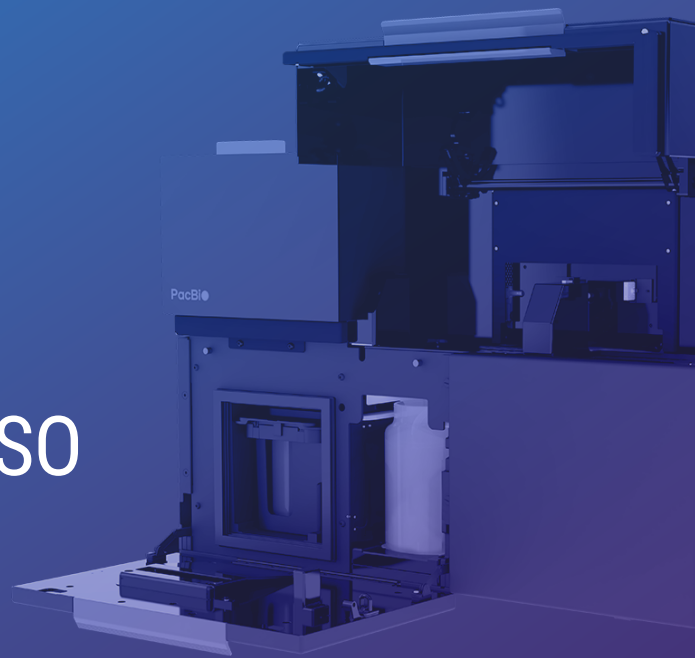# Product note

SEQUENCING BY BINDING (SBB) FOR USE ON THE ONSO SEQUENCING SYSTEM

Introduction

SBB® is an innovative sequencing technology that delivers unprecedented NGS accuracy. By incorporating native nucleotides and removing the need to cleave fluorescent modifications, the sequencing strand has minimal scarring and results in higher accuracy base calls than other sequencing chemistries.

Key features of SBB

- 90% of bases ≥Q40
- Industry-leading sensitivity
- High-confidence sequencing through repetitive regions
- Low duplication rate
- Minimal index-hopping
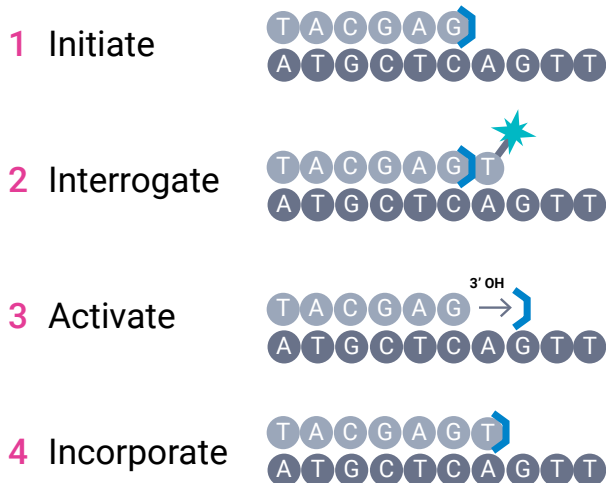
Sequencing by binding (SBB)

Figure 1. Conceptual diagram of SBB sequencing.

(1) Each cycle initiates with a 3' reversible blocked nucleotide. (2) Fluorescently labelled nucleotides are then flowed over the flow cell allowing for the appropriate base to bind. Unbound nucleotides are washed away so that the base can be interrogated with reduced background signal. (3) The 3' end of the nucleotide is activated via removal of the reversible terminator. (4) Native, unlabeled, reversible blocked nucleotides are flowed over the flow cell and the cognate base can incorporate into the growing strand. The process is then repeated for each new sequenced base.

By separating the interrogation and incorporation steps, and optimizing chemistries for each step, SBB chemistry enables high signal-to-noise levels to be achieved. Moreover, the lack of molecular scarring from the use of native nucleotides for incorporation drives industry-leading accuracy.

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	Paired end		Single end	
Lane	1	2	1	2
Number of reads	442 M	438 M	241 M	233 M
Q score (90%)	41.0	41.0	41.6	41.5
Read length	150/150	150/150	217	217
Yield	66.3 Gb	65.7 Gb	52.3 Gb	50.6 Gb

