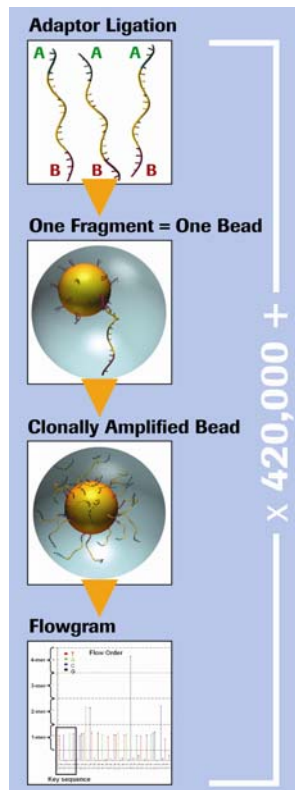


## How is genome sequencing done?

Using 454 Sequencing™ on the Genome Sequencer FLX™ System, DNA from a genome is converted into sequence data through four primary steps:



**Step One** – DNA sample preparation;

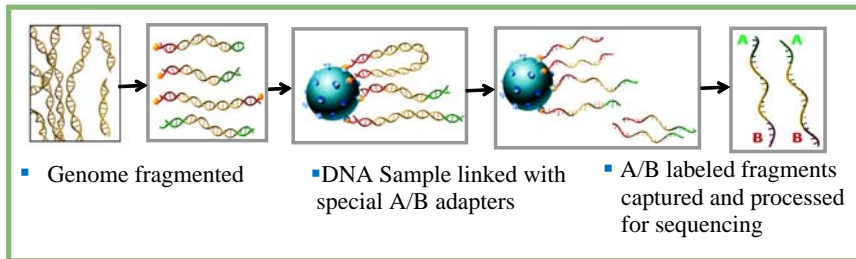
**Step Two** – Proprietary process to load DNA sample onto beads;

**Step Three** – Sequencing DNA on Genome Sequencer FLX instrument; and

**Step Four** – Analysis of the genome

## Step 1: Sample Preparation

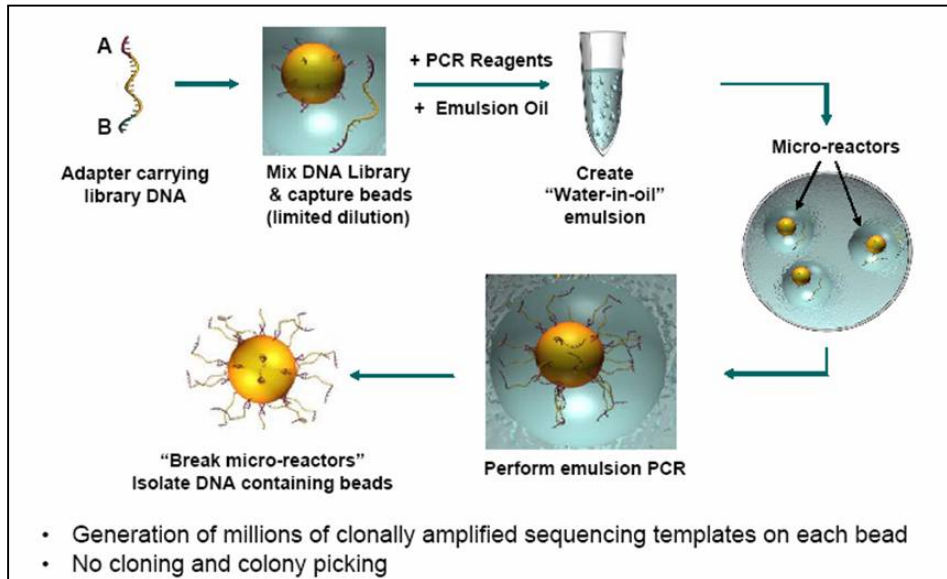
Starting with whole genome DNA or targeted gene fragments, the initial step in the process employed by our 454 Sequencing System is a universal library preparation for any sample. One library preparation is sufficient for sequencing any DNA sample from a virus to a bacteria to a human. The first step is to break the “double-helix” DNA ladder into shorter double-stranded fragments of approximately 400 to 600 base pairs. The next step is to attach adapters to the DNA fragments. Finally, the double-stranded DNA fragments are separated into single strands. Library preparation can be performed by one lab technician in an afternoon without special equipment. A single library preparation can currently supply enough material for numerous sequencing runs of the Genome Sequencer FLX™ System.



