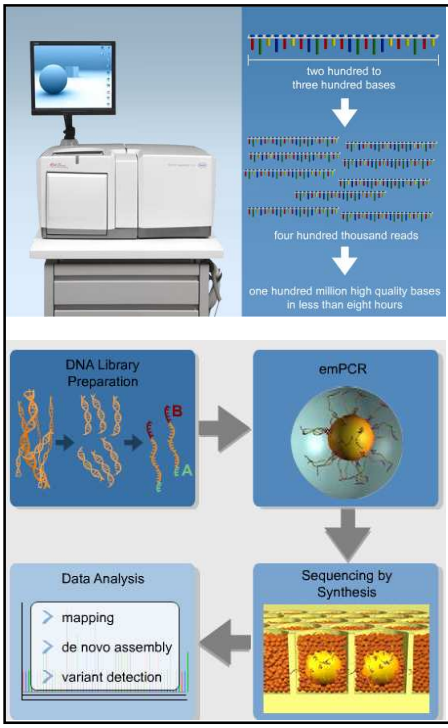
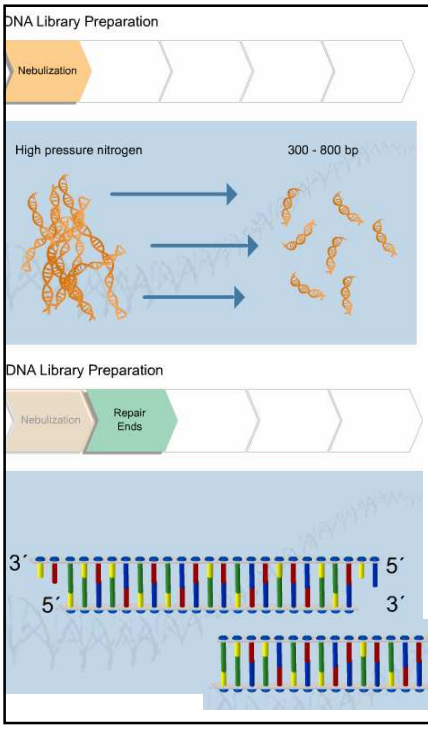


INTRO- Genome Sequencer FLX System from Roche Applied Science and 454 Life Sciences. With a read length of **200 to 300 bases** depending on organism and application - parallel sequencing of over **400 000 reads**, a single run typically yields over **100 M high quality bases in less than eight hours**.

DNA LIBRARY PREP- Four main steps comprise the complete sequencing workflow for the Genome Sequencer FLX system, from purified DNA through analyzed results. First, a **template library** consisting of single-stranded DNA is produced from a small sample of DNA, such as genomic DNA or PCR products. Second, the library is **amplified using emulsion-based, clonal amplification**, called emPCR. Third, **sequencing by synthesis on a PicoTiterPlate device** generates chemiluminescent signals, resulting in quality- filtered base sequence data.

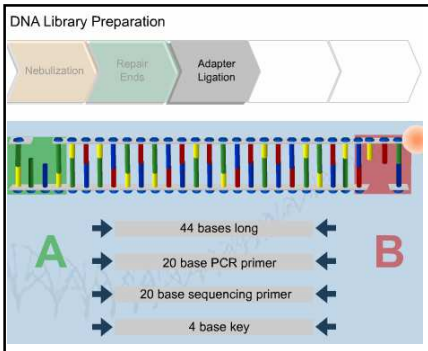


Preparation of the **DNA library** consists of a few simple steps.

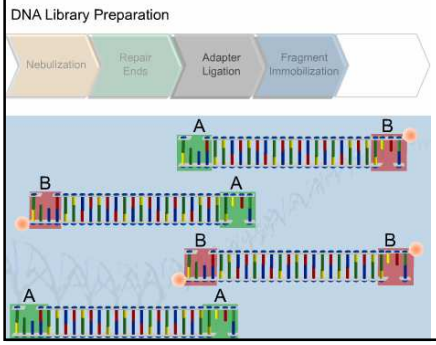
One preparation is sufficient for genomes of any size.

The library preparation protocol eliminates the need for expensive and time-consuming cloning and colony picking. To start, the DNA sample is **sheared into fragments by nebulization**.