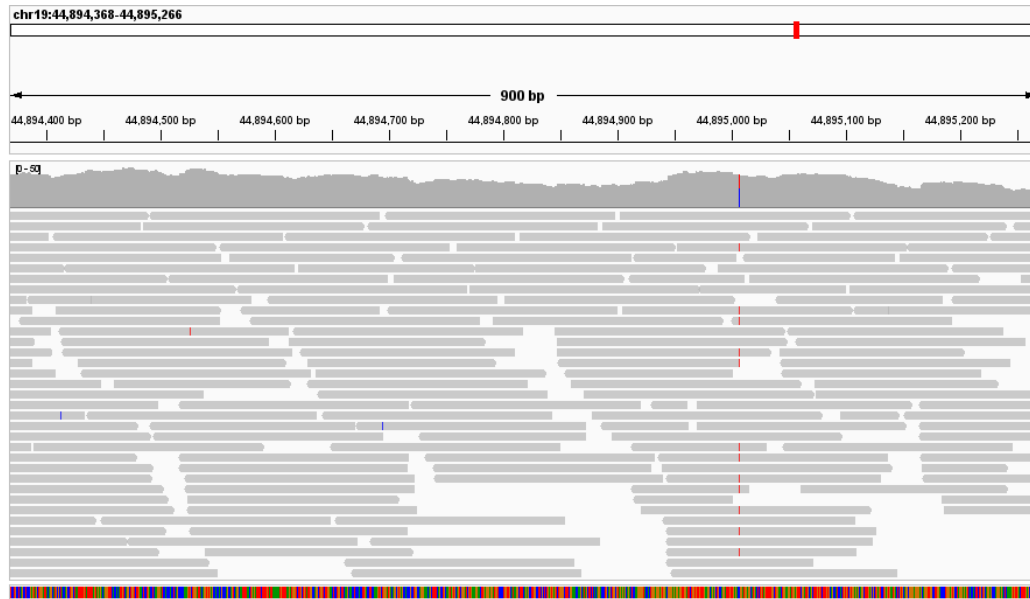Table 1. Sample output metrics from the Onso™ sequencing system.

Figure 2. SBB sequencing delivers near perfect reads with >90% of bases above Q40, low duplication rates, minimal index-hopping, and sequencing through difficult and repetitive regions.
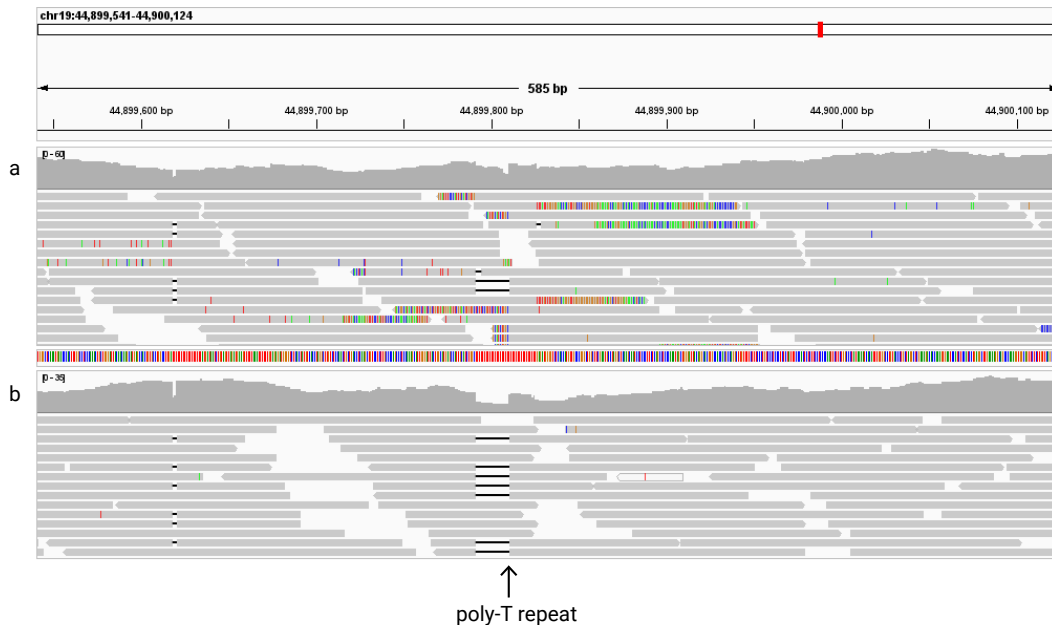

Figure 3. The Onso sequencing system produces near perfect reads through the variable poly-T region within the *TOMM40* gene. (a) SBS sequencing was unable to fully resolve the low-complexity region, with many false positive calls. (b) SBB sequencing was able to fully sequence through and confidently resolve this region, resulting in a call of T16 vs the original estimate of T35.

SBB complete workflow

Onso library prep

- Employ standardized NGS library prep optimized for SBB sequencing
- Create Onso libraries directly from samples
- Adapt existing SBS libraries to an Onso-compatible format

Onso sequencing

- Easily implement workflow into new and existing NGS labs
- Produce highly accurate reads with SBB chemistry
- Independently address flow cell lanes

Data analysis

- Generate industry-standard FASTQ output
- Easily integrate with currently available secondary analysis tools

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Explore the applications:
pacb.com/applications

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