

# PacBio® RS

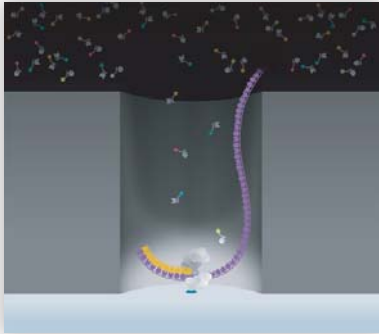
The world's first single molecule,  
real-time DNA sequencer



A revolutionary third generation DNA sequencing system incorporating novel single molecule sequencing with unprecedented readlengths to reveal new biological insights in real time.

# Key Advantages

## Single Molecule, Real-Time Analysis

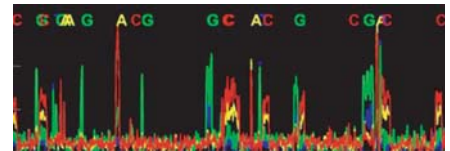


Single molecule, real-time (SMRT®) detection provides direct measurement of individual molecules, capturing multiple dimensions of data. Templates can be prepared for the PacBio *RS* without PCR amplification, resulting in more uniform sequence coverage across genomic regions regardless of GC content, facilitating the detection of minor variants in heterogeneous samples.

While observing single molecule sequencing by a highly processive strand-displacing DNA polymerase in real time, the system also records the kinetics of each nucleotide incorporation reaction, identifying base modifications of the native templates, such as DNA methylation.

Long readlengths simplify and improve genomic assembly by reducing the number of contigs, and producing superior consensus accuracy. With readlengths in the thousands of bases, the PacBio *RS* can resolve both SNPs and large-scale structural rearrangements. Long reads increase understanding of disease heritability through haplotype phasing.

## Longer Readlengths



Faster Time  
to Result  
**< 1 Day**

Read DNA at the speed of the polymerase in bases per second. With rapid sample preparation completed in 4-6 hours and short run times measured in minutes, you can obtain biologically meaningful answers within hours instead of a week or more. For data analysis, we offer a robust suite of SMRT Analysis tools to speed up your time from data to insights.

## Flexibility

Customize your experiments with granular, low cost consumables and adjustable run conditions. Multiple sequencing protocols provide maximum flexibility for a wide range of applications.



Prepare samples in a few hours without amplification. Monitor sequencing in real time, with feedback on the state of the PacBio *RS*, its contents, and your experiment through a touch-screen interface. Analyze data and view results in rich user interfaces built around industry standards. The entire workflow was designed with the user in mind – from sample preparation, to instrument use, to data analysis.

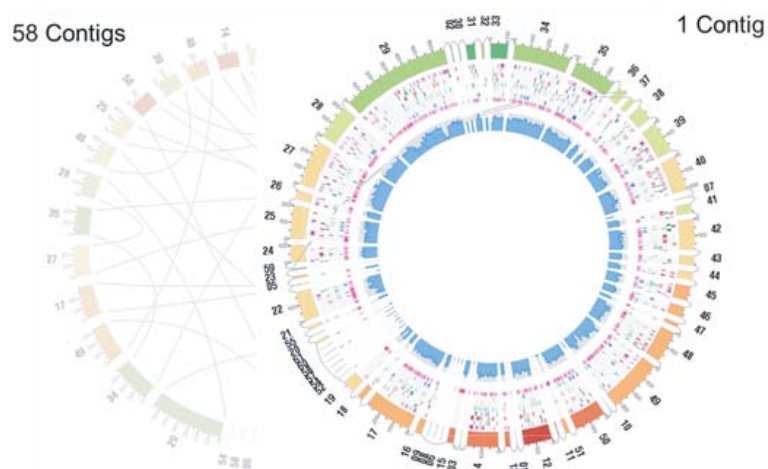
## Ease of Use

# Target Applications

## Targeted Sequencing

The PacBio *RS* is uniquely suited for variant detection due to high single molecule accuracy, extra long reads and granularity. It directly measures individual molecules, using long reads to fully characterize genetic complexity, including rare SNPs, indels, structural variants, haplotypes and phasing. Single molecule resolution allows comprehensive characterization of heterogeneous samples and identification of variation invisible to multi-molecule sequencing technologies.

