PACBIO PRODUCT RELEASES OVER THE LAST EIGHT YEARS

PacBio Long Read Technology

**Three sequencer generations

**Changing chemistry

May 2012
v1.3.1
- Epigenetic Modification

Aug 2012
v1.3.2
- MagBead Release

Feb 2012
v1.3.0
- C2 Launch

Nov 2012
v1.3.3
- XL Chemistry
- Stage Start

Jan 2013
v1.4
- HGAP/Quiver
- SMRT Cells v3

Apr 2013
RS II Product Release (v2.0)
- 75K to 150K ZMW
- 2x Throughput
- P4 release

Oct 2013
v2.1
- P5-C3 release
- HGAP 2.0

Mar 2014
v2.2
- Iso-Seq Method
- Long Amplicon Analysis

Apr 2015
Barcode Kits

Oct 2014
v2.3
- P6-C4 release

In Practice
**400-600 K reads

Oct 2015
Sequel System

Aug 2016
Sequel v3.1
- 1.2.1 Chemistry

Jan 2017
Sequel v4.0
- 2.0 Chemistry

Sept 2017
Sequel v5.0
- 2.1 Chemistry

Feb 2018
Sequel v5.1
- 2.1 Polymerase

Oct 2018
Sequel v6.0
- 3.0 Chemistry
- SMRT Cell 1M v3

Apr 2019
Sequel II v7.0
- 1.0 Chemistry
- SMRT Cell 8M

In Practice
**1.4-6.0 M reads

**1 Gb (99%)
**150k reads
**10 kb avg read

PacBio RS II System

**15 Gb (99%)
**500k reads
**30 kb avg read

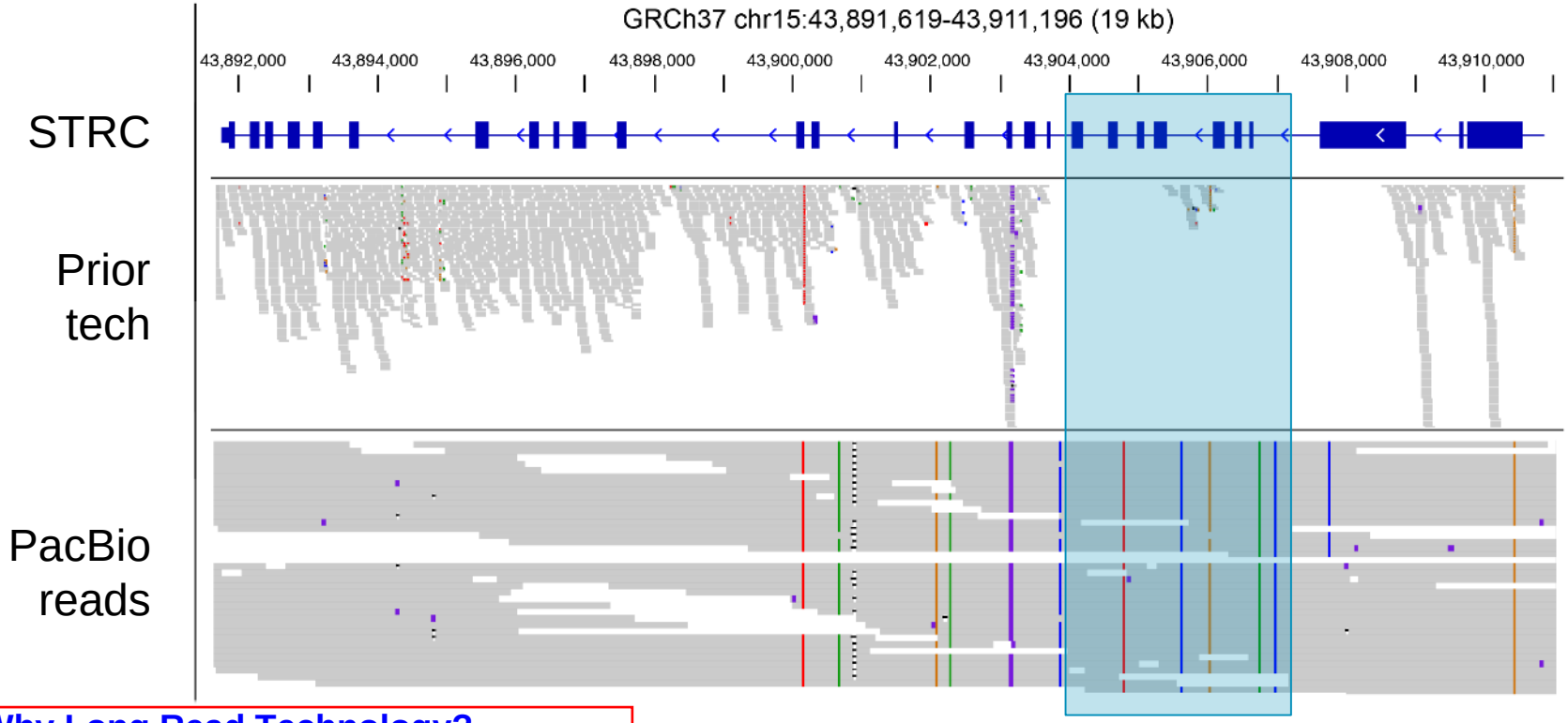
Sequel System

**100 Gb (99%)
**4M reads
**25 kb avg read

Increased throughput by over 10,000-fold

A COMPREHENSIVE VIEW OF THE GENOME

SMRT Sequencing provides even coverage across difficult to sequence regions of the genome.



