Next-level discovery
Setting a new standard for short reads with the Onso™ system

- Up to 15× higher accuracy than other benchtop sequencers
- Extraordinary sensitivity
- Reduced cost per sample
- Seamless workflow integration
What does Q40+ mean for you?

Powered by sequencing by binding (SBB™) chemistry, the Onso system delivers groundbreaking short-read sequencing performance with accuracy at 90% Q40+. This 15× improvement in accuracy translates to increased sensitivity needed for rare variant detection, significantly reduced sequencing requirements, and overall increased throughput at lower cost per sample.

![Empirical Q score diagram](image)

**Empirical Q score**
- **SBB**: ≥90% Q40+
- **SBS**: ≥85% Q30+

Figure 1. SBB consistently achieves Q40+ quality scores, relative to the diminishing scores of SBS to <Q30 after 100 cycles.

The heightened sensitivity enabled by the Q40+ accuracy of SBB technology allows for nearly twice the sensitivity for rare variant detection at equivalent sequencing depth compared to SBS (figure 2A) or better sensitivity with 4-fold less sequencing (figure 2B) compared to SBS.

![Variant detection performance](image)

**Figure 2.** Variant detection performance was tested for SBB and SBS sequencing with known variants in ten genes across five variant allele frequencies. (A) 6,000× SBB UMI- vs 6,000× SBS UMI-; (B) 6,000× SBB UMI+ vs 24,000× SBS UMI+. Adding UMI-based deduplication increases Onso performance even further (SBB in panel A vs in panel B).
What can you do with the Onso system?

**ctDNA detection for liquid biopsy research**

Liquid biopsy, a noninvasive assay for circulating tumor DNA (ctDNA) in blood and other fluids, holds the promise to revolutionize research on cancer detection and monitoring. Because ctDNA variants often occur at very low frequencies, their detection requires ultrasensitive technology that can best be provided by SBB chemistry.

**Distinguish the variant from the noise**

Since higher Q scores mean a higher signal-to-noise ratio, the Q40+ accuracy of the Onso system enables confident variant detection with fewer confounding errors. Sequencing errors in SBS sequencing make it almost impossible to distinguish the variant from the noise (figure 3A), whereas sequencing on the Onso system makes the true variant clear (figure 3B).

**What will you discover?**

**Cancer research**

Detect low-frequency mutations in cancer research applications, including in difficult-to-sequence regions

**Infectious disease**

Discover low-level drug-resistance mutations

**Gene editing research**

Identify novel biomarkers and confirm editing outcomes

**Single cell**

Understand cell heterogeneity with compatible 10x single cell DNA and RNA libraries

Figure 3. IGV output of SBS (A) and SBB (B) of example NRAS Q61R variant from the SeraCare ctDNA complete mutation mix at 6,000 coverage.
The difference is in the chemistry

Unlike traditional SBS technology, SBB uses optimized conditions for each phase of the sequencing cycle, nearly eliminating raw read errors. It is this difference in chemistry that constitutes a breakthrough in sequencing accuracy.

How does SBB technology work?

SBB consists of four primary steps: initiation, interrogation, activation, and incorporation. In a critical departure from SBS, SBB chemistry separates the binding and subsequent extension steps of the sequencing process which eliminates the errors introduced by molecular artifacts.

Initiate: Sequencing is initiated with a reversible blocker on the 3’ end to prevent additional bases from incorporating.

Interrogate: Fluorescently tagged bases flood the flow cell. Once the appropriate base binds, a signal is emitted and measured with the powerful optics of the Onso system.

Activate: Next, the 3’ end of the nucleotide is activated via the removal of the reversible blocker.

Incorporate: Finally, the complementary base is incorporated from unlabeled, blocked nucleotides that flood the flow cell. This blocks additional incorporation.

SBB features

- **No molecular scarring**: No residual linker arms left during incorporation
- **7x less sample input**: Use fewer of your valuable samples
- **Minimal duplication**: Fewer redundant sequences means more useful reads
- **Negligible index hopping**: Avoids library misassignments and increases usable reads
- **Q40+ accuracy**: Extraordinary error rate of only 1 in 10,000 bases or less

These aspects of SBB chemistry make Q40+ accuracy possible.
Library prep

The Onso library prep kits are used to create libraries that are optimized to support the Q40+ sequencing accuracy of the Onso system. These kits benefit from streamlined workflows, including the conversion of existing P5/P7 libraries, that generate complete libraries in as little as three hours.

Workflow

Workflows are available for high-molecular weight (HMW) DNA (figure 4A), and pre-fragmented or degraded DNA (figure 4B).

A. Onso fragmentation library prep workflow

B. Onso DNA library prep workflow

Figure 4. Workflows for the Onso fragmentation library prep kit and the DNA library prep kit.

