Whole Genome Sequencing Minimum Residual Disease Detection using Sequencing By Expansion (SBX)

Kendall Berg,[1] Thao Ho,[1] Abid Hasan,[2] Alan Kimura,[1] Alberto Gatto,[2] Alec Sautter,[1] Alex Lehmann,[1] Anasha Arryman,[1] Chen Zhao,[2] Cynthia Cech,[1] Dinendra Abeyawardhane,[1] Emily Ormbrek,[1] Fan Song,[2] Grant Kingsley,[1] Jagadeeswaran Chandrasekar,[1] Joanne Leadbetter,[1] John Mannion,[2] Lacey McGee,[1] Mahdi Golkaram,[2] Marc Prindle,[1] Matthew Lopez,[1] McKenna Osentowski,[1] Megan Freer,[1] Megan LeProwse,[1] Melud Nabavi,[1] Robert Busam,[1] Ron Cicero,[2] Ryan Toma,[1] Taylor Lehmann,[1] Thomas Reid,[1] Upneet Bala,[1] Mark Kokoris[1]

[1]Roche Sequencing Solutions, Inc, Seattle, WA, USA; [2]Roche Sequencing Solutions, Inc, Santa Clara, CA, USA

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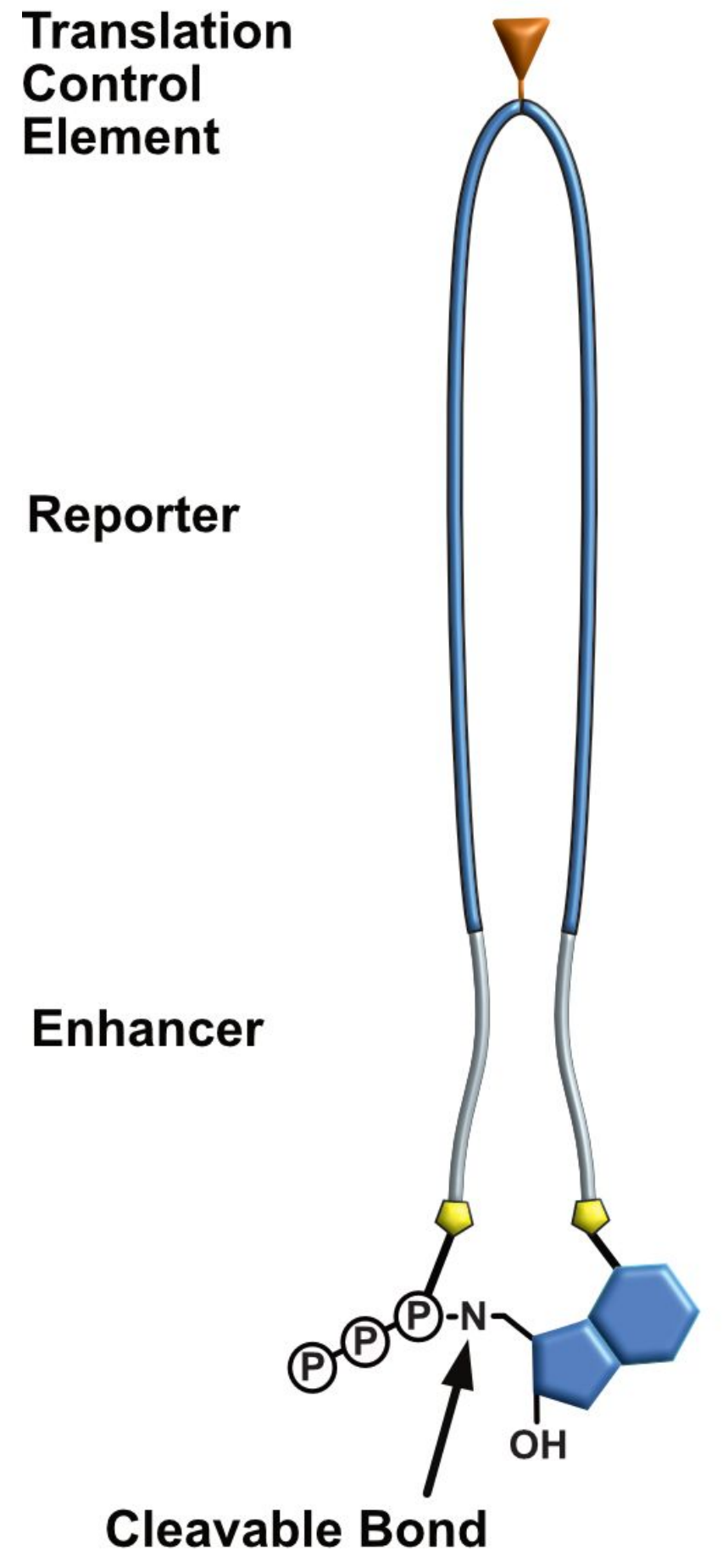
Background

