PACIFIC BIOSCIENCES®

PacBio *RS*

REVEAL THE TRUE BIOLOGY

A revolutionary DNA sequencer advancing discovery in *de novo* assembly, targeted sequencing, and base modification applications

Single Molecule, Real-Time Sequencing

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:

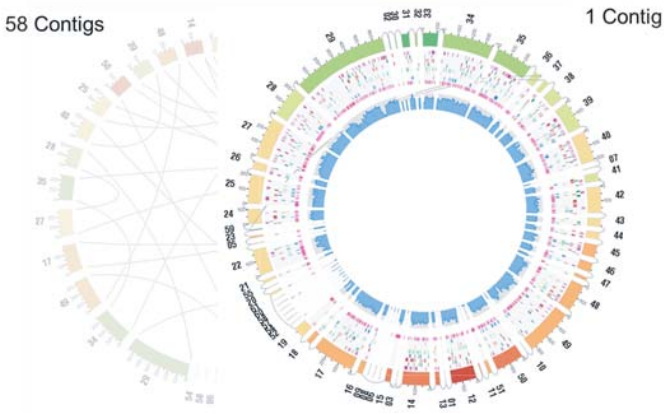
- Finish genomes and comprehensively characterize genetic variation with **very long read lengths.**
- Confirm discoveries with **high single molecule and consensus accuracy.**
- Obtain deep insights into base modifications with **unique kinetic information.**
- Complete projects quickly and efficiently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

De Novo Assembly

Generate finished genome assemblies

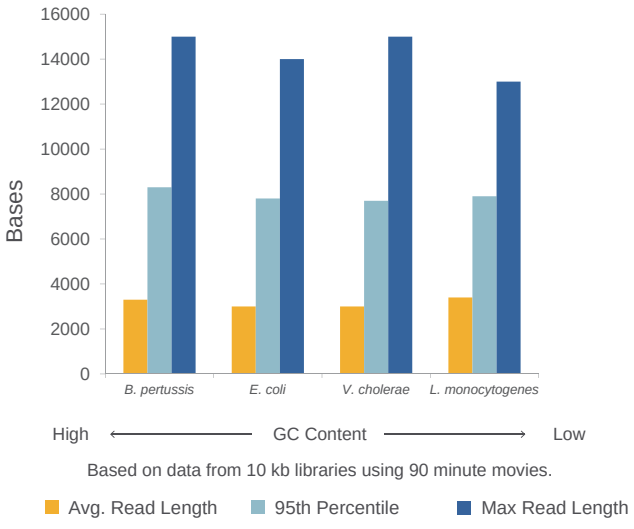
The PacBio *RS* is the only microbial sequencing platform that finishes genomes, determines structure and resolves strains. Combine long read PacBio data with short read second generation data or use PacBio data exclusively.

- **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

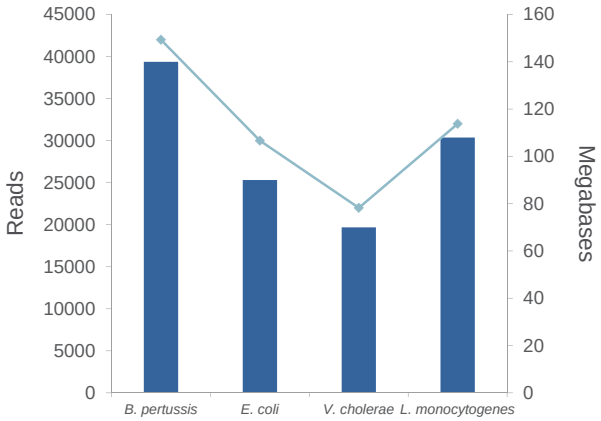
Read Length



Based on data from 10 kb libraries using 90 minute movies.