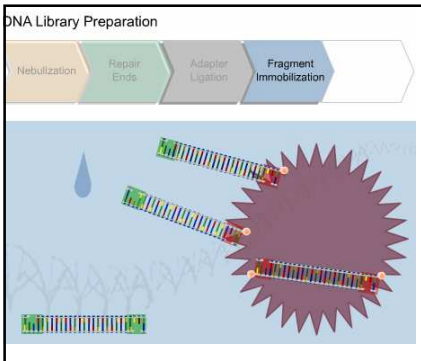
These double-stranded DNA fragments are **purified, blunt- ended and phosphorylated**.



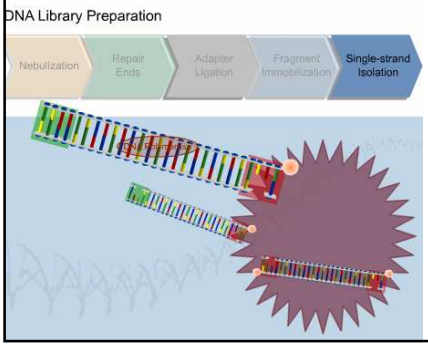
Two short adaptors, an A- adaptor and a B-adaptor are then ligated to the fragments, one to each end. The adaptors provide **priming sites for both amplification and sequencing** of the sample library fragments, as well as **a special key sequence**. The B- adaptor also contains a **five- prime biotin tag that enables selective immobilization** of the library in a later step. The **key sequence** is a short section of **four nucleotides** in a specific order,and is used by the system to **calibrate base- calling** and recognize legitimate library fragments.



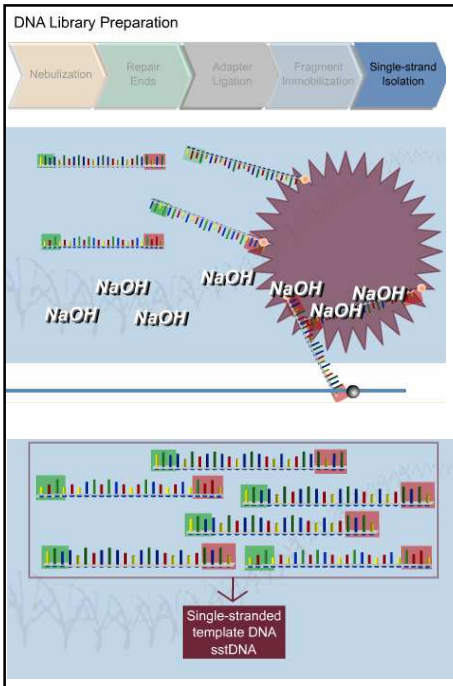
Four types of products are generated from the adapter ligation step.



Products with one or two biotinylated B-adaptors are captured on **streptavidin-coated magnetic beads**. Products with two A-adaptors do not bind to the beads and are washed away.

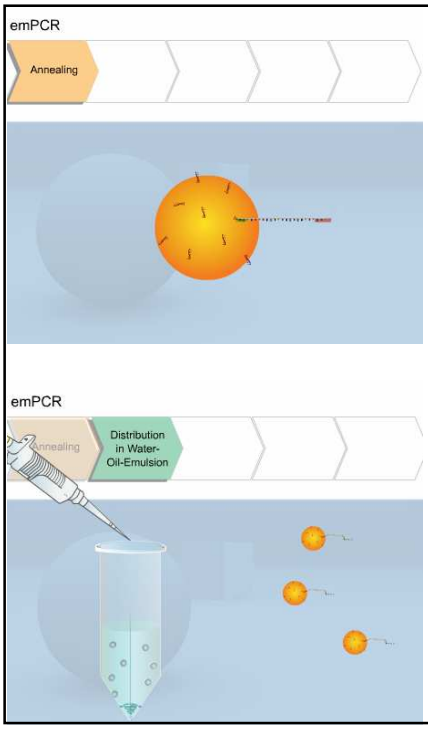


Since the DNA oligonucleotides used for the Adaptors are not phosphorylated, gaps will be present at the 3'-junctions of the DNA fragments. These gaps are **repaired** using a strand-displacing DNA polymerase.



The double- stranded products bound to the bead are then **denatured** to release the complementary non- biotinylated strands containing both an A- and a B- adaptor sequences.