Detection of minimal residual disease (MRD) is one of the strongest predictors of cancer relapse post treatment, and therefore a critical component of cancer management. While WGS of cfDNA offers a non-invasive and comprehensive view, it is limited by the error rate and sensitivity required to detect mutations especially in low tumor burden conditions.[1] Here, we assess the performance of WGS workflow for MRD detection using Sequencing by Expansion (SBX).

Introduction to SBX

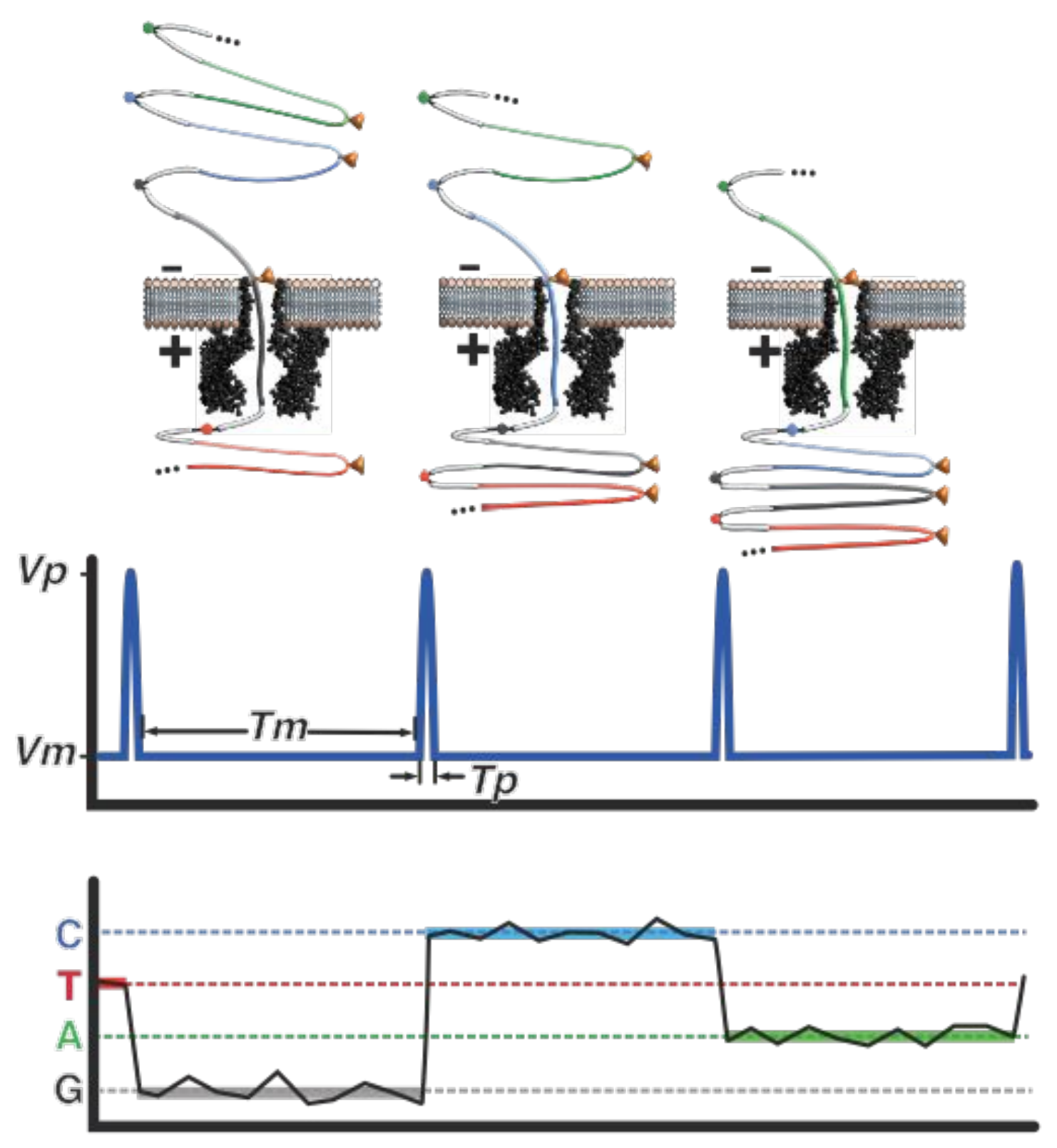
Sequencing By Expansion (SBX) technology is a novel sequencing approach that uses a biochemical process to encode the sequence of a target nucleic acid molecule into a measurable surrogate polymer called an Xpandomer.[2]