## *De Novo* Assembly

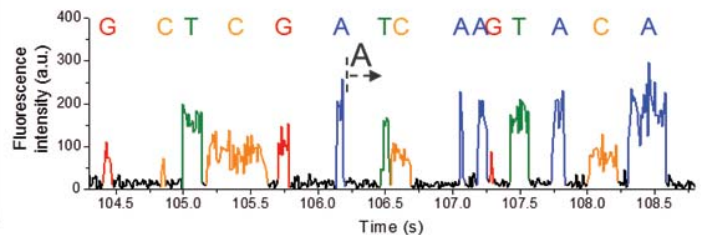
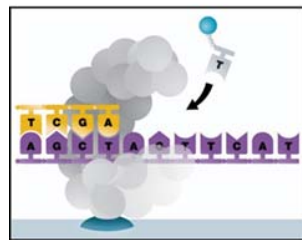
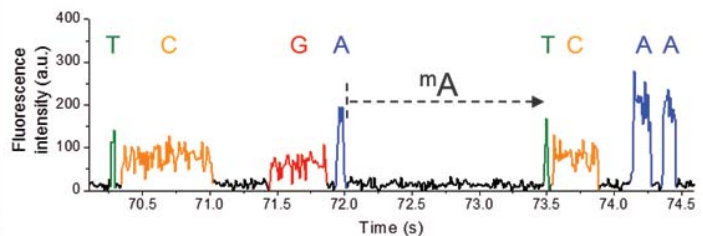
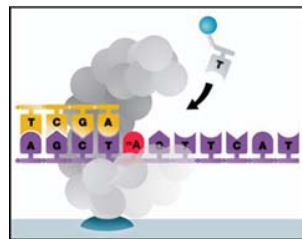


Hybrid assembly: Finish genomes and reduce contigs at a lower cost and with higher quality by combining long read PacBio® data with short read second generation sequencing data.

Solo assembly: Assemble microbial genomes with PacBio reads alone.

## Base Modification Detection

DNA base modifications are key components of many important biological processes. 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 will affect the kinetics of the polymerization reaction 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.

# SMRT<sup>®</sup> Sequencing

## Workflow

The PacBio *RS* offers a streamlined workflow from template preparation to data analysis. World-class partner products from our Partner Program help accelerate your time to results.



