

Whole Genome Precision at Scale: Sequencing by Expansion for Cancer Genomics Research

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Background

Whole genome sequencing (WGS) offers a more comprehensive view of the cancer genome compared to targeted methods, enabling the detection of several genetic alterations in a single test. However, the substantial sequencing requirements, challenges in achieving high sensitivity while maintaining low false positive rates—particularly in low-quality samples like Formalin-Fixed Paraffin-Embedded (FFPE) tissues—and long turnaround times have hindered broader adoption of WGS.¹

Sequencing By Expansion (SBX) offers flexible, ultra-high-throughput sequencing with rapid turnaround.² Here, we show that hairpin duplex SBX (SBX-D) can overcome these barriers, enabling broader WGS adoption in oncology research. We present advanced bioinformatics algorithms for accurate detection of somatic variants, including SNVs, INDELs, copy number alterations (gain, loss, LOH), structural variants, and biomarkers of interest such as tumor mutational burden, mutational signatures, HLA typing.

Materials and Methods

In this study, we performed 70–100x for both SBX (mean) and Illumina WGS (mean) on 20 FFPE tissues using 100 ng DNA across a range of sample qualities to benchmark SBX variant calling against Illumina NGS.

Illumina samples were sequenced via NovaSeq 6000 S4 and processed through DRAGEN 4.3 using Illumina recommended parameters.³ SBX-D data were processed through SBX-optimized open source (XOOS) variant callers.⁴⁻⁵

Overall, SBX-D approach shows higher SNP and INDEL accuracy compared to Illumina. Better SNV and INDEL accuracy could be attributed to duplex sequencing and replacing PCR with linear amplification which can significantly reduce long HP error rate (Fig. 1).

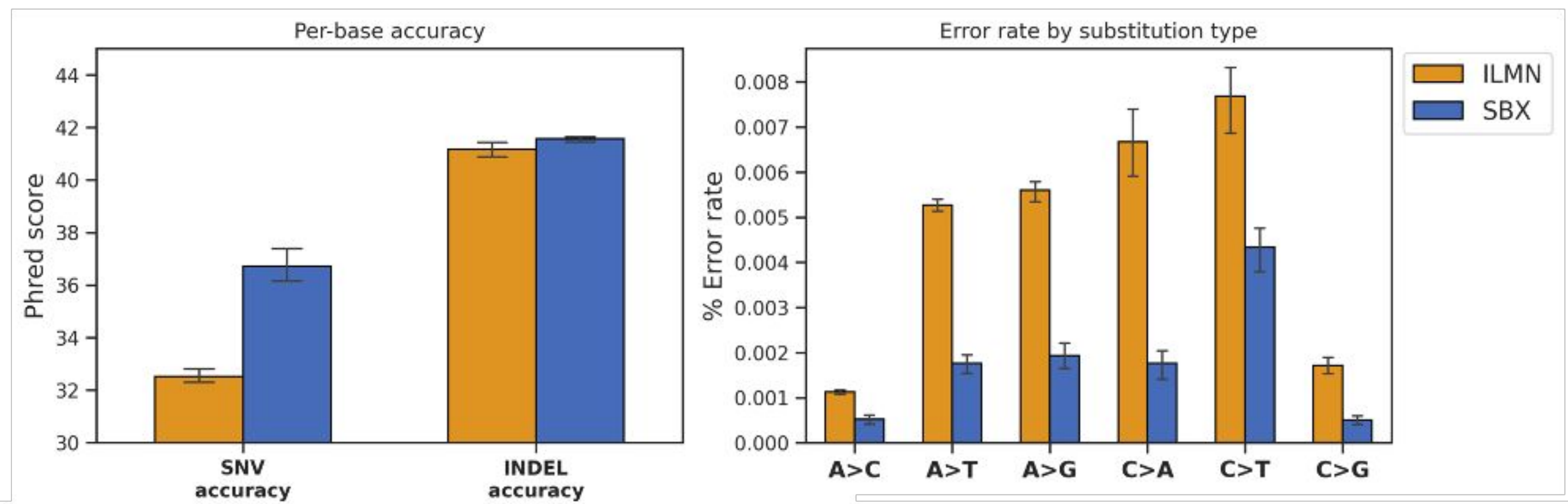


Figure 1. Comparison of SNV and INDEL accuracy between Illumina and SBX-D sequencing of FFPE tissues. Duplex sequencing and linear amplification contribute to higher SNV and INDEL accuracy in SBX-D.