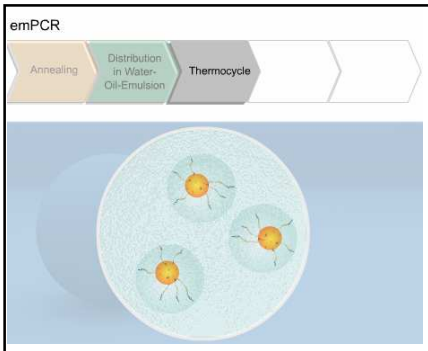
These denatured strands form the **single-stranded template DNA library.**



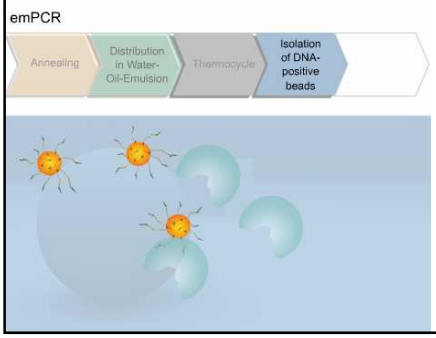
emPCR - For DNA amplification, the Genome Sequencer FLX system employs **emulsion-based clonal amplification**, called emPCR. The emPCR step eliminates the need for expensive and time- consuming cloning and colony picking.

After **purification** and **quantification** of the fragments, the single-stranded DNA library is **immobilized** by hybridization onto **primer-coated capture beads**. The process is optimized to produce beads, where **a single library fragment is bound to each bead**. This forms the basis for clonal sequencing, where each bead will correspond to a single read.

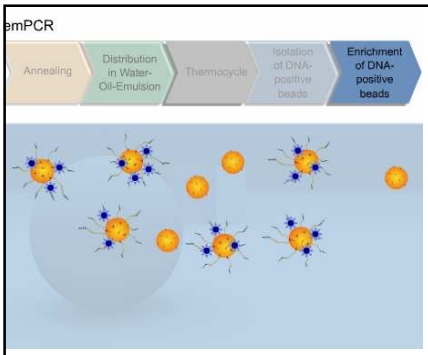
The bead- bound library is emulsified along with the amplification reagents in a water-in-oil mixture. Each bead, with a single library fragment is **captured within its own emulsion microreactor**, where independent clonal amplification takes place.



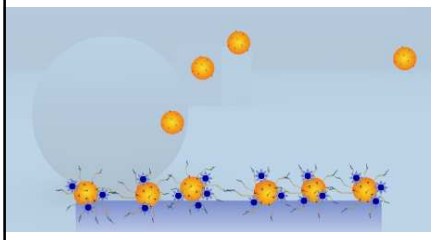
The **forward PCR primer is biotinylated** for use later during the enrichment step. All microreactors within the emulsion are processed in parallel, resulting in millions of beads, **each with its own unique clonally amplified DNA fragment**.



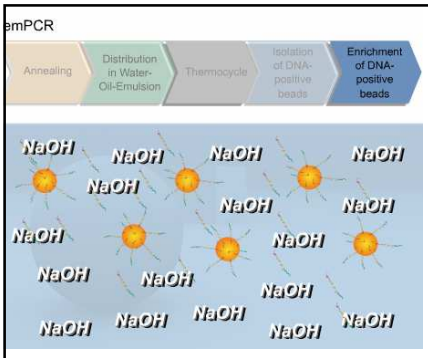
After amplification, the microreactors are broken, releasing the DNA-positive beads for the next step, which is enrichment.



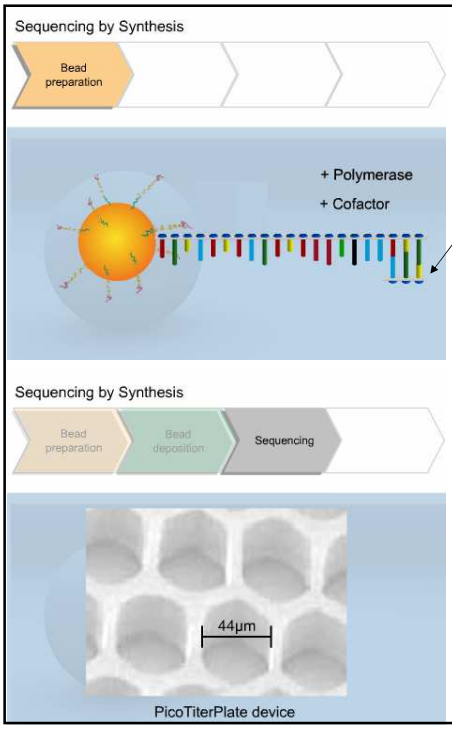
Streptavidin-coated magnetic enrichment beads are added to **capture the successfully amplified fragments**, binding only to those which have incorporated the biotinylated primer.



