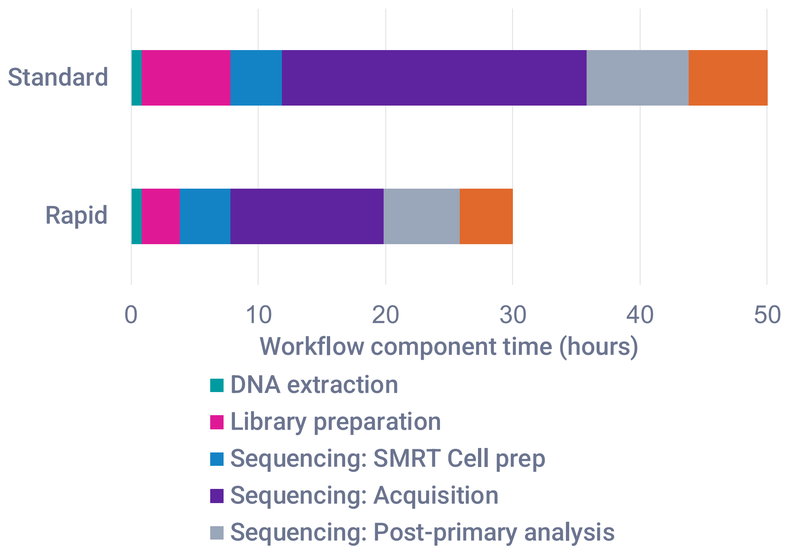

Figure 1. Workflow timing estimates of standard HiFi WGS workflow vs accelerated protocol.

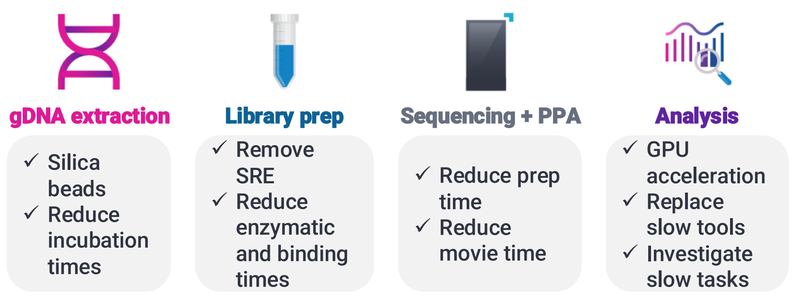

Figure 2. Workflow component accelerations. Overview of changes to rapid protocol to decrease overall workflow turnaround time.

Results

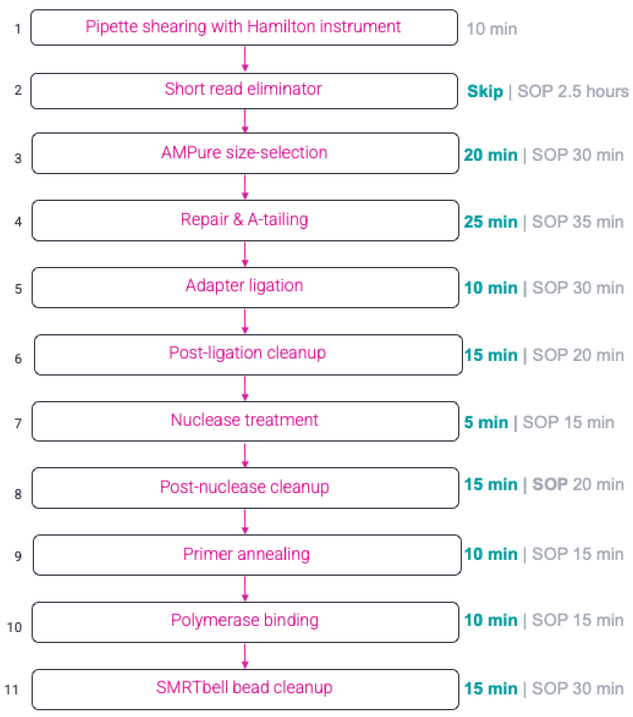

Figure 3. rWGS library preparation workflow. Timing modifications for rapid processing are shown in teal. Standard operating procedure (SOP) times are shown in grey.

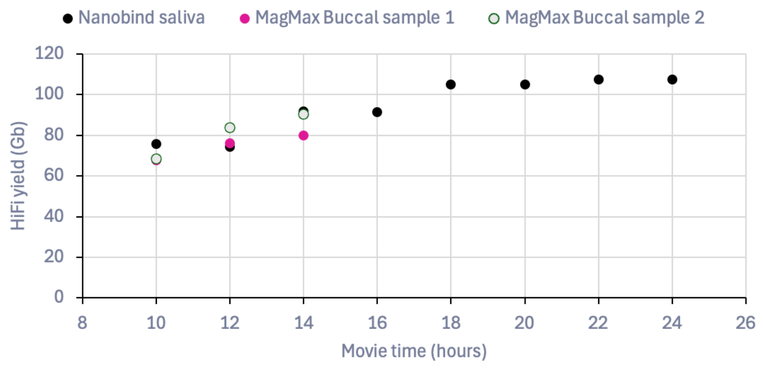

Figure 4. HiFi yield vs sequencing movie time for saliva and buccal samples. One Nanobind extracted saliva library and 2 MagMax extracted buccal libraries were loaded across multiple cells with varied movie times from 10 hours up to 24 hours.

