Towards rapid HiFi long-read whole genome sequencing for time-critical rare disease applications

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Introduction

Rapid whole genome sequencing (rWGS) can support time-sensitive genomic investigations in rare disease programs and NICU settings. Current rWGS workflows based on short-read technology may miss complex genomic regions such as structural variants, repeat expansions, and phased haplotypes. HiFi long-read sequencing can detect these variant classes in a single genome-wide assay but has not been widely adapted for rapid WGS applications.

Here, we developed an accelerated HiFi WGS workflow on Revio with SPRQ-Nx chemistry integrating shortened library preparation, 12-hour sequencing, and GPU-accelerated analysis, enabling estimated DNA extraction-to-VCF turnaround in under 30 hours for one 20× genome across blood and saliva specimens.

Methods

Wet lab workflow

Thirty-one samples with variants previously-characterized by GeneDx were evaluated (23 saliva, 8 whole blood specimens). Saliva samples were collected with DNA Genotek ORAcollect OCD100 collection devices and extracted with Thermo Fisher MagMAX kits. Whole blood specimens were extracted with Revvity Chemagic or bead-based Nanobind extraction. The samples represented positive controls for SNVs, indels, structural/copy-number variants, tandem repeat expansions, and complex haplotypes.

Rapid whole genome libraries were prepared at PacBio and GeneDx using an accelerated HiFi prep kit 96 workflow with automated pipette shearing targeting 10–15 kb fragments. Short Read Eliminator (SRE) size selection was omitted, and enzymatic incubation and bead-binding times were reduced, decreasing library preparation from 7-8 to less than 4 hours.

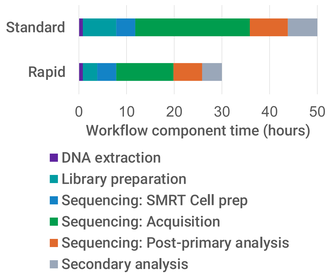


Figure 1. **Workflow timing estimates** of standard HiFi WGS workflow vs accelerated protocol.

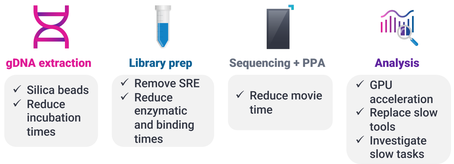


Figure 2. **Workflow component accelerations.** Overview of changes to rapid protocol to decrease overall workflow turnaround time.

Sequencing and performance metrics

Libraries were sequenced at PacBio and GeneDx on the PacBio Revio system with SPRQ-Nx chemistry using 15-hour movies for saliva and 12-hour movies for whole blood. HG002 (cells extracted with Lucigen MasterPure Complete DNA and RNA) was used to assess workflow reproducibility across independent laboratory sites (Figure 3).

Secondary analysis

HG002 was used to benchmark GPU-accelerated HiFi WGS Variant pipeline on a local HPC and AWS HealthOmics as a proof of concept for rapid secondary analysis*.

For local deployment, rapid analysis is recommended on HPC nodes with ≥48 CPUs, 192 GiB RAM, and 4 NVIDIA GPUs; AWS HealthOmics can alternatively run the workflow using a 4-GPU Parabricks configuration.

Results

HiFi Yield, Gb (S.D.)	Mean Read Length, bp (S.D.)	Mean QV	% Q30+ (S.D.)	Mean Human Coverage (blood samples)	Mean Human Coverage (saliva samples)	Mean % Mapping to Human Genome (saliva samples)
76.2 (10.4)	9,151 (1,422)	Q35	92.1% (1.0)	23.6×	21.5×	91%

Table 1. Sequencing performance of samples processed with the rapid HiFi WGS workflow. Summary of sequencing metrics for the 31 samples, combined across specimen types unless otherwise indicated.

The rapid workflow consistently generated >20× human genome coverage across specimen types while maintaining high-quality HiFi sequencing data.

Variant class	Samples, n	Concordant, n	Example loci
SNVs / indels	16	16	*DMD, CFTR, HBB, ACADM, TTN, PLEC, SGCD*
Tandem repeat expansions	8	7	*FMR1, DMPK, CNBP*
SVs / CNVs / complex copy number	7	6	*DMD*, chr13, chr17, chrX mosaic, *SMN1/SMN2*, multi-gene
Total	**31**	**29**	—

Table 2. Detection of previously characterized variants across variant classes. Samples processed with the rapid workflow were concordant in 29 of 31 samples based on orthogonal clinical assays. Discordant results included one *CNBP* repeat expansion in a sample with low human genome coverage and one *SMN2* copy-number result that remains under investigation.

*For analysis of the 31 previously characterized samples, the standard (non-rapid) HiFi WGS variant calling pipeline was used.

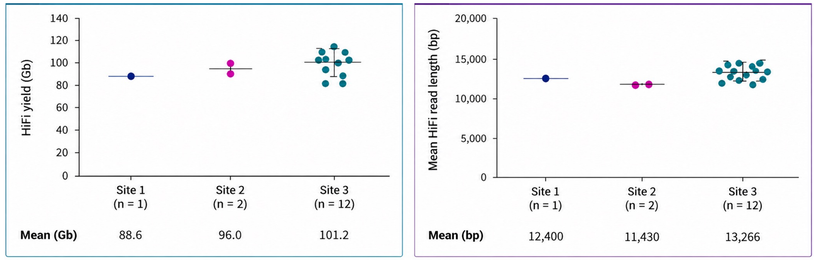


Figure 3. Reproducibility of rapid HiFi WGS sequencing performance across independent sites. HiFi yield (left) and mean HiFi read length (right) for HG002 processed and sequenced independently at three sites. Points represent individual sequencing runs; horizontal lines indicate mean for each site.

Rapid analysis

Parallelized alignment, GPU-enabled small-variant calling with Parabricks DeepVariant, and optimization of phasing, SV, and tandem repeat analysis reduced secondary analysis wall time. Observed analysis times are configuration-dependent and may vary with compute resources, workflow settings, dataset characteristics, and cluster or cloud queue times.

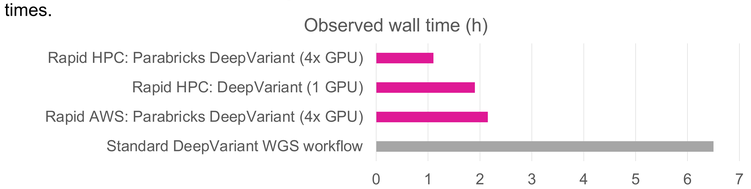


Figure 4. Proof-of-concept acceleration of HiFi WGS secondary analysis. Representative standard HiFi WGS analysis (through VCF) time is shown alongside observed wall times for an accelerated PacBio WGS Variant Pipeline using a HG002 on various local HPC and AWS HealthOmics (us-east-1) configurations.

Conclusion

These results demonstrate the potential for rapid long-read sequencing to provide comprehensive genomic insights while reducing workflow complexity and turnaround time.

- **Rapid HiFi WGS generated >20× human genome coverage across blood and saliva** specimens using shortened library preparation and 12–15-hour sequencing.
- Previously characterized complex variants were detected in 29/31 samples; with the 2 discordant results associated with low coverage or an unresolved copy-number discrepancy.
- **Reproducible HG002 sequencing across three independent sites and GPU-accelerated analysis benchmarks support the feasibility of an end-to-end HiFi WGS workflow in less than 30 hours.**

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