

## **W1-1**

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### **High-throughput Pyrosequencing (to use GS 20; whole genome sequencer)**

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The Genome sequencer FLX system, developed by 454 Life Science Corporation, is an ultra-high-throughput automatic DNA sequencing system capable carrying out and monitoring sequencing reactions in a massively parallel fashion. Hundreds of thousands of simultaneous reactions, in the “well” of a PicoTiterPlate device. The basic chemistry utilize the release of pyrophosphate (PPi) that occurs with each nucleotide addition during DNA-directed DNA synthesis to generate an amount of light commensurate with the amount of PPi released; this light is captured by a charge-coupled device camera and converted into a digital signal.

The raw data resulting from a sequencing run consists of a series of digital images captured by the camera, where the images are a representation of the surface of the PicoTiterPlate device over which the sequencing reaction are taking place; and each image corresponding to one reagent flow over that surface, as defined by the Run script. If the sample DNA fragment present in a given PicoTiterPlate well is extended during a nucleotide flow, light is emitted from the well and captured on the image corresponding to that flow. Furthermore, the amount of light emitted is proportional to the number of nucleotide extended. Knowledge of the nucleotide flowed while each image is being captured, of location on the PicoTiterPlate device where light is being emitted during each flow allows the software to identify PicoTiterPlate wells that contain a DNA library fragment and determine the sequence of the DNA fragments present in each well.