# Demonstrating the versatility, accuracy, and throughput of Sequencing By Expansion (SBX)

## Ultra fast whole genome sequencing from sample prep through variant analysis in less than 4 hours

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## Introduction to SBX-Fast

Sequencing By Expansion (SBX) technology is a novel sequencing approach that uses a biochemical process to encode the sequence of a target nucleic acid molecule into a measurable surrogate polymer called an Xpandomer.[1] SBX-Fast is duplex-based, amplification-free research workflow, designed with the intent to provide a deployable solution for rapid sequencing applications where time to result is important. This workflow produces high-accuracy results by linking both strands of the target DNA in a single sequencing read, enabling rapid and accurate identification of InDels, SNVs, STRs, and CNVs.

We evaluated SBX-Fast's variant-calling performance across 41 diverse cell lines from the Coriell Institute for Medical Research, which encompass a wide array of genetic conditions. Furthermore, in a speed trial to determine the minimum turnaround time, we successfully demonstrated the complete process—from library preparation to the final VCF variant file—in under four hours (3 hours, 59 minutes), using the HG002 reference sample.

## Materials and Methods

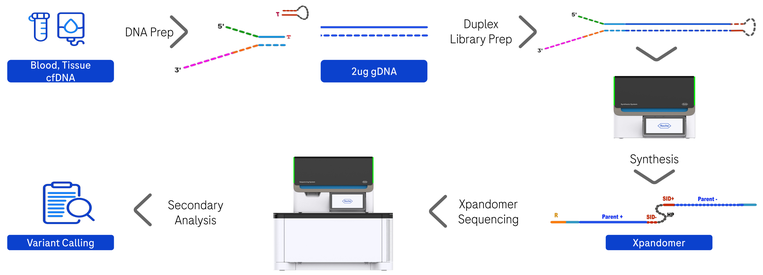
### Reference Benchmarking

2 µg of unsheared genomic DNA from each sample was input into the SBX-Fast workflow. Samples were sequenced as a trio for 1 hr.

### Time Trial

2 ng of unsheared genomic DNA from HG002 was input into a time optimized SBX-Fast workflow, and sequenced solo for 20 minutes to target >30x coverage.

**Figure 1. SBX-Fast Workflow**




SBX-D data were processed through SBX-optimized open source (XOOS) variant callers.[2-3]

## Accelerated Data Processing Overview

Raw sequencing data is processed (basecalling, demultiplexing, intramolecular consensus) in real-time on the sequencing system's integrated hardware. These consensus reads are then continuously mapped to the reference genome. Once the target coverage depth is achieved for a sample, a merged and sorted BAM file is generated. After the BAM files are generated, the Roche SBX Optimized Open Source (XOOS) tools are used for variant detection (SNVs, INDELs, CNVs, and STRs).

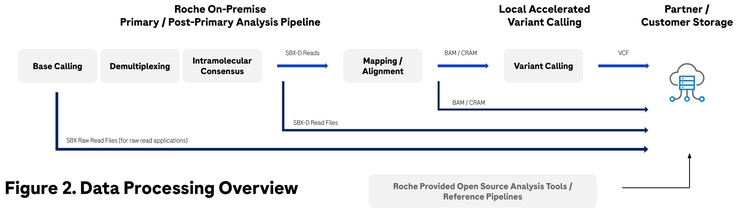



**Figure 2. Data Processing Overview**

## Reference Benchmarking (Results)

As shown in the table below, we successfully called the expected variant in all reference samples.

| Sample ID | Description | Variant Type | |
|---|---|---|---|
| NA25495 | Choroideremia | CNV | ✅ |
| NA23710 | Epileptic encephalopathy | CNV | ✅ |
| NA02325 | Translocated chromosome | CNV | ✅ |
| NA21698 | Chromosome deletion & CNV Reference | CNV | ✅ |
| NA08618 | Chromosome deletion | CNV | ✅ |
| NA03330 | Trisomy 13, Patau syndrome | CNV | ✅ |
| NA13480 | Williams–Beuren syndrome | CNV | ✅ |
| NA10283 | Hyperglycerolemia | CNV | ✅ |
| NA05117 | Duchenne muscular dystrophy | CNV | ✅ |
| NA22208 | Propionic acidemia | CNV | ✅ |
| NA04520 | Tuberous Sclerosis Complex 2 | CNV | ✅ |
| NA06151 | Machado-Joseph Disease | STR | ✅ |
| NA04079 | Friedreich's Ataxia | STR | ✅ |
| NA06894 | Fragile X mental retardation | STR | ✅ |
| NA03756 | Myotonic Dystrophy | STR | ✅ |
| NA13716 | Dentatorubral-Pallidoluysian Atrophy | STR | ✅ |
| NA23709 | Spinal and bulbar muscular atrophy | STR | ✅ |
| GM28741 | Homocystinuria-megaloblastic anemia | CNV | ✅ |
| GM27903 | Cerebral creatine deficiency syndrome 1 | SNV/INDEL | ✅ |
| GM28606 | Shwachman-Diamond syndrome | SNV/INDEL | ✅ |