Figure 1. X-NTP Structure



The Expandable Nucleotide Triphosphate (X-NTP) is the building block of the Xpandomer. There are 4 different XNTPs, one for each nucleotide. Highly designed components of the XNTP allow for high signal to noise and non-stochastic translocation control.

Figure 2. Xpandomer Measurement



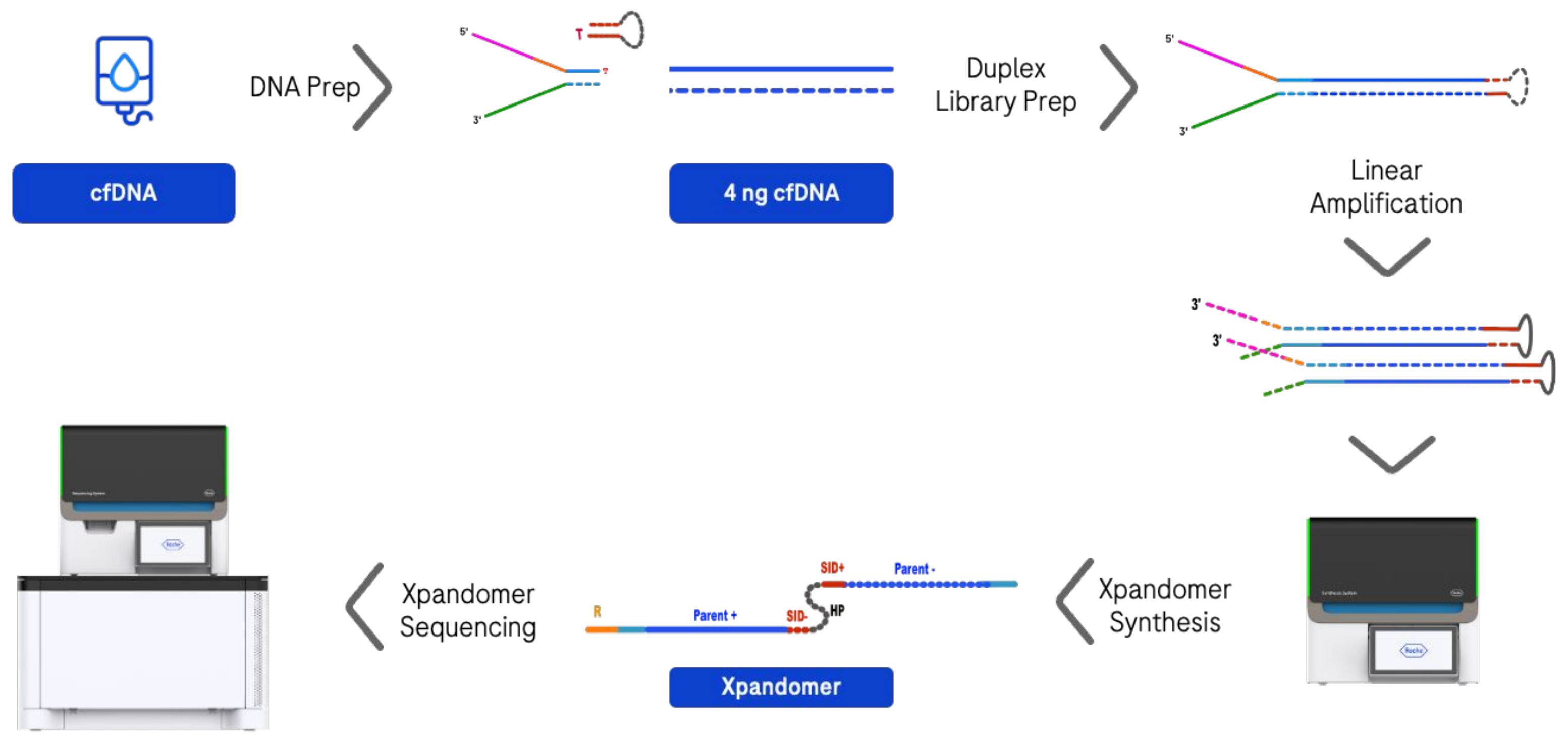
The SBX workflow has two components: synthesis and measurement. During synthesis, X-NTPs are used to enzymatically transcribe the template DNA into an Xpandomer molecule.

