PacBio RS third generation DNA sequencer

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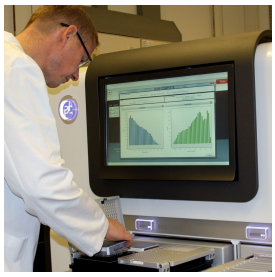

Brand

Pacific Biosciences

Type

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Organisation

Plant Sciences Group

Department

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Description

CAT-AgroFood sequencing facilities offer the PacBio RS II system, a third generation DNA sequencer that provides analysis of Single Molecule in Real Time (SMRT).
PacBio RS II with SMRT technology is able to generate very long reads from single DNA molecules without prior DNA amplification. These multiple kilobases long sequence reads are essential for assembly of large and complex genomes or discovery of large structural variations.
Key advantages of the PacBio RS II over other NGS platforms include:

• Single molecule real time detection
• Long reads (average > 10 Kb)
• Amplification free sample preparation

Mechanism
The SMRT technology harnesses the natural process of DNA replication, which is a highly efficient and accurate process. The enzyme responsible for replicating DNA in nature is called the DNA polymerase. The DNA polymerase attaches itself to a strand of DNA to be replicated, examines the individual base at the point it is attached, and then determines which of four building blocks, or nucleotides, is required to replicate that individual base. After determining which nucleotide is required, the polymerase incorporates that nucleotide into the growing strand that is being produced. After incorporation, the enzyme advances to the next base to be replicated and the process is repeated. This SMRT technology enables the observation of DNA synthesis as it occurs in real time. To overcome the challenges inherent in observing an enzyme that is 15 nanometers in diameter running in real time, three key innovations were developed by Pacific BioSciences:

• The SMRT Cell
• Phospholinked nucleotides
• The PacBio RS II

Single molecule real time sequencing utilizes so called zero-mode waveguides (ZMWs) located on a chip in the SMRT cells that can be placed into the sequencer in packs of eight. A single DNA polymerase enzyme is fixed at the bottom of a ZMW with a single molecule of DNA as a template. The ZMW is a structure that creates an illuminated observation volume that is small enough to observe only a single nucleotide of DNA being incorporated by DNA polymerase. Each of the four DNA bases is attached to one of four different fluorescent dyes. When a nucleotide is incorporated by the DNA polymerase, the fluorescent tag is cleaved off and diffuses out of the observation area of the ZMW where its fluorescence is no longer observable. A very sensitive detector detects the fluorescent signal of the nucleotide incorporation, and the base call is made according to the corresponding fluorescence of the dye.
Library preparation
A Blue Pippin is available for preparing high quality libraries. This instrument selects genome fragments according to size, to make sure that only the long fragments will be subsequently sequenced by the PacBio RS II.

Technical Details

Per run (based on the C4/P6 chemistry release)

- 8 SMRT cells per run
- Total number of raw reads ~30.000-50.000/ SMRT cell
- Average raw read length > 10 Kb
- Total output of 0.3 to 1 Gb per SMRT cell
- Raw read single-pass accuracy of ~ 85%; depending on the insert size, circular consensus sequencing can increase this to 99.99%