Disclosures

This study was funded by Roche Diagnostics International Ltd (Rotkreuz, Switzerland)
All authors are employees of Roche Sequencing Solutions, Inc and may hold non-voting equity securities in F. Hoffmann-La Roche Ltd.

Results - Variant Calling

Overall, we observed high alignment between Illumina and SBX-based variant calling (Fig. 2). Duplex sequencing and the replacement of PCR with linear amplification in SBX reduced SNV and INDEL error rates, improving sensitivity for detection of subclonal variants and variants in challenging genomic context, such as low-complexity regions. These gains were achieved while maintaining the same low false positive rates as Illumina (0.13 SNV FP/Mbp and 0.08 INDEL FP/Mbp).

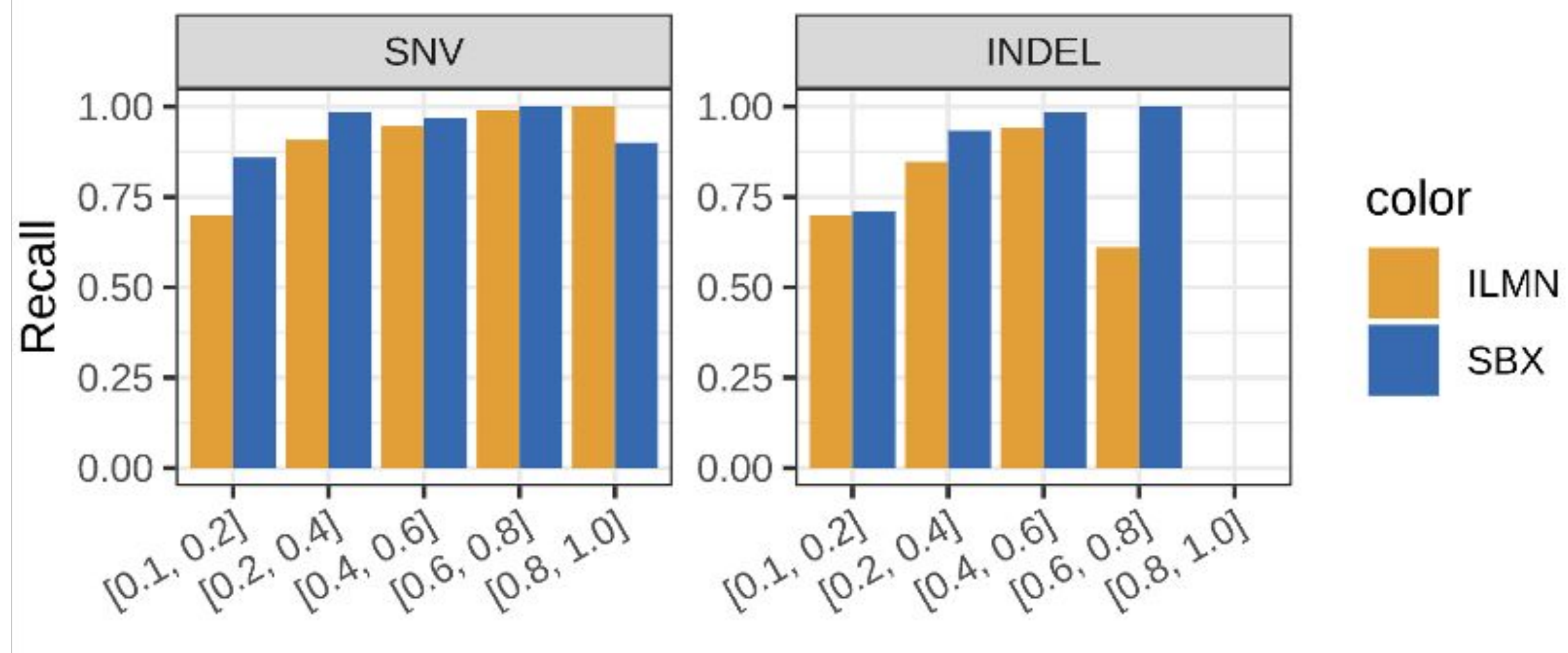


Figure 2. Performance of SNV and INDEL calling in SBX-D sequencing of FFPE tissues. Duplex sequencing and linear amplification contribute to high SNV and INDEL accuracy in SBX-D

In addition to small variant calling, next we evaluated the performance of SBX-D based sequencing to perform somatic allele specific copy number variant calling, somatic structural variant calling and HLA typing. Overall, we observed >99% concordance in our cohort consisting of 20 T/N pairs (Fig. 3).