## Step 2: Loading DNA Sample onto Beads

Through our proprietary process of emulsion-based clonal amplification, or emPCR, the DNA library fragments are put onto micron-sized beads. As a result of the amplification of the DNA fragments, the signals produced during the sequencing step are easily detectable.

**This process takes approximately eight hours.** Using the current Sanger method of cloning DNA in bacteria, the amplification process currently takes approximately three weeks and also introduces bias in the DNA samples.



In the initial phase of the amplification process, the DNA library fragments along with capture beads and enzyme reagents in a water mixture, are injected into small, cylindrical plastic containers containing a synthetic oil. The combination of these materials and vigorous shaking causes the water mixture to form droplets around the beads, called an emulsion. Typically, most droplets that

contain DNA will contain only one DNA fragment. The water mixture includes an enzyme that causes the single and isolated DNA fragment in each droplet to be amplified into millions of copies of DNA. This reaction is also known as a polymerase chain reaction, or PCR. Through this reaction, a single DNA fragment is amplified into approximately ten million identical copies that are immobilized on the capture beads. When the PCR reaction is complete, the beads are screened from the oil and cleaned. Those beads that do not hold DNA are eliminated. Those beads that hold more than one type of DNA fragment are readily filtered out during sequencing signal processing.

### Step 3: Sequencing

The 454 Sequencing™ process uses a sequencing by synthesis approach to generate sequence data. In sequencing by synthesis, a single-stranded DNA fragment is copied with the use of an enzyme making the fragment double stranded. Starting at one end of the DNA fragment, the enzyme sequentially adds a single nucleotide that is the match of the nucleotide on the single strand. Nucleotides are paired one by one as the enzyme moves down the single stranded fragment to extend the double-helix ladder structure.

Following the separation and amplification of DNA strands with our library preparation and emPCR kits, the DNA-capture beads are placed on our PicoTiterPlate™ for sequencing. We believe that the PicoTiterPlate is a major

technological advancement because it enables the miniaturization of sequencing with our technology. One side of the PicoTiterPlate is polished and the other side contains wells that are 75 picoliters in volume. Each PicoTiterPlate comprises 1.6 million wells. The diameter of the wells is designed so that only a single capture bead will fit into each well.

**Depositing DNA Beads into the PicoTiter™ Plate**

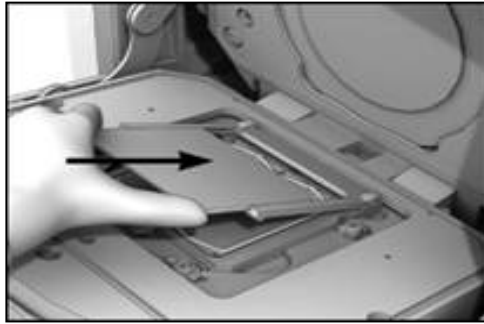
Load beads into PicoTiter™ Plate → 
 Load Enzyme Beads → 
 Centrifuge Step

150 micron ~ Tip of a human hair

Each plate has 1.6 Million wells

After the wells on the PicoTiterPlate are filled with capture beads containing the fractured and amplified DNA strands along with many small enzyme beads, the plate is placed into the 454 Sequencing System instrument. Our instrument includes a fluidics system capable of washing the PicoTiterPlate with various reagents including the A, C, G and T nucleotides. The four nucleotides are flowed sequentially in four washes over the PicoTiterPlate. When these nucleotides are incorporated onto the DNA strands, the bead-bound enzymes contained in each PicoTiterPlate well convert the chemicals generated during nucleotide incorporation into light in a chemi-luminescent reaction similar to that used by a firefly.

