# Improved liquid biopsy assay performance using sequencing by binding (SBB)

Daniel Nasko, Phillip Pham, Stuti Joshi, Kristi Kim, Nairi Pezeshkian, Christopher Kingsley, Daniel Portik, Young Kim, Alex Sockell, and Jonas Korlach
PacBio, 1305 O'Brien Drive, Menlo Park, CA 94025

## Introduction

Liquid biopsy is revolutionizing the field of early cancer detection research through non-invasive detection of tumor DNA in the blood. However, existing liquid biopsy assays are limited in their sensitivity for ctDNA detection at low variant allele frequencies (VAFs). Here we describe the application of the PacBio Onso short-read sequencing system to enable detection of ctDNA at low VAFs using the SeraCare Complete ctDNA Mutation Mix reference sample.

## Improved raw accuracy with SBB

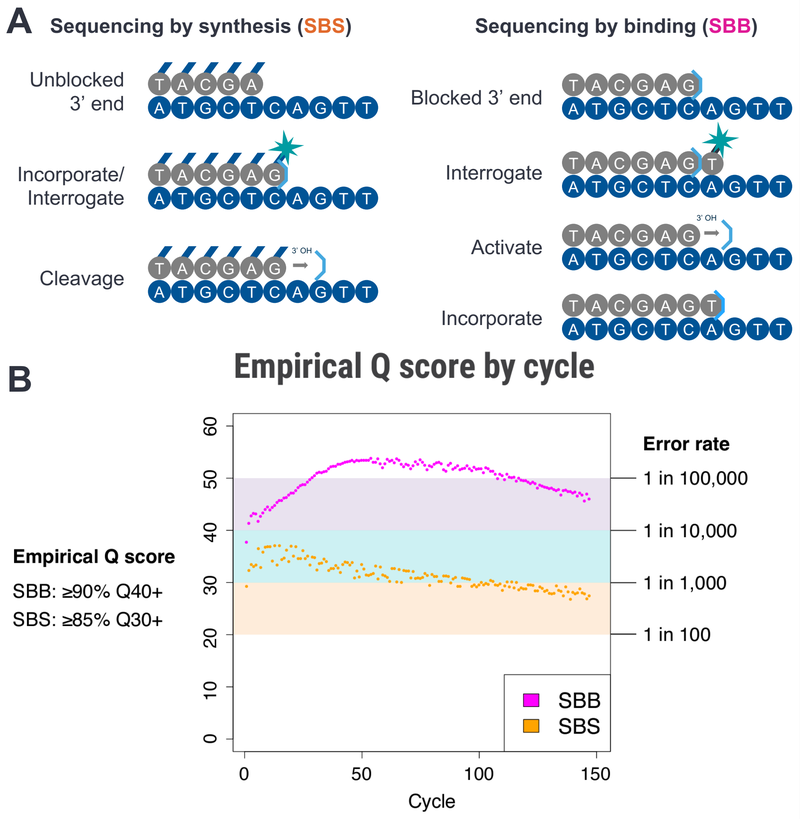



**Figure 1. (A)** SBB results in improved empirical Q scores compared to SBS due to decoupling of the interrogation and incorporation steps during each sequencing cycle. **(B)** Empirical Q score for top 99% of bases with SBB vs. SBS.