Sample 4 (Ovarian Cancer)

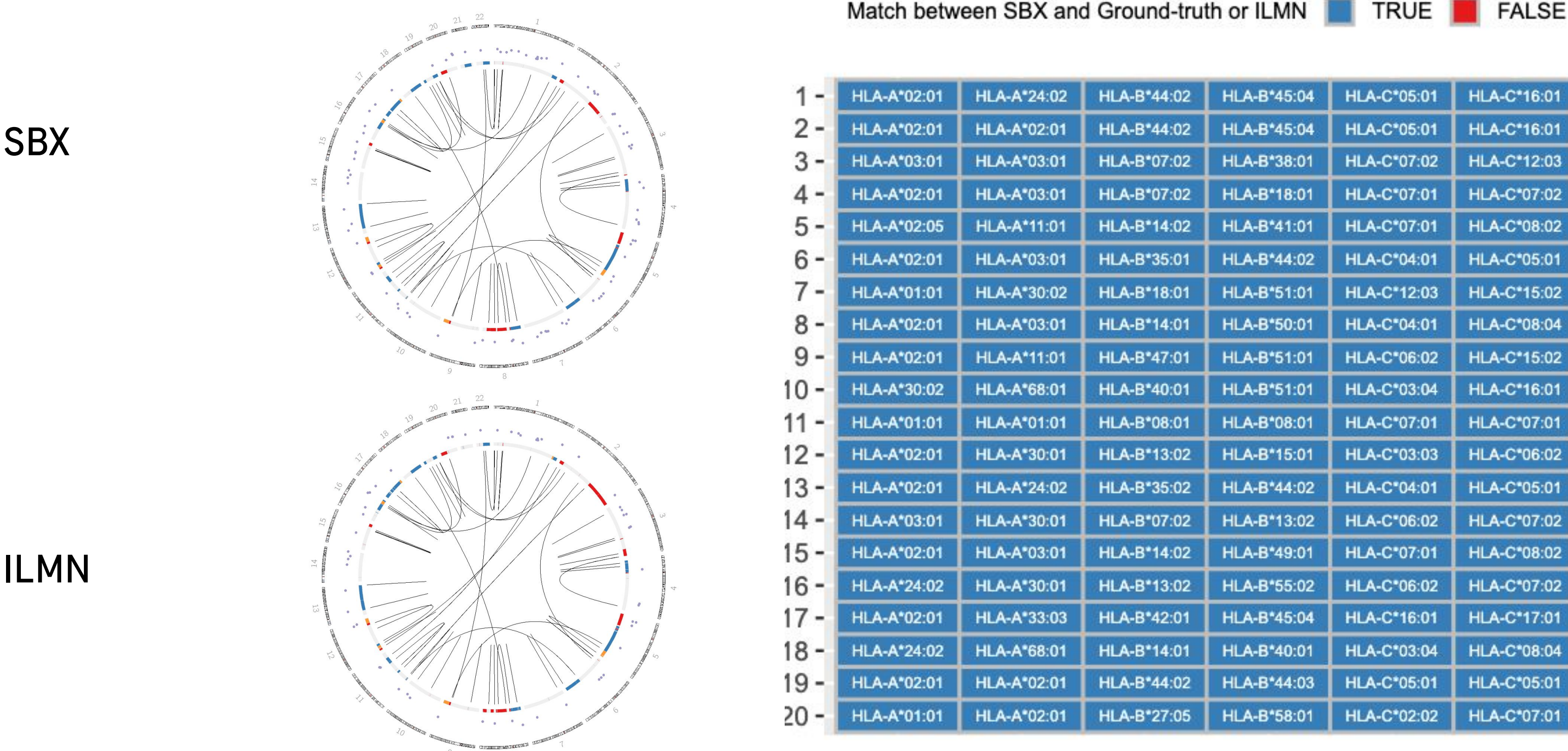


Figure 3. Benchmarking of comprehensive genomic profiling using SBX-D including small variant calling (SNV/INDEL), allele specific copy number variants (CNV), structural variants (SV) and HLA typing. Circos plots display COSMIC small variants using purple dots, CNVs on inner tracks displayed with red (gain), blue (loss), orange (LOH), and curved arcs to represent larger SVs such as translocations, inversions, insertions, deletions, or duplications across genomic regions.

Results - Biomarkers

Finally, we evaluated the performance of SBX-D in detection of biomarkers. Overall, SBX-D shows a strong performance across all measured biomarkers suggesting SBX-D as a reliable approach for biomarker research studies (Fig. 4).