Avg. Read Length | 95th Percentile | Max Read Length

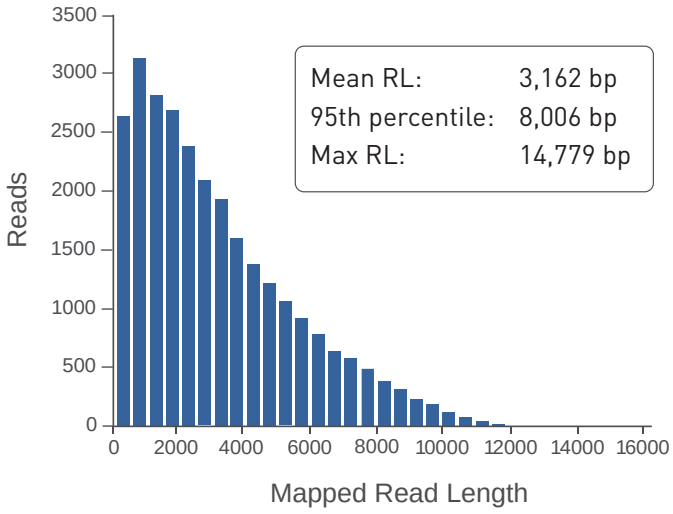
Output per SMRT® Cell



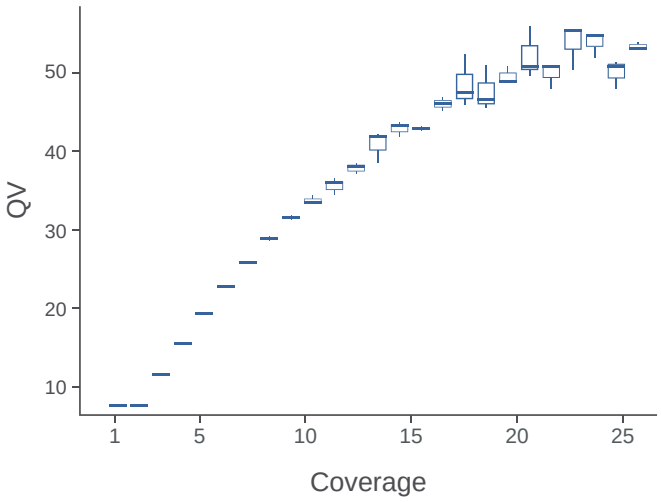
Based on data from 10 kb libraries using 90 minute movies.

Mapped Bases | Mapped Reads

Read Length Distribution



Accuracy



Based on data from *E. coli* with 10 kb libraries using a 90 minute movie.

www.pacb.com/denovo

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

Movie Time	2 x 30 mins
Insert Size	250 bp
Mapped Reads	53K
Mapped Bases	120 Mb
Avg. Read Length	2.2 Kb
Avg. Subread Length	200 bp
95% ile Read Length	4.3 Kb

		NA17316				NA17317			
Exon	Position	Sanger	RS 1	RS 2	Validated	Sanger	RS 1	RS 2	Validated
EGFR4	90	-	-	-	CONFIRM	T	T	T	CONFIRM
EGFR4	185	-	-	-	CONFIRM	A/G	A/G	A/G	CONFIRM
EGFR13	104	A/G	A/G	A/G	CONFIRM	A/G	A/G	A/G	CONFIRM
EGFR16	28	A/T	A/T	A/T	CONFIRM	A/T	A/T	A/T	CONFIRM
EGFR18	182	-	-	-	CONFIRM	G/A	G/A	G/A	CONFIRM
EGFR20	108	-	-	-	CONFIRM	A	A	A	CONFIRM
EGFR23	48	C	C	C	CONFIRM	C	C	C	CONFIRM
EGFR25	63	-	-	-	CONFIRM	C/T	C/T	C/T	CONFIRM
Met19	94	-	-	-	CONFIRM	C/T	C/T	C/T	CONFIRM
Met20	129	-	-	-	CONFIRM	G/A	G/A	G/A	CONFIRM
Met20	204	-	-	-	CONFIRM	G/A	G/A	G/A	CONFIRM