After synthesis, the resulting Xpandomer is sequenced by stepwise translocation through a nanopore.

SBX-D Workflow

SBX-D is a library prep and linear amplification sequencing workflow that achieves high accuracy by linking both strands of the target DNA within a single sequencing read. This allows for single-strand errors that arise on separate strands of the DNA molecule to be resolved, thereby achieving higher accuracy.

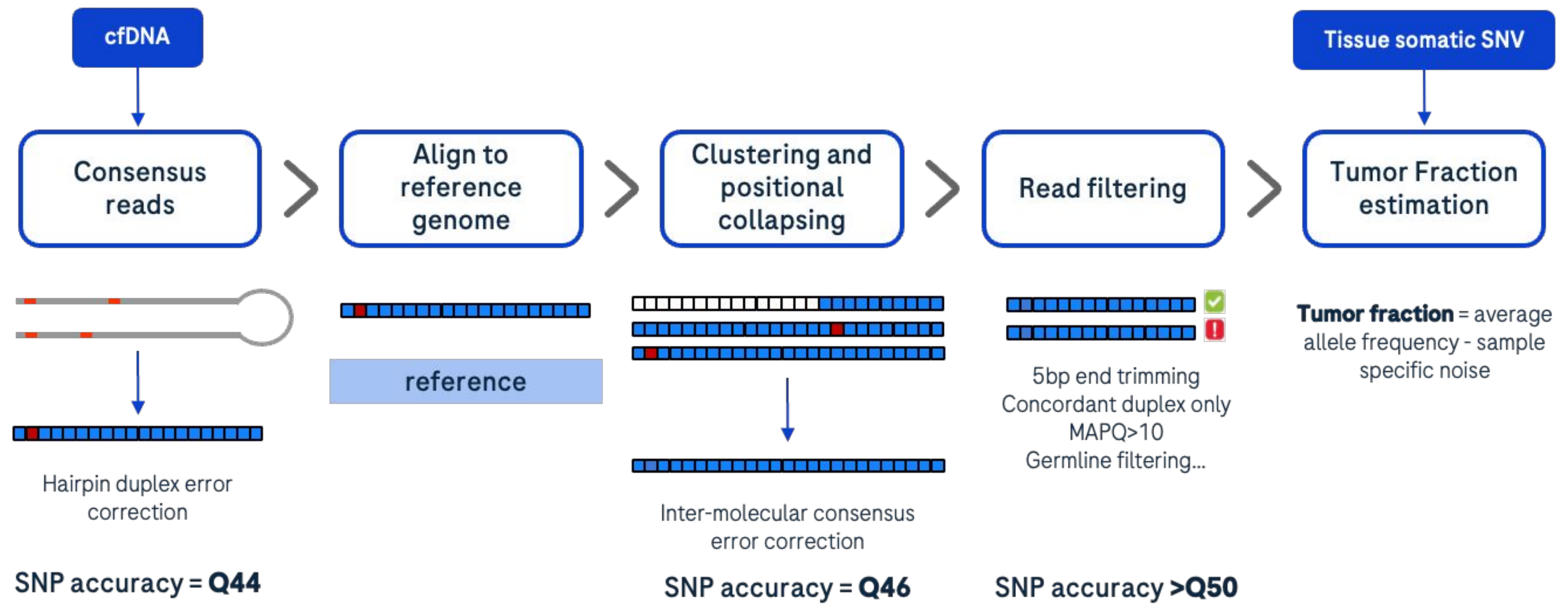
Fig 3. SBX-D workflow for MRD



Materials & Methods

Fifteen (15) healthy and fifteen (15) cancerous cfDNA research samples from a diverse cohort of cancer diagnoses and stages were used to benchmark MRD detection performance. Four nanograms (4 ng) of DNA was input into the SBX-D library prep workflow and sequenced targeting 100x coverage. SBX data were processed through SBX-optimized open source (XOOS) variant callers.[3]