## Experimental design

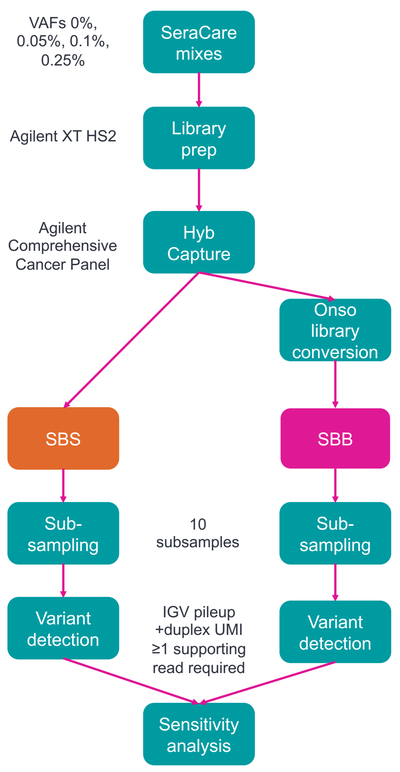
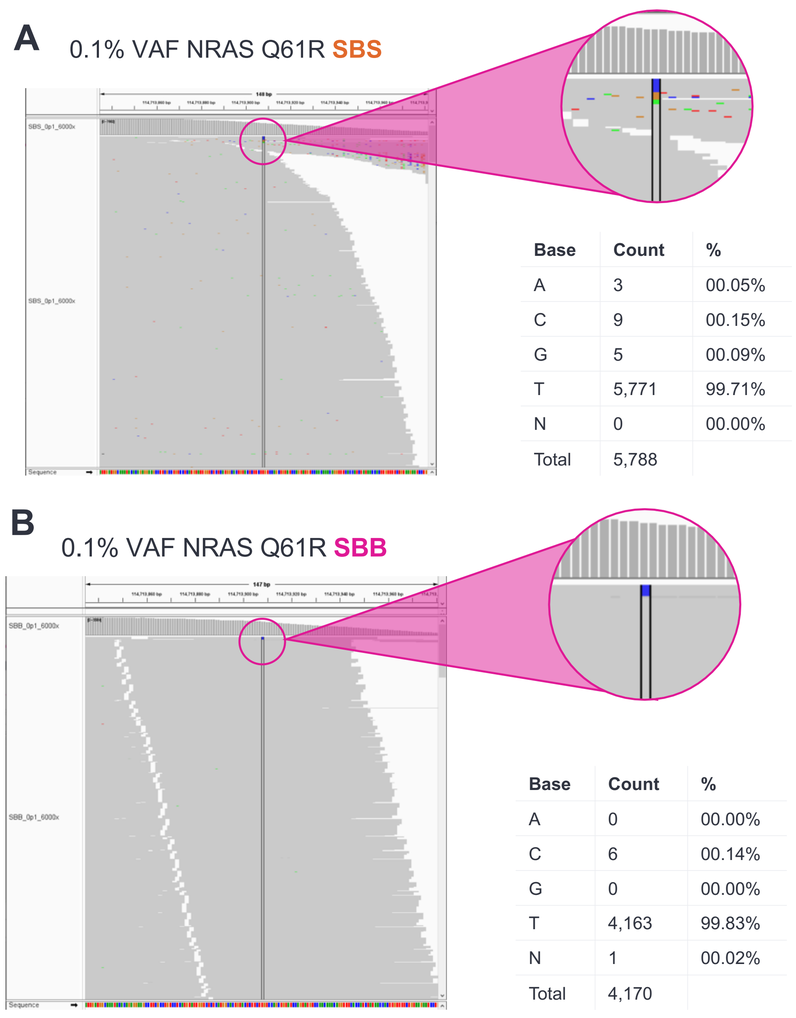



**Figure 2.** Overview of experimental workflow. Post-capture libraries were split into 2 aliquots for the comparison, one of which was converted using the Onso library conversion kit to add Onso-compatible adapter sequences.

## Decreased noise at variant sites

Increased sequencing accuracy achieved by SBB reduces noise due to errors at variant sites. In SBS data, all 3 alternative alleles have a similar number of base calls, obscuring the true variant. In SBB data, the lack of errors reveals the variant is a T→C.




| Base | Count | % |
|---|---|---|
| A | 3 | 00.05% |
| C | 9 | 00.15% |
| G | 5 | 00.09% |
| T | 5,771 | 99.71% |
| N | 0 | 00.00% |
| Total | 5,788 | |

| Base | Count | % |
|---|---|---|
| A | 0 | 00.00% |
| C | 6 | 00.14% |
| G | 0 | 00.00% |
| T | 4,163 | 99.83% |
| N | 1 | 00.02% |
| Total | 4,170 | |

**Figure 3.** IGV plots (left) and base counts (right) for SBS **(A)** and SBB **(B).**

## Reduction in false positives

Reduced false positive variant calls in the WT sample for SBB lowers the limit of detection and improves recall when specificity is set at 100%.

