

Pacific Biosciences (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 (Continuous Long Read)

“**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

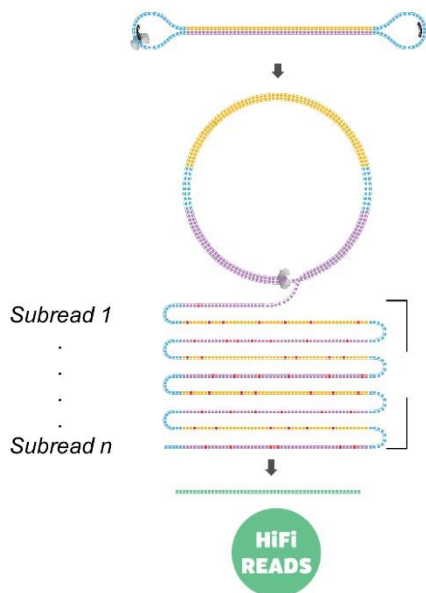
CCS vs. CLR Modes



TWO MODES OF SMRT SEQUENCING

Circular Consensus Sequencing (CCS) Mode

Inserts 10-20 kb



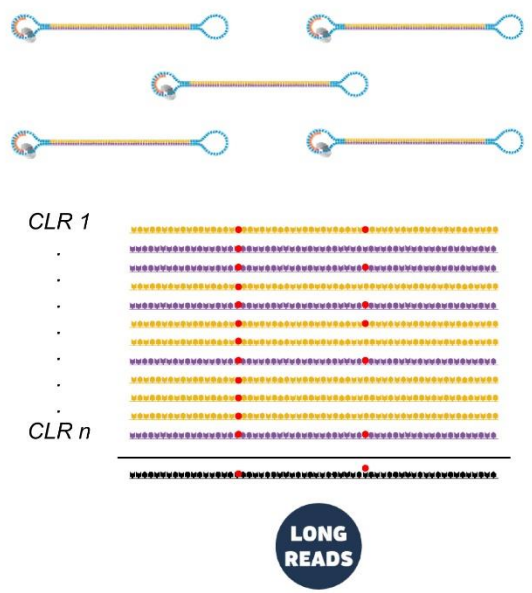
Single-molecule consensus sequence

99% Accurate

Single molecule;
multiple reads

Continuous Long Read (CLR) Sequencing Mode

Inserts >25 kb, up to 175 kb



Multi-molecule consensus sequence

90% Accurate

Multiple molecules;
single reads