PacBio RS
REVEAL THE TRUE BIOLOGY

A revolutionary DNA sequencer advancing discovery in *de novo* assembly, targeted sequencing, and base modification applications
The PacBio RS system is a third-generation DNA sequencer that provides real-time analysis of biomolecules with single molecule resolution. SMRT® sequencing technology allows users to:

- Finish genomes and comprehensively characterize genetic variation with very long read lengths.
- Confirm discoveries with high single molecule and consensus accuracy.
- Obtain deep insights into base modifications with unique kinetic information.
- Complete projects quickly and efficiently with a simple, fast workflow.

**De Novo Assembly**

Generate finished genome assemblies

The PacBio RS is the only microbial sequencing platform that finishes genomes, determines structure and resolves strains. Combine long read PacBio data with short read second generation data or use PacBio data exclusively.

- **Complete genome assemblies** – long read lengths combine with high accuracy to produce high-quality, finished genomes
- **Accurate characterization of large structural variations** – long read lengths uniquely provide the ability to sequence large repeat regions and resolve complex structure
- **Unbiased genome coverage** – balanced coverage and minimal GC-bias for high-quality assembly of high or low GC content organisms or regions
- **Cost-effective and fast** – approximately 10x reduction in finishing costs and results in less than 10 hours
Typical Results

Read Length Distribution

Mean RL: 3,162 bp
95th percentile: 8,006 bp
Max RL: 14,779 bp

Accuracy

QV vs Coverage

Based on data from E. coli with 10 kb libraries using a 90 minute movie.
Targeted Sequencing
Comprehensively characterize genomic variation

The PacBio RS provides long reads to fully characterize genetic complexity, including rare SNPs, indels, structural variants, and haplotypes. Long reads are required because variant calling with short reads is limited by mapping errors and imprecision. For example, a sample may contain sequence that is divergent from the reference or the reference itself may be incomplete. In such cases, short reads are likely to map incorrectly, potentially leading to false variant calls. In cases of larger structural variants, short reads cannot determine the exact location, size or allelic sequence.

Long reads coupled with single molecule resolution allow comprehensive characterization of heterogeneous samples and identification of variation invisible to short read multi-molecule sequencing technologies.

- **Reduced false positives** – little systematic bias provides confidence in results and higher positive predictive value
- **Observation of structural variants** – location, size, and allelic sequence information enabled by long reads
- **Ability to resolve phasing of mutations** – observation of haplotypes and correlation to phenotypes or drug response
- **Access to the entire genome** – flexibility to sequence through repetitive and GC-rich regions

### Typical Results

**EGFR-MET Amplicon Panel**

<table>
<thead>
<tr>
<th></th>
<th>NA17316</th>
<th>NA17317</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Movie Time</strong></td>
<td>2 x 30 mins</td>
<td></td>
</tr>
<tr>
<td><strong>Insert Size</strong></td>
<td>250 bp</td>
<td></td>
</tr>
<tr>
<td><strong>Mapped Reads</strong></td>
<td>53K</td>
<td></td>
</tr>
<tr>
<td><strong>Mapped Bases</strong></td>
<td>120 Mb</td>
<td></td>
</tr>
<tr>
<td><strong>Avg. Read Length</strong></td>
<td>2.2 Kb</td>
<td></td>
</tr>
<tr>
<td><strong>Avg. Subread Length</strong></td>
<td>200 bp</td>
<td></td>
</tr>
<tr>
<td><strong>95% ile Read Length</strong></td>
<td>4.3 Kb</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of Sanger and PacBio RS variant calls. All 4 homozygous SNP calls (1 in NA 17316 and 3 in NA17317) and 10 heterozygous SNP calls (2 in NA17316 and 8 in NA17317) were in perfect agreement.

www.pacb.com/target
Base modifications, such as DNA methylation, are key components of biological processes such as gene expression, host-pathogen interactions, DNA damage and DNA repair. The PacBio RS detects single nucleotide additions in real time, measuring the kinetic properties of base incorporation during the sequencing process. These kinetic measurements can be used for direct detection of a variety of base modifications. Unlike other techniques, no genetic alterations to the source material are required in order to view the modifications.

Base modifications affect the kinetics of polymerization during the normal course of sequencing. In this example, a methylated adenine in the template (top) slows the incorporation of a thymine in the replicating strand of DNA. The rate of incorporation can be compared to an unmodified version of the same template (bottom) which has a much faster thymine addition. Differences between the modified and unmodified incorporation rates indicate potential sites of modified bases. These differences often span multiple bases, creating a distinctive signature.

Products and Workflow

The PacBio RS includes a comprehensive suite of products that deliver a simple, fast workflow from template preparation to data analysis. Compatible products from our Partner Program address application-specific needs in an integrated workflow.

PacBio’s End-to-End Solution

Time to Result in <10 hours
Circular consensus provides multiple subreads on shorter insert sizes. Typically, each library prep can be distributed across $\geq 35$ SMRT Cells.

**Key Workflow Parameters:**

<table>
<thead>
<tr>
<th>Insert Size (bp)</th>
<th>Input DNA required per library prep (ng)</th>
<th>Sequencing protocols per insert size</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>250</td>
<td>Up to 1 x 90 mins</td>
</tr>
<tr>
<td>500</td>
<td>250</td>
<td>Up to 2 x 45 mins</td>
</tr>
<tr>
<td>1,000</td>
<td>500</td>
<td>Circular consensus provides multiple subreads on shorter insert sizes.</td>
</tr>
<tr>
<td>2,000</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>5,000</td>
<td>2,000</td>
<td>Standard sequencing provides a single pass read on longer insert sizes.</td>
</tr>
<tr>
<td>10,000</td>
<td>5,000</td>
<td></td>
</tr>
</tbody>
</table>

**Movie Times**
- Up to 1 x 90 mins
- Up to 2 x 45 mins

Compatible products complement and enhance key aspects of the PacBio RS sequencing workflow. For an updated list, please see [www.pacb.com/compatible](http://www.pacb.com/compatible).
PacBio RS System Specifications

PacBio® RS Specifications and Operating Environment

Instrument and environmental cabinet

- Power requirements: 208 – 240 VAC. UPS recommended
- Operating temperature: 15°C – 25°C (59°F – 77°F) ± 2°C per hour
- Humidity: 20% – 80%, noncondensing
- Ventilation: HVAC capacity of up to 22,720 BTU (6654 Watts)
- Nitrogen: 90 – 125 PSI (4,654 – 6,464 torr)
- WxDxH: 78.9in x 30.3in x 62.2in (200.4cm x 77.0cm x 158.0cm)
- Weight: 2,405lb (1,091kg)

Blade center

- Includes integrated computation and storage for performing single molecule, real-time sequencing, kinetic data generation, basecalling and quality assessment.
- WxDxH: 27.5in x 27in x 39.2in (69.9cm x 68.6cm x 99.6cm)
- Weight: 250lb (113kg)