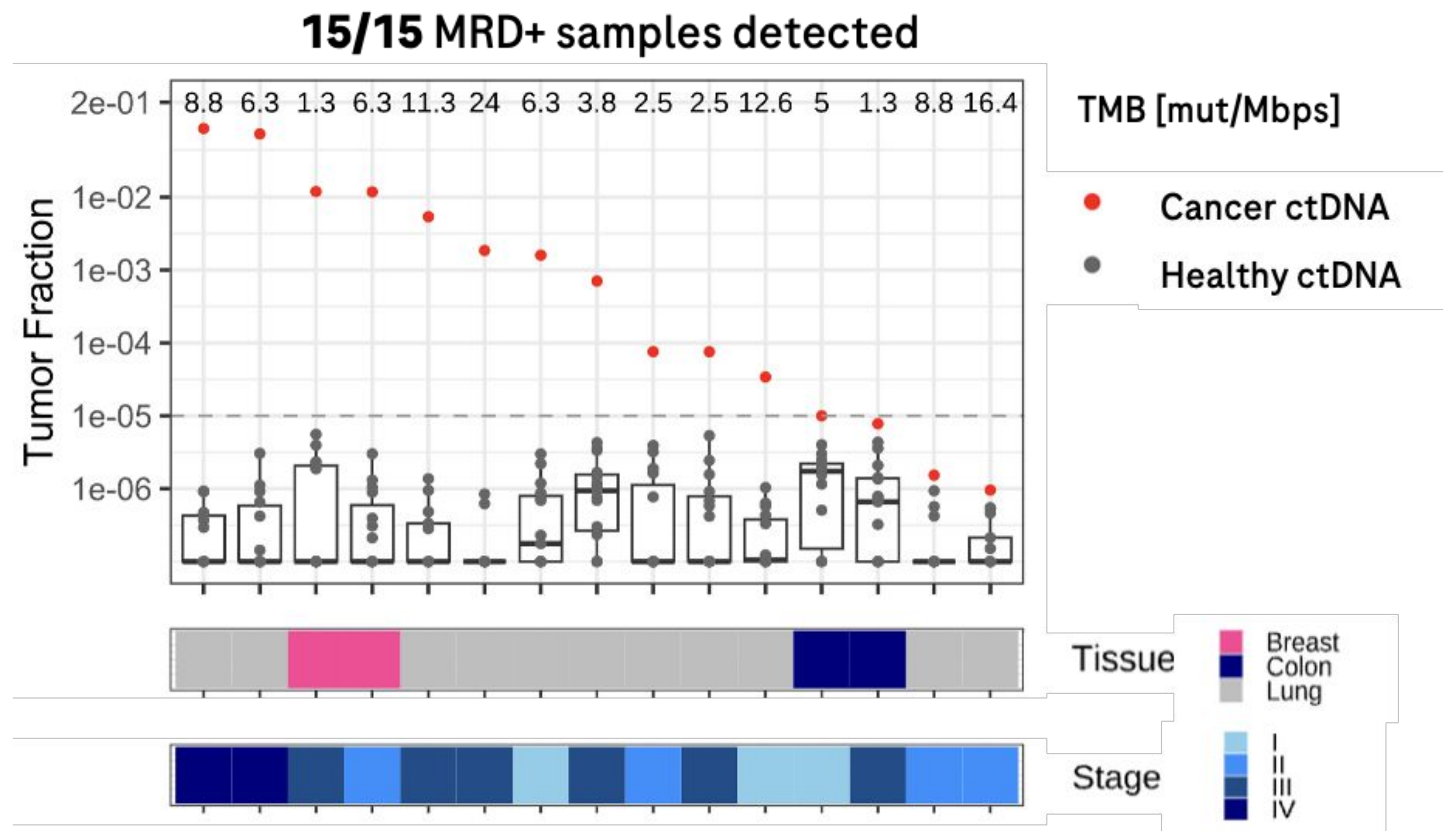
Fig 4. Read Processing and Analysis



Results

MRD signal was detected for all cancer sample driven cfDNA. Using inter-molecular consensus formation on duplex reads and bioinformatic error correction, we observed SNP accuracy >Q50, and detected low frequency variants indicative of MRD in all cancer samples, even in a sample with tumor fraction as low as 1x10-6. Average measured tumor fraction in healthy cfDNA samples was measured to be 6x10-7.

Fig 5. MRD detection



Conclusion

This preliminary result demonstrates the potential of SBX-D for minimal residual disease (MRD) applications, even with challenging samples. This enhanced sensitivity could enable earlier and more precise identification, which may lead to more timely and tailored treatment to improve long-term outcomes.[1]

Disclosures

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

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