### Products

Template Prep Kit

RS Remote

Binding Kit

Sequencing Kit  
SMRT Cells  
RS Touch  
RS Remote

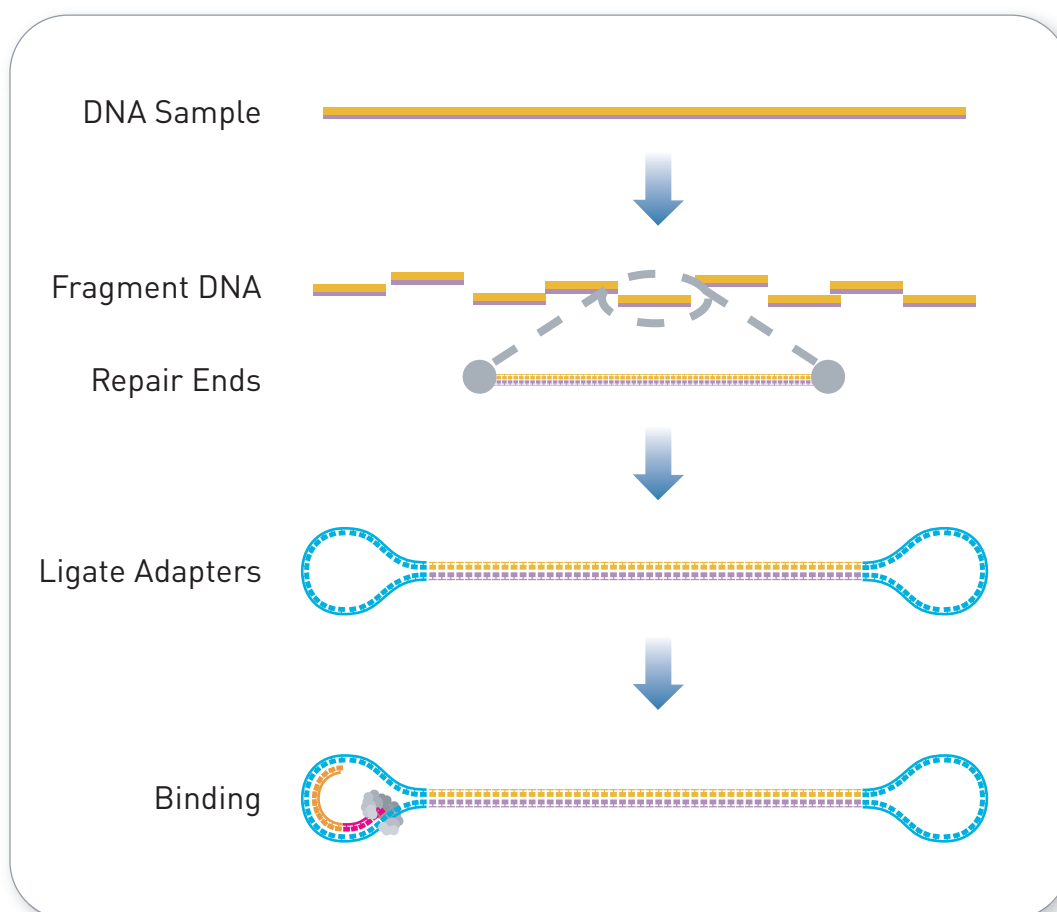
Blade Center

SMRT Portal  
SMRT View

DevNet

## Template Preparation

Single molecule, real-time sequencing begins with preparation of circular SMRTbell<sup>™</sup> templates that support multiple sequencing protocols.

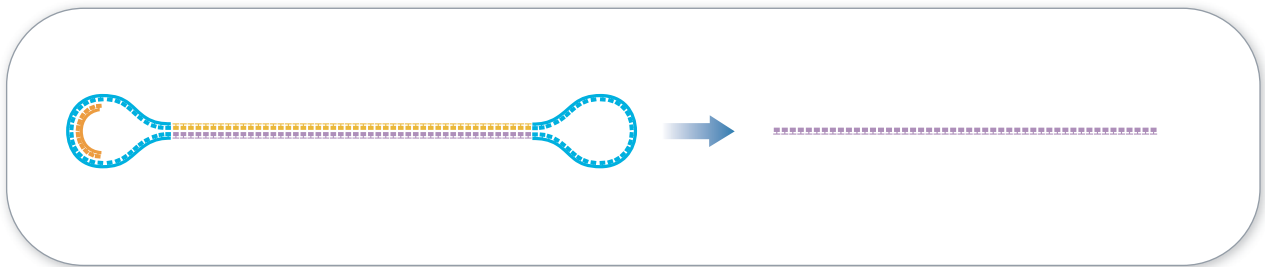


## Protocols

Two sequencing protocols are available, both utilizing the long readlength capabilities in different ways to provide maximum flexibility for a range of project types and applications.

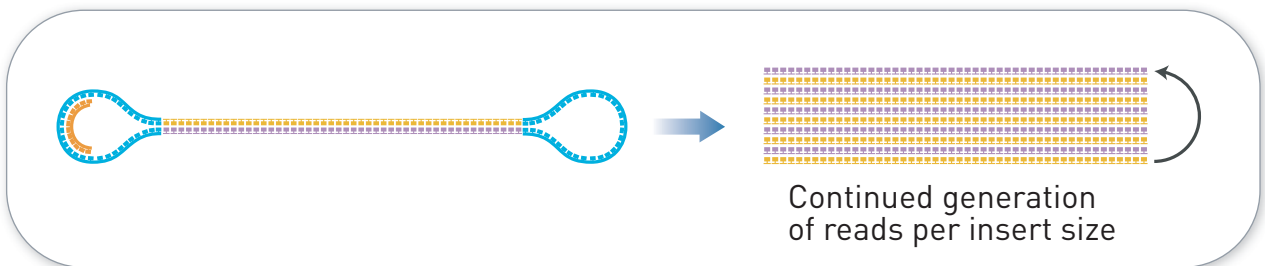
### Standard

- Generate single pass long readlengths in the thousands of bases
- Insert size: 1,000 – 10,000 bases
- Ideal for targeted sequencing and *de novo* applications
- Flexibility to collect for different time periods