Key benefits

- **Performance**
  - Libraries optimized for Q40+ sequencing accuracy
  - Higher conversion efficiency than ligation-based approaches

- **Ease of use**
  - Optimized workflow for complete library prep with a single kit in as few as three hours

- **Flexibility**
  - Accommodates a wide range of sample types (e.g., fragmented or HMW DNA) and input amounts (10–1,000 ng)

- **Compatibility**
  - Supports major short-read applications
  - Library conversion kit enables existing P5/P7 libraries to be sequenced on the Onso system
  - Seamless integration of Onso libraries with PacBio Compatible partners across the sequencing workflow

Libraries optimized for Q40+ accuracy

Sequencing reads generated from Onso library prep kits benefit from greater accuracy than those generated through standard short-read library prep methods (figure 5).

Figure 5. Improved read accuracy with Onso library prep vs competitor kits (competitors K and N).
Fits into your workflow

Onso sequencing consumables and ancillary kits enable a seamless workflow for today’s NGS laboratories.

Onso reagent pack, flow cell, and clustering plate.

Three-step loading process

1. Load flow cell into cluster generator
2. Insert reagent pack into sequencer
3. Insert flow cell into sequencer

Minimal hands-on time required for setup
Intuitive user interface allows step-by-step guidance through workflow
Individually accessible flow cell lanes for added loading flexibility

Application specifications

The Onso system is a mid-range short-read sequencer capable of supporting a wide range of applications required for most laboratories.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Read format</th>
<th>Number of samples/run†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target enrichment panel (1 Mb, 6,000× mean depth)</td>
<td>2 × 150</td>
<td>20–25</td>
</tr>
<tr>
<td>Single-cell RNA-Seq (10K cells, 20K reads/cell)</td>
<td>2 × 100*</td>
<td>2–3</td>
</tr>
<tr>
<td>Targeted amplicon panel (160 genes/500× mean depth)</td>
<td>2 × 150</td>
<td>48–60</td>
</tr>
</tbody>
</table>

* Application-specific read format varies; supported by Onso 200-cycle sequencing kit
† Numbers shown are estimates based on expected output per kit. Actual number of samples will vary depending on sample type, quality, and experimental objectives.
The Onso system

The Onso system and cluster generator offer a scalable and flexible benchtop platform that gives you remarkable accuracy and the capability to integrate with existing short-read tools.

Sequencing specifications

<table>
<thead>
<tr>
<th>Onso reagents</th>
<th>Read length</th>
<th>Reads</th>
<th>Output (Gb)</th>
<th>Run time</th>
<th>Quality score</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 cycle sequencing kit</td>
<td>2 × 100 bp 1 × 200 bp</td>
<td>400–500 M</td>
<td>100</td>
<td>32 hours</td>
<td>≥90% Q40</td>
</tr>
<tr>
<td>300 cycle sequencing kit</td>
<td>2 × 150 bp</td>
<td>400–500 M</td>
<td>150</td>
<td>48 hours</td>
<td>≥90% Q40</td>
</tr>
</tbody>
</table>

Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>Part number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onso fragmentation DNA library prep kit</td>
<td>102-499-100</td>
</tr>
<tr>
<td>Onso DNA library prep kit</td>
<td>102-431-400</td>
</tr>
<tr>
<td>Onso indexed adapter kit</td>
<td>102-431-700</td>
</tr>
<tr>
<td>Onso library amp kit</td>
<td>102-410-800</td>
</tr>
<tr>
<td>Onso library quant kit</td>
<td>102-431-800</td>
</tr>
<tr>
<td>Onso blocking oligo kit</td>
<td>102-431-600</td>
</tr>
<tr>
<td>Onso library conversion kit</td>
<td>102-529-500</td>
</tr>
<tr>
<td>Onso indexed library control kit</td>
<td>102-529-900</td>
</tr>
<tr>
<td>Onso system</td>
<td>102-837-000</td>
</tr>
<tr>
<td>Onso 200 cycle sequencing kit</td>
<td>102-860-100</td>
</tr>
<tr>
<td>Onso 300 cycle sequencing kit</td>
<td>102-860-300</td>
</tr>
</tbody>
</table>
We have offices in countries around the world. Visit pacb.com/contact for contact info.

READY TO GET STARTED WITH THE ONSO SYSTEM?

Products and services
pacb.com/products

Documentation
pacb.com/documentation

Application-specific workflows
pacb.com/applications

Publications using SMRT® sequencing
pacb.com/pubs

Connect with PacBio
North America: nasales@pacb.com
South America: sasales@pacb.com
EMEA: emea@pacb.com
Asia Pacific: apsales@pacb.com

Contact a certified service provider
pacb.com/CSP

Research use only. Not for use in diagnostic procedures. © 2023 Pacific Biosciences of California, Inc. (“PacBio”). All rights reserved. Information in this document is subject to change without notice. PacBio assumes no responsibility for any errors or omissions in this document. Certain notices, terms, conditions and/or use restrictions may pertain to your use of PacBio products and/or third-party products. Refer to the applicable PacBio terms and conditions of sale and to the applicable license terms at pacb.com/license. Pacific Biosciences, the PacBio logo, PacBio, Circulomics, Omniome, SMRT, SMRTbell, Iso-Seq, Sequel, Nanobind, SBB, Revio, and Onso are trademarks of PacBio.