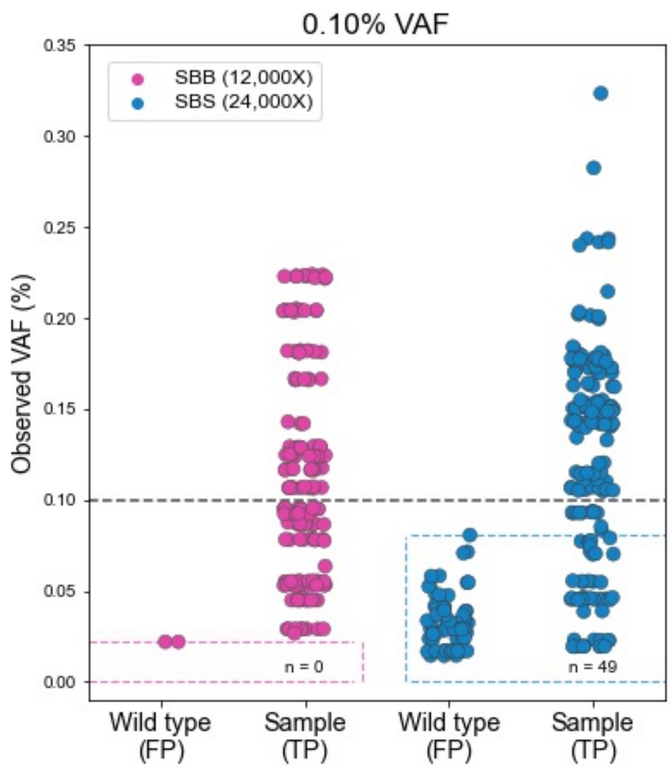



**Figure 4.** Observed VAFs for WT sample (false positives, FP) and 0.1% VAF sample (true positives, TP). Dashed box indicates variants that would be excluded with this specificity cutoff for each technology.

## More accurate VAF estimation

SBB also performs better at estimating the true VAF than SBS with only half the sequencing depth, with less variability in allele frequency estimates.

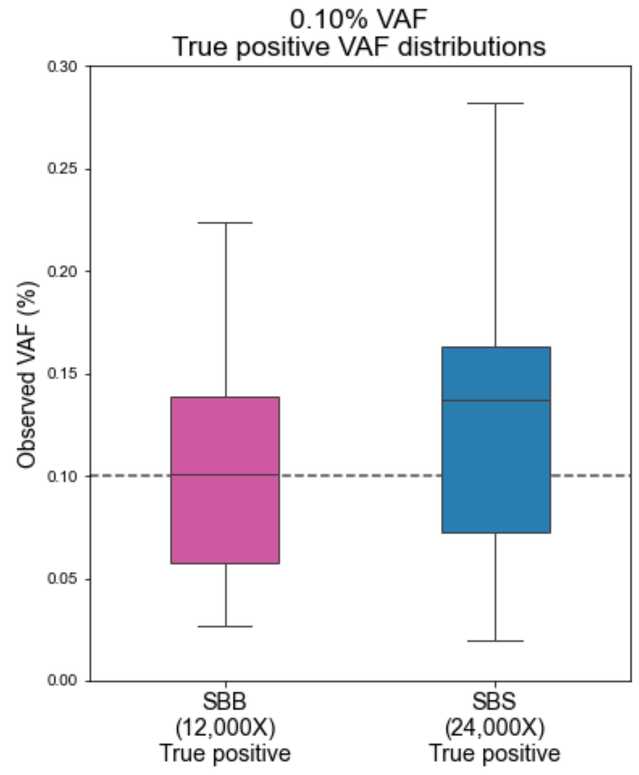



**Figure 5.** Distribution of observed VAFs for true positive variants in the 0.1% VAF sample for SBB at 12,000X and SBS at 24,000X.

## Improved recall at low VAFs

Improved accuracy with SBB results in improved recall for low frequency VAFs (0.05%, 0.1%) compared to SBS, even with half the sequencing depth.

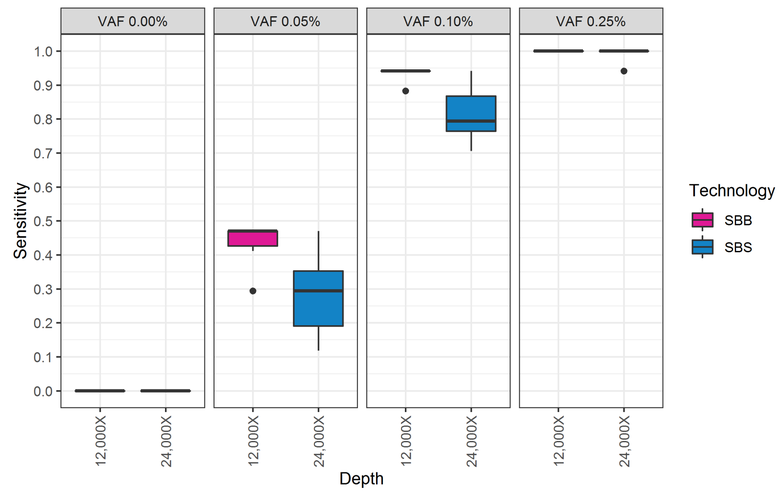



**Figure 6.** Sensitivity for variant detection at VAFs 0.0% (WT), 0.05%, 0.1%, and 0.25% for SBB at 12,000X and SBS at 24,000X.

## Conclusions

We observe superior sensitivity for ctDNA detection using SBB compared to SBS at low VAFs (0.05%, 0.10%) at comparable sequencing depth. Furthermore, SBB requires significantly less sequencing to achieve comparable sensitivity results to SBS. Taken together, our results demonstrate the potential of SBB to improve upon existing methods of liquid biopsy and better enable research on early cancer detection.