### Circular Consensus

- Achieve unprecedented single molecule consensus accuracy greater than 99% by sequencing in multiple passes around a circular SMRTbell template
- Insert size: 250 – 1,000 bases
- Ideal for rare variant detection and validation



# Performance Specifications: Projected Q1 2012

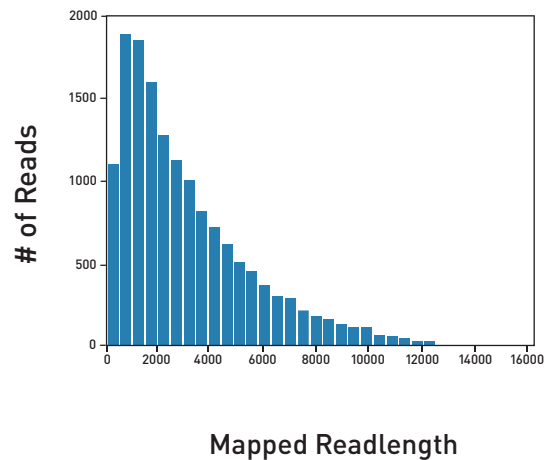
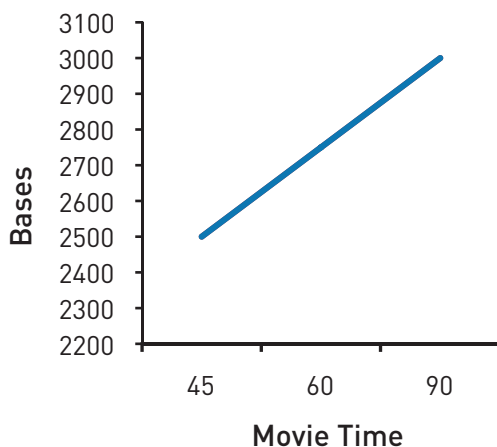
Description	X-long	Long
<b>Readlength distribution</b>		
Average	3,000bp	2,500bp
95%ile	8,500bp	6,000bp
<b>Time to result</b>		
Movie Time	90 min	45 min (x 2)
Time / SMRT Cell (after initial setup)		2 hrs
Template preparation to basecalls		<1 day
<b>Throughput</b>		
Reads / SMRT Cell	≥ 22,000	≥ 35,000
Mappable data / SMRT Cell	65Mb	90Mb
SMRT Cells / day		12
<b>Accuracy</b>		
Single Molecule		99%@5X CCS
Consensus*		99.999%@30X
<b>Sample preparation</b>		
Minimum input DNA		500ng**
Sample prep time		4–6 hours

Based on *Enterobacteria phage λ*. Performance may vary with sample types.

\*Based on data from *Escherichia coli* K12 strain. Performance may vary with sample types.

\*\*Depends on insert size.

## Readlength



24 SMRT Cells/day can be run at 20 minute movie times.  
For longer movies of 45 minutes, 12 SMRT Cells can be run per day.

Based on a 90 minute movie of an *Enterobacteria phage*.  
Performance may vary with sample types.

# PacBio<sup>®</sup> RS System Specifications

## PacBio RS Specifications and Operating Environment

000-693-241 PacBio RS with touch screen LCD

Instrument and environmental cabinet

Power requirements: 208 – 240 VAC. UPS recommended.

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)

