Pyrosequencing

Why/When: *To identify single base-pair changes in short sequences of targeted DNA. This technique is used to identify the presence of mutations that may confer antiviral resistance or possibly indicate potential new viral recombinants. These results are reported directly to the CDC.*

The technique is based on the "sequencing by synthesis" principle. It differs from Sanger sequencing, in that it relies on the detection of light as pyrophosphates are released during nucleotide incorporation. As a nucleotide is added to the synthesizing sequence, light is emitted by a chemiluminescent reaction. The dispensation order of the bases is known (programmed into the instrument), and based on the intensity of light emitted, the instrument can determine how many of each base is added at each dispensation. Pyro sequencing is effective for very short DNA strands (300-500 bp) whereas Sanger sequencing can sequence much larger segments.