| Sample ID | Description | Variant Type | |
|---|---|---|---|
| NA04327 | Duchenne muscular dystrophy | CNV | ✅ |
| NA23127 | Muscular Dystrophy, Becker Type | CNV | ✅ |
| NA06804 | Lesch-Nyhan syndrome | CNV | ✅ |
| NA09834 | Basal Cell Nevus Syndrome | CNV | ✅ |
| NA12214 | Charcot-Marie-Tooth disease type 1A | CNV | ✅ |
| NA05876 | DiGeorge syndrome | CNV | ✅ |
| NA22010 | Propionic Acidemia, clinically affected | SNV | ✅ |
| NA22011 | Clinically unaffected mother | SNV | ✅ |
| NA22012 | Clinically unaffected father | SNV | ✅ |
| NA14553 | Arterial Calcification | SNV | ✅ |
| NA00882 | Fabry Disease | SNV | ✅ |
| NA00372 | Gaucher Disease, Type I | SNV/InDel | ✅ |
| NA22113 | Propionic Acidemia clinically affected sister 1 | Delins | ✅ |
| NA22112 | Propionic Acidemia clinically affected sister 2 | Delins | ✅ |
| NA22111 | Clinically unaffected mother | Delins | ✅ |
| NA22110 | Clinically unaffected father | Delins | ✅ |
| NA11195 | Phenylketonuria | SNV/InDel | ✅ |
| NA23391 | Myotonic Dystrophy | STR | ✅ |
| NA05131 | Fragile X | STR | ✅ |
| NA16202 | Clinically unaffected mother | STR | ✅ |
| NA16203 | Friedreich's Ataxia | STR | ✅ |

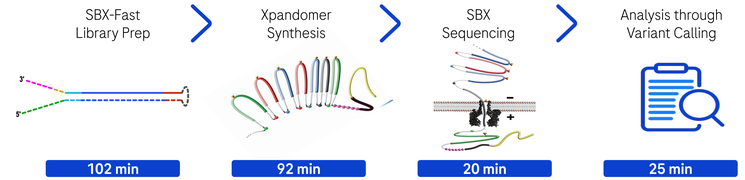
**Table 1. Reference Samples Results**

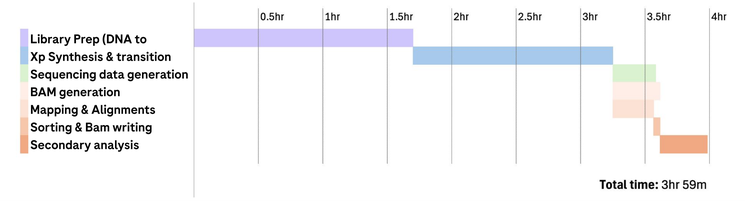
## Time Trial (Results)

Using HG002, we demonstrated DNA to VCF in 3 hours, 59 minutes.

Library prep through VCF in **3 hours, 59 minutes**

| Sample | Total Duplex Reads (B) | Mean Insert Read Length | Coverage | % genome at >10x coverage |
|---|---|---|---|---|
| HG002 | 1.06 | 158 | 33x | 99.5 |




**Total time:** 3hr 59m

## Conclusion

For settings requiring rapid genomic analysis, SBX-Fast has the potential to deliver timely information that could impact decisions to improve outcomes.

## Disclosures

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All authors are employees of Roche Sequencing Solutions, Inc. or Roche Diagnostics GMBH and may hold non-voting equity securities in F. Hoffmann-La Roche Ltd.

## References

1. https://doi.org/10.1101/2025.02.19.639056
2. https://roche-axelios.gitbook.io/xoos
3. "Enabling rare disease research with rapid workflows by SBX technology and the AVENIO Edge automated KAPA HTyperExome V2 solution", European Human Genetics Conference in Milan, Italy, May 2025