## Using the Genome Sequencer FLX™ System

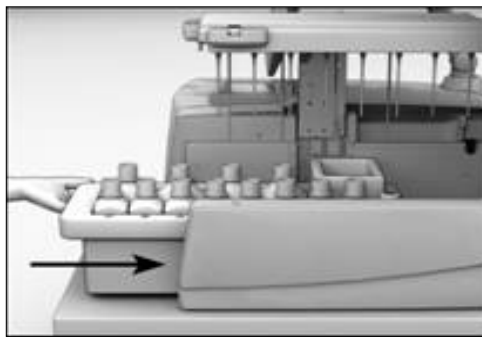


Load PicoTiterPlate  
device on instrument

Open instrument



Load reagents

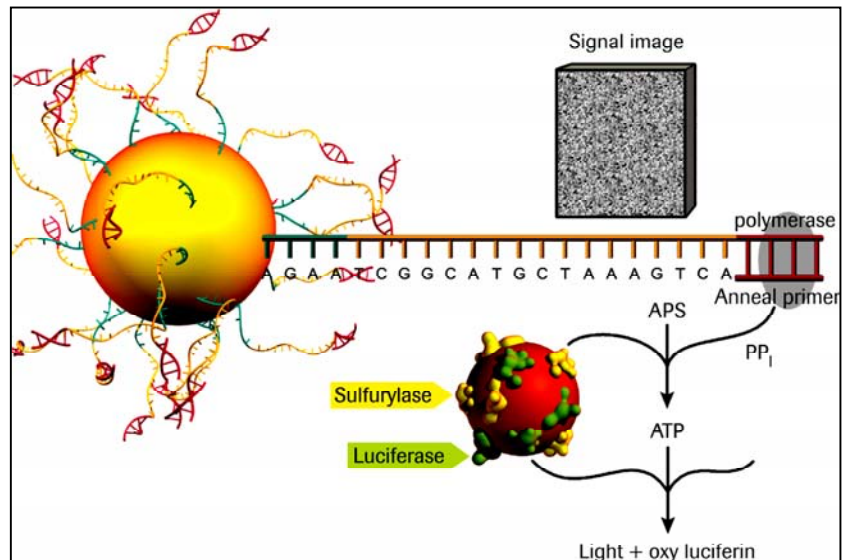


**Press START for Sequencing!**

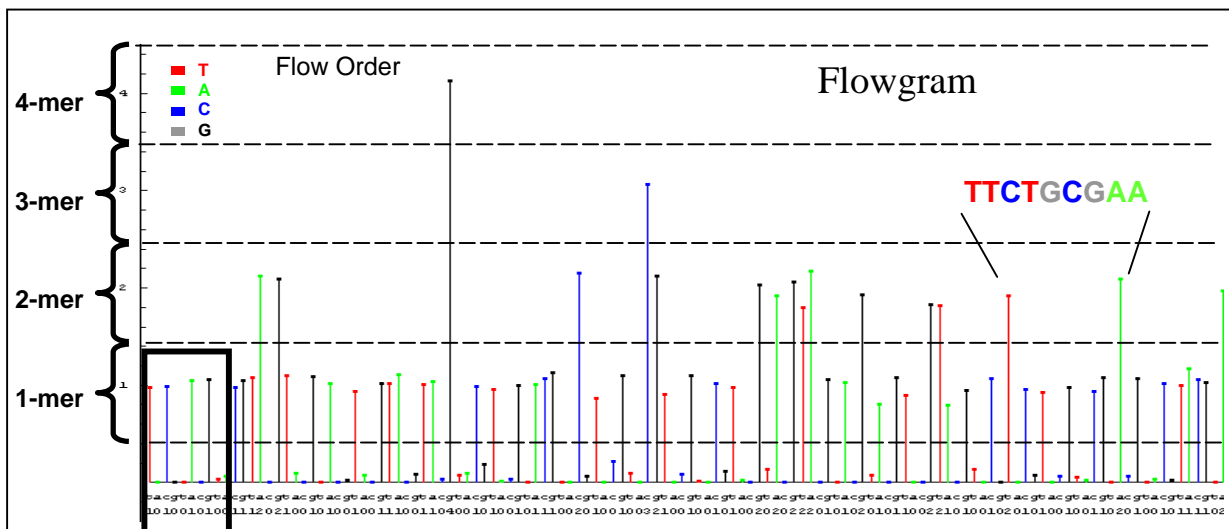


## How the 454 Sequencing™ process works

- Bases (TACG) are flow sequentially and always in the same order (100 times for a large FLX run) across the PicoTiterPlate during a sequencing run
- A nucleotide complementary to the template strand generates a light signal
- The light signal is recorded by the CCD camera
- The signal strength is proportional to the number of nucleotide incorporated



The chemi-luminescent signal produced in this reaction is detected by the CCD camera assembly included in the instrument. A CCD camera uses a small, rectangular piece of silicon rather than a piece of film to receive incoming light. This is a special piece of silicon called a charge-coupled device, or CCD. The intensity of light generated during the flow of a single nucleotide varies proportionately with the consecutive number of complementary nucleotides on the single-stranded DNA fragment being analyzed. For example, if there are three consecutive A's in the single-stranded fragment, the amount of light generated would be three times that of a single A in the fragment. The signals created in the sequencing process are then analyzed by the 454 Sequencing System's software to generate millions of sequenced bases per hour from a single run.