Why Long Read Technology?

**Obtain sequence data not available from Illumina

**Repeat structure

**Unsequenced regions

Almost no coverage with prior tech

PacBio reads sequence straight through and detect variants, some falling in coding regions

CHOOSE THE SEQUENCING MODE THAT'S RIGHT FOR YOUR PROJECT

Sequel II System

**Newest release

**Flexible solutions

HiFi READS

- Highly accurate long reads with minimum accuracy of Q20 (99%)
- Small file sizes and fast analysis time
- Assemble up to a 2 Gb genome in a single SMRT Cell 8M*
- Run up to 200 samples (2 Gb) per year, per system*

Plant genome size

LONG READS

- Longest reads, with half of data >50 kb and maximum read lengths up to 175 kb
- Short sequencing run times
- Assemble up to a 3 Gb genome in a single SMRT Cell 8M*
- Run up to 400 samples (3 Gb) per year, per system*

Human genome size

*Read lengths, reads/data per SMRT Cell 8M, and other sequencing performance results vary based on sample quality/type and insert size. Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II System and does not include instrument amortization or other reagents.

WHOLE GENOME SEQUENCING (WGS) APPLICATIONS

Sequel II System
**Newest release
**Flexible solutions

HiFi READS

- Comprehensive detection of variants (SNVs, SVs, CNVs)
- High-quality, phased genome assembly

VARIANT DETECTION

ASSEMBLY

LONG READS

- Detection of structural variants (SVs)
- Assembly of very large genomes

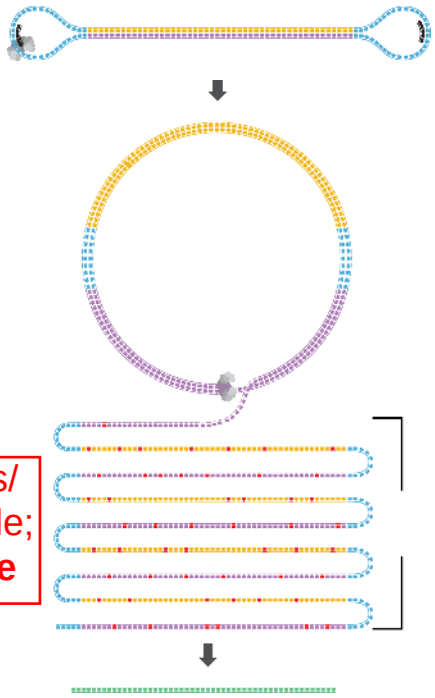
STRUCTURAL VARIANT DETECTION

ASSEMBLY

TWO MODES OF SMRT SEQUENCING

Circular Consensus Sequencing (CCS) Mode

Inserts 10-20 kb



Subread 1

Multiple reads/ Single molecule; **99% accurate**

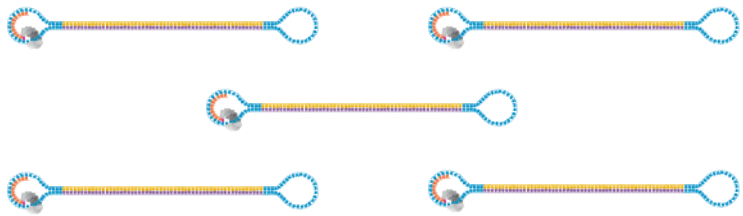
Subread n

HiFi READS

Single-molecule consensus sequence

Continuous Long Read (CLR) Sequencing Mode

Inserts >25 kb, up to 175 kb



CLR 1

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Single read/ Multiple molecules; **90% accurate**

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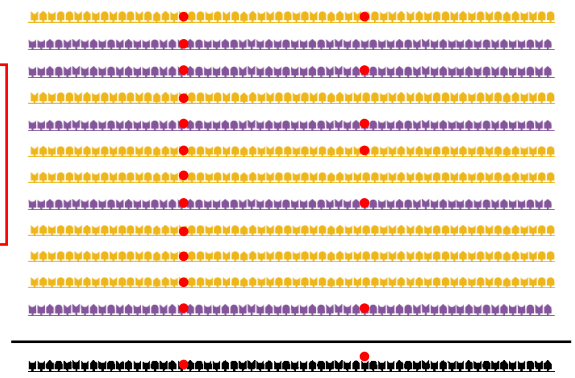
CLR n



LONG READS

Multi-molecule consensus sequence

Pacific Biosciencs (PacBio) Sequel II Sequencing

Steps

1. Fractionate the DNA template
2. Add adapters to create
    - **"SMRTbell**: A double-stranded DNA template capped by hairpin adapters (i.e., SMRTbell adapters) at both ends. A SMRTbell template is topologically circular and structurally linear" PacBio definition

CCS Mode (Circular Consensus Sequencing)

"Circular consensus sequencing (CCS) read: The consensus sequence resulting from alignment between subreads taken from a single ZMW. ***Requires at least two full-pass subreads from the insert***. CCS reads are advantageous for amplicon and RNA sequencing projects and are highly accurate (>99% accuracy, Q>20)." PacBio definition

Insert Size: **10-20 kb**

CLR Mode (**C**ontinuous **L**ong **R**ead)

"Continuous long reads (CLR) read: Reads with a subread length approximately equivalent to the polymerase read length indicating that the ***sequence is generated from a single continuous template from start to finish***. The CLR sequencing mode emphasizes the longest possible reads." PacBio definition

Insert Size: **25-175 kb**