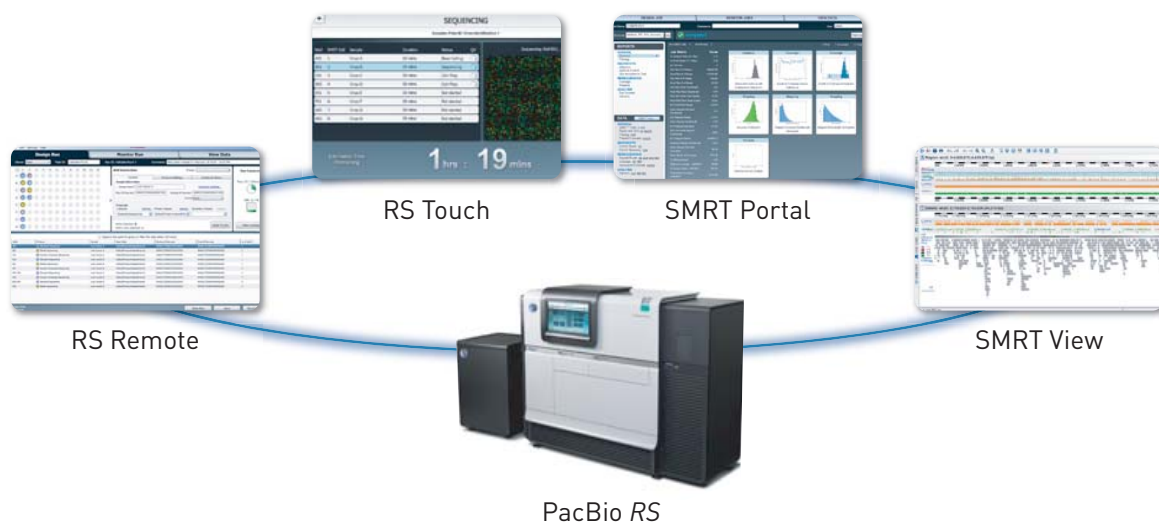
Weight: 250lb (113kg)

# PacBio *RS* Software and Data Analysis

## Software

The PacBio *RS* system includes an integrated software solution from beginning to end to support the key benefits of third generation sequencing data: fast time to result, long readlengths, flexible and granular runs, and kinetic information.

- Fully automated analysis from run setup, with the option to manually run later
- Efficient integration with LIMS and third party analysis tools
- User-friendly UI design for advanced informatics researchers as well as biologists and clinicians
- Industry-standard output formats: FASTA, FASTQ, SAM/BAM, VCF, etc.
- Open source through our developers' network



## PacBio *RS* Instrument Software

### RS Remote

Design and monitor instrument runs directly from your desktop, with constant feedback on time and consumables, using an intuitive and rich interface.

### RS Touch

The integrated touchscreen application on the PacBio *RS* instrument guides you through instrument loading and sequencing initiation, and provides real-time videos and feedback on sequencing and time-to-completion.

### Primary Analysis

The PacBio *RS* Blade Center processes images in real-time, calling bases and assessing quality. Results stream directly to secondary analysis and are available through RS Touch and RS Remote.

# SMRT Analysis Software

## SMRT Pipe

- BLASR** Map reads against a reference sequence with blazing speed with our BLASR algorithm. BLASR searches against pre-computed suffix arrays, then refines with banded Smith-Waterman and computes the statistical significance.
- ALLORA** Iteratively assemble long raw reads with ALLORA, “a long read assembler” based on the open source *de novo* assembler AMOS.
- AHA** Finish genomes and reduce the number of contigs by combining PacBio’s standard long reads with second generation short reads with AHA, “a hybrid assembler”.
- EviCons** Achieve superior consensus accuracy using evidence-based consensus (EviCons) with conditional probabilities, likelihood ratios and the Steiner method.

## SMRT Portal

SMRT Portal is your window to the power of single molecule sequencing. View and download variants, assemblies and other secondary analysis results. Monitor analysis jobs and manage protocols, all from a rich web-based interface.

## SMRT View

SMRT View is a high-performance interactive genome browser for exploration, interpretation and verification of analysis results, displaying circular consensus sequences and kinetics.

## Downstream Analysis of SMRT Sequencing Data

The PacBio *RS* embraces open standards and open source with a broad array of APIs and file formats for convenient integration with common applications and development of novel solutions.

## DevNet

Join the SMRT community at [pacbiodevnet.com](http://pacbiodevnet.com). DevNet gives you access to the latest software releases, source code, datasets and documentation. You can contribute to the SMRT community with your ideas and knowledge, and benefit from what others have shared. Partner products and solutions are listed as well.