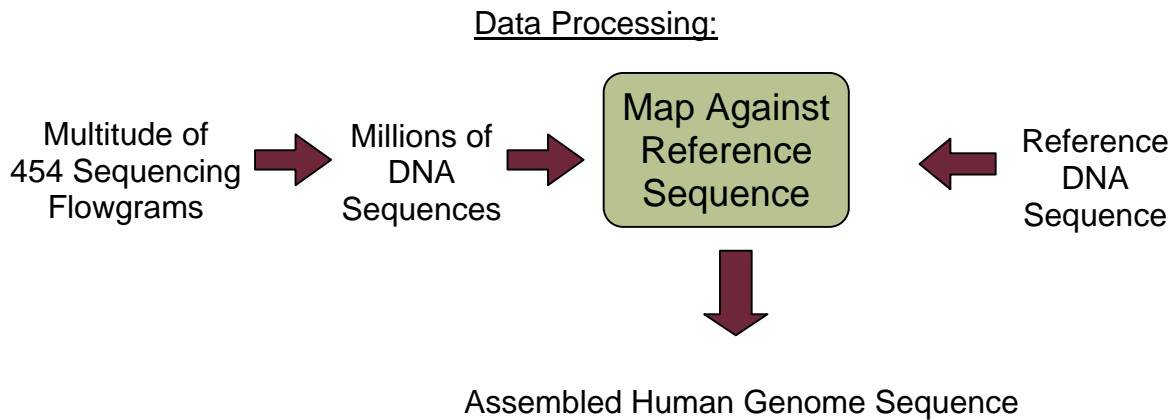


Key sequence = TCAG for signal calibration

Based on the chemi-luminescent signal, our software generates a bar graph of light intensities called a "flowgram" for each well contained on the PicoTiterPlate™. The signal strength is proportional to the number of nucleotide incorporated.

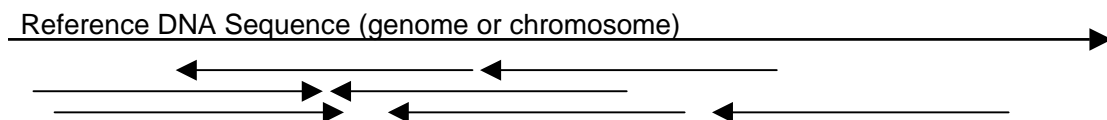
## Step 4: Analysis of the Genome

Data generated by 454 Sequencing™ on the Genome Sequencer FLX has the unique advantage of high throughput combined with longer read length to create a more complete picture of the human genome. By eliminating bias from sample preparation known to exist from traditional sequencing technologies and speeding up the time, quality and depth of sequencing results per run, one is able to now tackle the analysis of an entire individual's genome. Results of each GS FLX run (a multitude of flowgrams) are collected and compared to the reference genome, such as that generated from the Human Genome Project, to detect regions of exact match and differences.



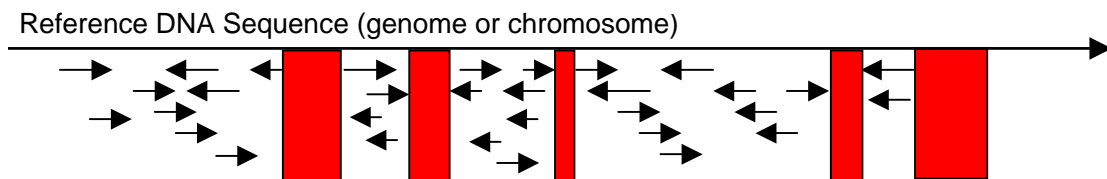
### 454 Sequencing™ Delivers a Complete Picture of the Genome

- Millions of DNA Sequences – Long Reads with 454 Sequencing™



- Millions of DNA Sequences – Short Reads via Alternative Technologies

█ = gaps in the Genome sequence



Generating sequences of high quantity is not enough. Longer read lengths from 454 Sequencing™ allows more overlap among the millions pieces of sequence information and create a more complete picture of an individual's genome. These differences could include insertions or deletions of one or several bases or more extensive DNA rearrangements. This information is critical for understanding what makes each individual unique and offers new understanding for improving human health.

**Source: 454 Life Sciences**

454 Life Sciences Corporation  
20 Commercial St.  
Branford, CT 06510  
203-871-2300  
[www.454.com](http://www.454.com)