HiFi Yield, Gb (S.D.)	Mean Read Length, bp (S.D.)	% Q30+ (S.D.)
77 (11.7)	8,895 (1,294)	93% (0.8%)

Mean Human Coverage	Mean % Mapping to Human Genome	Mean Mito Coverage
21.5x	91%	868x

Table 1. Sequencing performance metrics. Summary of output from buccal/saliva samples with 15-hour movie times.

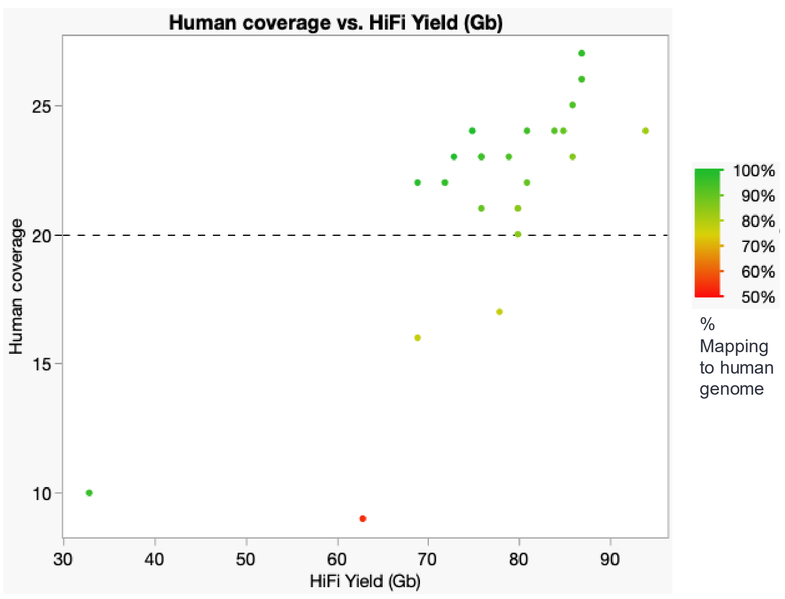

Figure 5. WGS coverage depends on total HiFi yield and %mapping to human (for non-blood samples). 4 samples failed to reach 20x human coverage, 1 due to poor DNA quality and subsequently low HiFi yield, 3 due to lower percentage human mapping.

With 15-hour movie times, greater than 20x coverage was achieved for buccal samples, with over ~70 Gb in HiFi yield and >85% mapping to the human genome.

Specimen type	Gene(s) / Region	Variant Class	Human coverage	Called?
Buccal/saliva	*DMD*	SNP	21	✓
Blood	*DMD*	Indel	23	✓
Buccal/saliva	*CFTR*	Indel, SNP	24	✓
Buccal/saliva	*CFTR*, *IRAK4*	Indel, SNP	20	✓
Buccal/saliva	*HEXA*, *ATM*	SNPs	23	✓
Buccal/saliva	*HBB*, *CAMTA1*	SNPs	24	✓
Buccal/saliva	*HBB*	Indel	17	✓
Buccal/saliva	*ABCD1*	SNP	24	✓
Buccal/saliva	*ABCD1*	SNP	26	✓
Buccal/saliva	*ACADM*	SNP	24	✓
Buccal/saliva	*ACADM*	Indel, SNV	16	✓
Buccal/saliva	*VHL*	SNP	22	✓
Buccal/saliva	*VWF*	SNP	23	✓
Buccal/saliva	*VWF*	SNP	23	✓
Buccal/saliva	*FMR1*	Tandem Repeat	22	✓
Buccal/saliva	*DMPK*	Tandem Repeat	23	✓
Buccal/saliva	*DMPK*	Tandem Repeat	10	✓
Buccal/saliva	Chr X	SV, Mosaic	21	✓
Buccal/saliva	*DMD*	SV	22	✓
Buccal/saliva	*CNBP*	Tandem Repeat	24	✓
Buccal/saliva	*CNBP*	Tandem Repeat	9	✗
Buccal/saliva	Chr 13	SV	23	✓
Buccal/saliva	Chr 13	SV	27	✓
Buccal/saliva	Multi-gene	SNPs	25	✓

Table 2. Detection of previously characterized pathogenic variants. The cohort included multiple variant types across blood and buccal/saliva specimens, previously characterized by orthogonal clinical assays. The rapid WGS protocol achieved 96% concordance with orthogonal clinical testing methods across 24 samples.

Comprehensive variant detection was achieved across both blood and buccal/saliva specimens, with successful calls observed at human genome coverages as low as 10x for several variant types.

The single missed *CNBP* repeat expansion buccal sample showed low mapping to the human genome, resulting in insufficient spanning read depth across the repeat locus.

Conclusion

HiFi long-read sequencing can be incorporated into an accelerated WGS workflow for enabling time-critical applications. These results demonstrate the potential for rapid long-read sequencing to provide comprehensive genomic insights while reducing workflow complexity and turnaround time for rare disease and urgent genetic testing applications.

- The rapid workflow supports multiple specimen types, including blood, buccal, and saliva. Greater than 20x coverage was achieved for buccal samples with ~70+ Gb in HiFi yield and >85% mapping to the human genome.
- This workflow achieved 96% detection of difficult, previously characterized pathogenic variants spanning SNVs, indels, SVs, and repeat expansions,
- Accelerated library preparation, reduced sequencing run times, and GPU-enabled analysis enabled generation of genome-wide variant calls in <30 hours from extraction to VCF.

*For analysis of the 24 samples shown here, the standard (non-rapid) HiFi WGS variant calling pipeline was used.