## IT Requirements

Network: 1GbE connection required; 2GbE recommended. Four (4) static IP addresses. Network address, subnet mask, default gateway. DNS servers, DNS default search domain, NTP servers, outbound NAT address or range, outbound internet, ssh/scp access, port 3306 open  
Compute cluster: 5 nodes x 8 cores/node. 2GB RAM per core. 250GB/node  
Storage: Minimum 10TB recommended

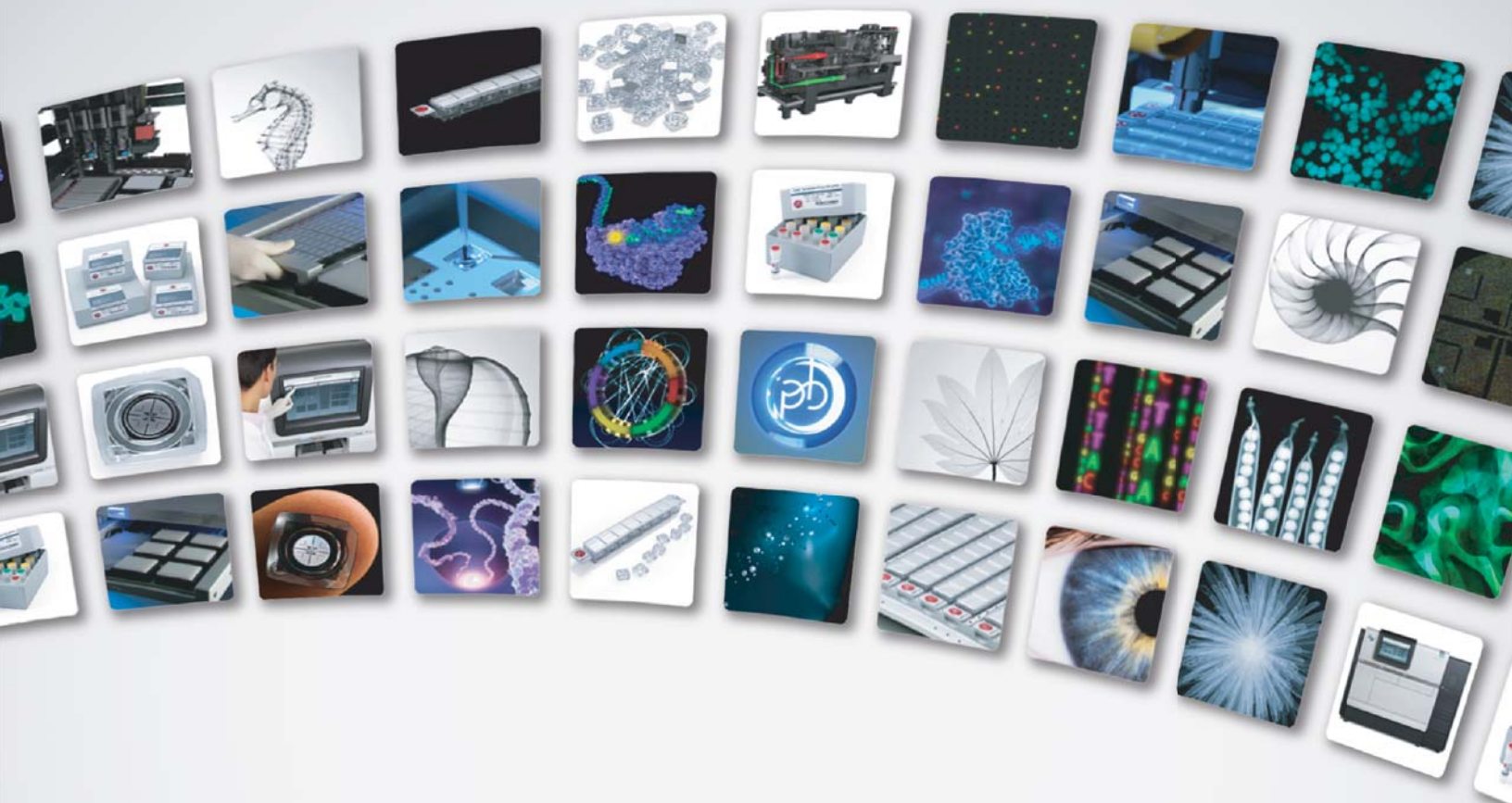
## File Formats and Sizes

Primary analysis	Base calls and quality values	HDF5*, FASTQ, FASTA
Primary analysis	Kinetics	HDF5*
Resequencing	Aligned reads and consensus calls	HDF5, SAM/BAM
Resequencing	Variants	GFF, VCF
<i>De novo</i> assembly	Assembled reads	HDF5, ACE, SAM/BAM

\*Base calls, quality values and kinetics are stored in the same file

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# REVEAL THE TRUE BIOLOGY



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