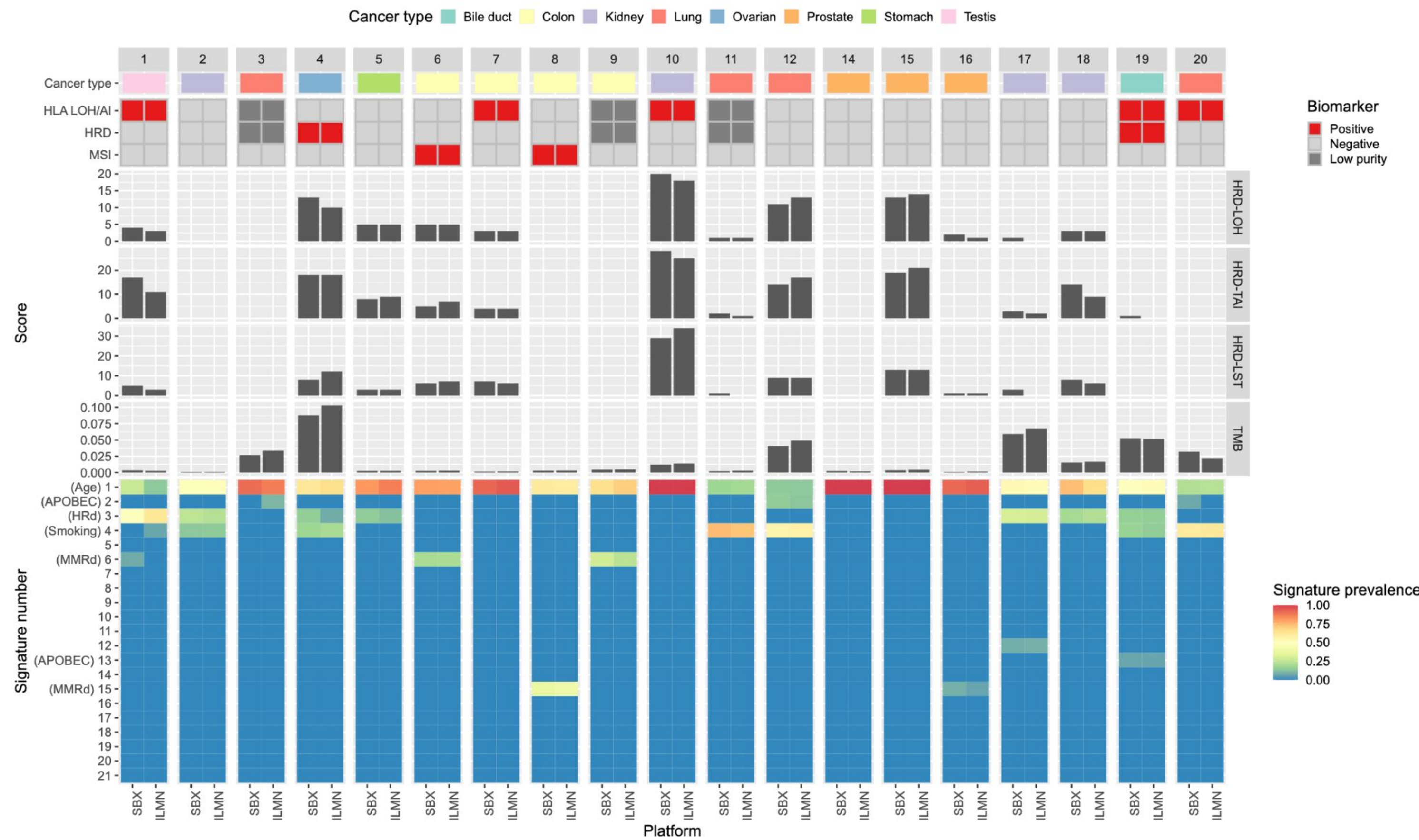


Figure 4. Comprehensive benchmarking of SBX-D base oncology biomarkers from WGS of FFPE samples. Results show a high concordance across a variety of biomarkers including tumor mutational burden (TMB), microsatellite instability (MSI), homologous recombination deficiency (HRD), HLA loss or allelic imbalance (LOH/AI), and mutational signatures.

Conclusion

Here, we benchmarked comprehensive genomic profiling and biomarker measurements using WGS of FFPE tissues using SBX-D technology. Overall, SBX-D showed highly consistent results across all benchmarking samples and variant types, while also detecting low AF subclonal events due to a high base accuracy.

SBX-D demonstrates reasonable sensitivity and turnaround times, offering a promising approach for accurate whole genome sequencing in oncology research applications.

References

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