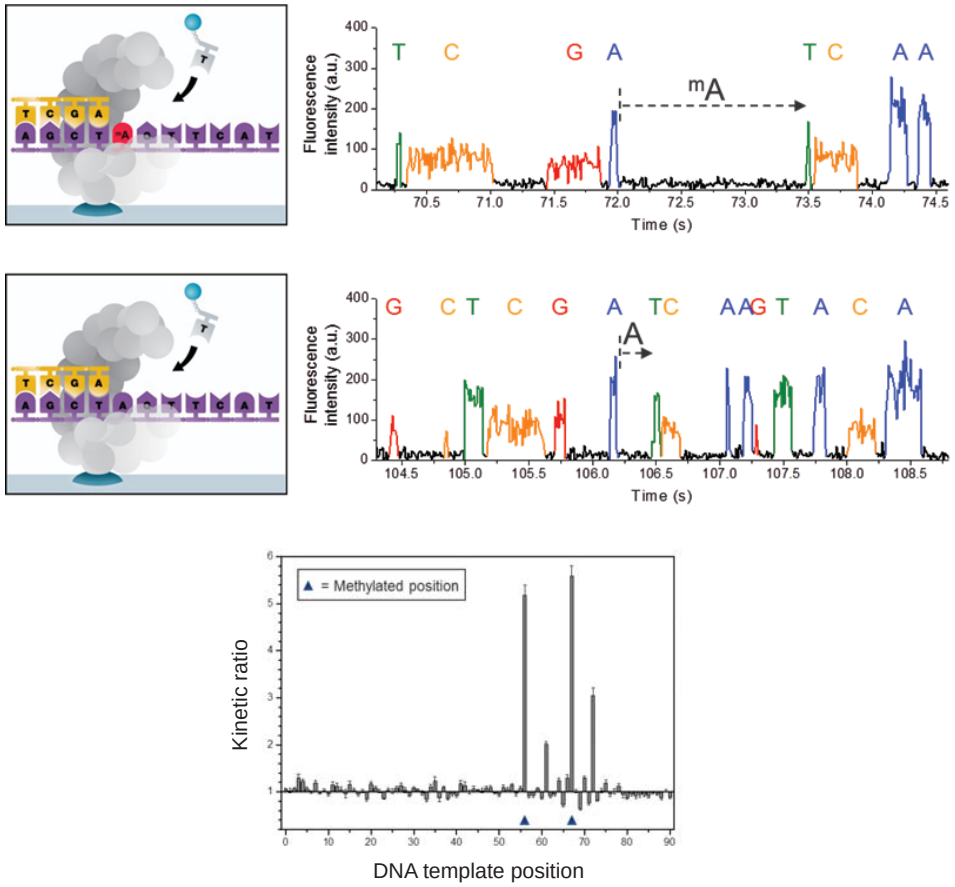
Comparison of Sanger and PacBio *RS* variant calls. All 4 homozygous SNP calls (1 in NA 17316 and 3 in NA17317) and 10 heterozygous SNP calls (2 in NA17316 and 8 in NA17317) were in perfect agreement.

www.pacb.com/target

Base Modification Detection

Application in development

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.



Flusberg et al. (2010) Nature Methods 7: 461-465

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.

www.pacb.com/basemod

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

PacBio *RS* Instrument with Touchscreen Controls

SMRT® Cell 8Pac with Tray

DNA Template Prep Kit
DNA/Polymerase Binding Kit
DNA Sequencing Kit

Primary Analysis, Secondary Analysis, and Visualization Tools

Time to Result in <10 hours

PacBio Products	Template Prep Kit	RS Remote	Binding Kit	Sequencing Kit SMRT Cells RS Touch RS Remote	Blade Center	SMRT Portal SMRT View	DevNet Website

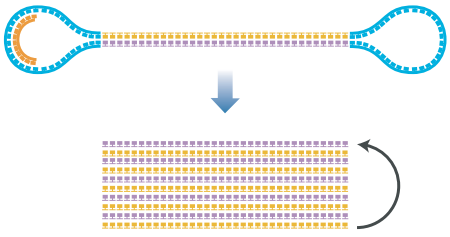
Partner Products

Compatible products complement and enhance key aspects of the PacBio *RS* sequencing workflow. For an updated list, please see **www.pacb.com/compatible**.

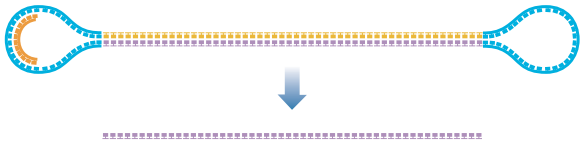
Key Workflow Parameters:

Insert Size (bp)	Input DNA required per library prep (ng)
250	250
500	250
1,000	500
2,000	500
5,000	2,000
10,000	5,000

Sequencing protocols per insert size



Circular consensus provides multiple subreads on shorter insert sizes.



Standard sequencing provides a single pass read on longer insert sizes.

Movie Times

Up to 1 x 90 mins
Up to 2 x 45 mins

Typically, each library prep can be distributed across ≥ 35 SMRT Cells .

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)

Pacific Biosciences
1380 Willow Road
Menlo Park, CA 94025
650.521.8000

sales@pacificbiosciences.com
www.pacificbiosciences.com

PACIFIC BIOSCIENCES®