Enabling Ultra-Rapid Whole Genome Sequencing for Same-Day Return of Results

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Introduction

Whole genome sequencing (WGS) is an essential tool for diagnosing critically ill infants in NICU and PICU settings, often uncovering actionable findings that directly influence care and improve outcomes. Despite this, the fastest typical clinical WGS workflows can require several days from sample receipt to result delivery. This may limit usefulness for urgent decision-making.

Speed is critical

- In the hospital setting, routine tests results are expected in days, not weeks. (Blood tests, imaging, karyotyping, CMA, metabolic testing)
- Clinicians expect speed to guide treatment
- Timing impacts duration of hospital stays

WGS provides precise results for ambiguous symptoms

- Genetic conditions are leading cause of NICU and PICU mortality
- Many conditions cause overlapping non-specific phenotypes
- Clinical WGS results reduce costs per patient by eliminating unnecessary testing or treatments
- Clinical WGS results reduce length of inpatient stay

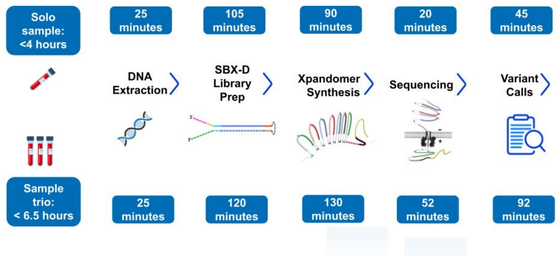


Figure 1. End-to-end workflow featuring approximate timing for each step. Top: the time required to process a single sample. Bottom: the time for a trio of samples.

Broad Clinical Labs identified a potential method to establish an ultra-rapid WGS workflow in our CAP/CLIA certified lab. Roche Sequencing Solutions has developed a simplified workflow, based on Sequencing by Expansion (SBX) chemistry, called SBX-FAST, that is capable of delivering variant call files (VCFs) from whole blood samples within a few hours. The protocol can support either single samples or multiplex trios.

Our team set out to test the speed and quality of this workflow, and whether a single technician could run it end-to-end in a routine setting.

Materials & Methods

A typical clinical WGS process requires multiple days due to:

- Batching requirements - massive throughput workflows require a minimum number of samples for cost effective processing
- Sequencing time - standard sequencing technologies take many hours to generate >30X WGS data
- Analysis - producing a VCF from hundreds of Gb of sequencing reads and identifying relevant variants takes many hours or even days

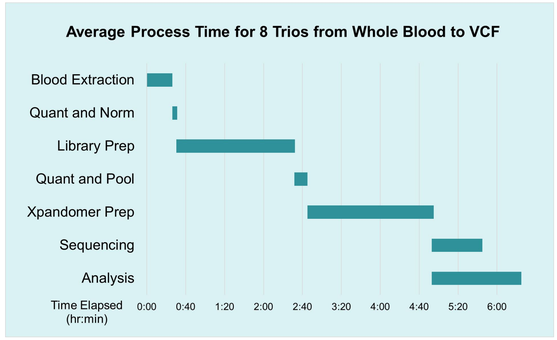


Figure 2. Average timing across 8 runs for workflow steps in our pilot project. WGS data was produced for 24 healthy donors, processed using the trio lab workflow.

This innovative workflow addresses each of these challenges.

- Genomic DNA is isolated from 200 uL of whole blood. Libraries are prepared using enzymatic fragmentation, hairpin adapter ligation, and Xpandomer synthesis.

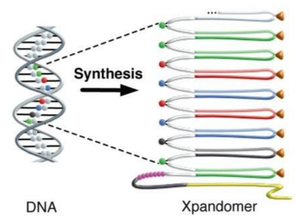


Figure 3. Xpandomer synthesis illustration

- Libraries are sequenced on the Roche prototype SBX platform using rapid nanopore sequencing to generate >30X genome coverage per sample for a trio within 40 minutes
- VCFs are produced less than 2 hours after sequencing starts, allowing for a complete turnaround from blood to variant calls in under seven hours.

Results

BCL evaluated the reproducibility of the workflow across eight independent runs. All runs yielded high-coverage data suitable for downstream tertiary analysis.

Library performance

- A rapid DNA extraction optimized for speed produces 5 ug of DNA from 200 uL blood. 2 ug is required for library preparation.
- Libraries are normalized before Xpandomer synthesis to ensure even representation
- 100% of samples met minimum 30X coverage, with mean insert size 178 and Phred quality score 39.4.

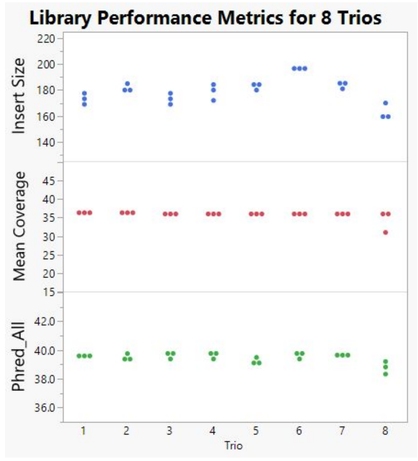


Figure 4. Library performance metrics for 8 healthy donor trios

Accuracy and Precision

- We also performed benchmarking using replicates of HG002 to ensure high quality precision and recall.
- We were able to identify variants of interest* including SNVs, insertions, deletions, duplications, and repeat expansions.

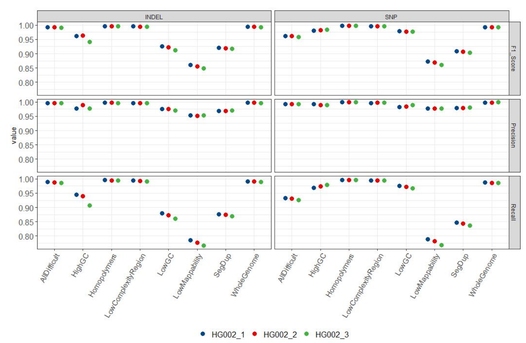


Figure 5. Precision and recall for HG002 in triplicate

Discussion and Conclusion

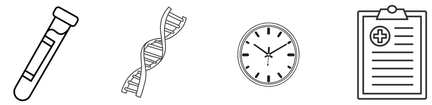
WGS is uniquely suited to provide diagnostic answers in challenging cases. This ultra-rapid WGS workflow shows the potential for same-day sequencing results to become routine.

Simplicity:

- A single technician can conduct the hands-on portion of the workflow from end to end.
- Next steps will include further automation of the liquid handling as well as the downstream variant analysis and interpretation
- The most rapid results would be enabled by adoption of this technology in point-of-care settings, eliminating transport times

Flexibility:

- In addition to this pilot with 24 healthy donor blood samples, our lab produced same day return of results for 10 single samples, including 6 NICU patients enrolled in our research pilot
- No batching is required, samples can be run on demand or batched in sets of up to 12
- The hands-on portion of the workflow is under 4 hours, making multiple runs per day a possibility.



By dramatically shortening turnaround time, this workflow has the potential to make rapid genomic testing more accessible for critically ill patients, enabling precision medicine interventions within a clinically meaningful timeframe.

References

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**SBX was not used for diagnosis, research only.*