The enrichment beads are then immobilized on a **magnetic solid** support for separation and extraction.



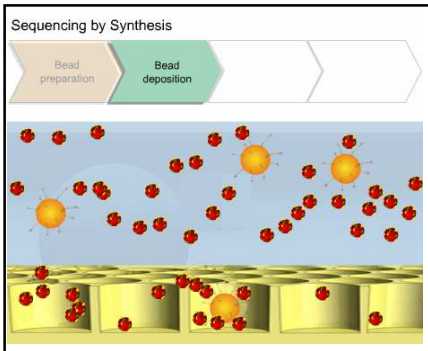
The double- stranded DNA fragments, bound to the enrichment beads **are made single-stranded** by the addition of sodium hydroxide. Now enriched in single- stranded DNA-carrying beads, the amplified library is ready for sequencing



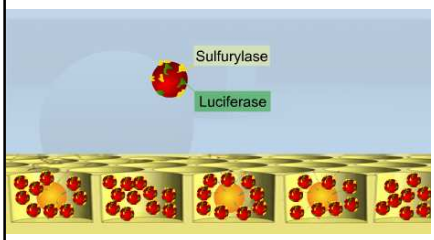
SEQUENCING- To prepare the DNA beads to be sequenced, a **sequencing primer** is annealed to the single-stranded DNA fragments and the beads are mixed with polymerase and cofactor.

Sequencing is performed simultaneously in all wells of the fiber-optic **PicoTiterPlate device**. A full run will yield, on average, more than **400 000** high-quality sequencing reads. The controlled diameter of the wells inhibits the co-location of more than one DNA bead within any given well.

Sequencing by Synthesis

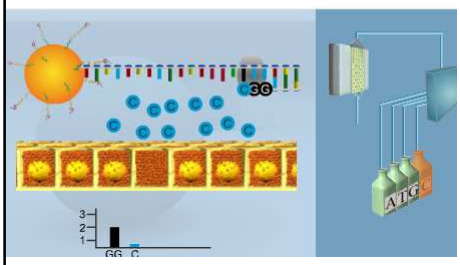


The DNA beads are layered onto a PicoTiterPlate device, depositing the beads into the wells, followed by enzyme beads and packing beads. The bead deposition process optimizes the number of wells that contain a single amplified library bead.

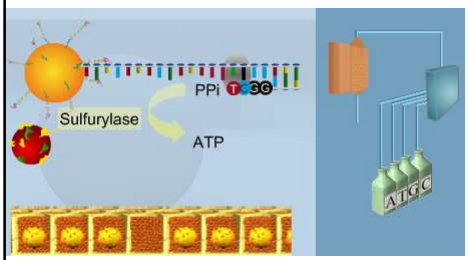


The enzyme beads contain **sulfurylase and luciferase**, key components of the sequencing reaction, while the packing beads ensure that the DNA beads remain positioned in the wells during that sequencing reaction.

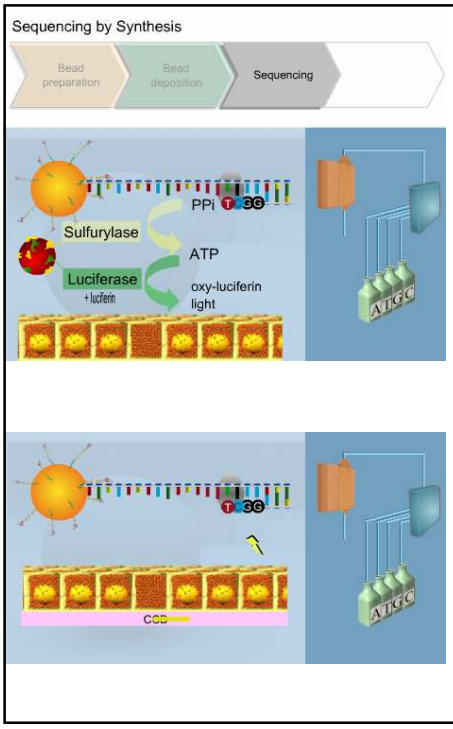
Sequencing by Synthesis



The fluidics sub-system delivers sequencing reagents, containing buffers and nucleotides, by flowing them across the wells of the plate. Nucleotides are flowed **sequentially in a specific order** over the PicoTiterPlate device.

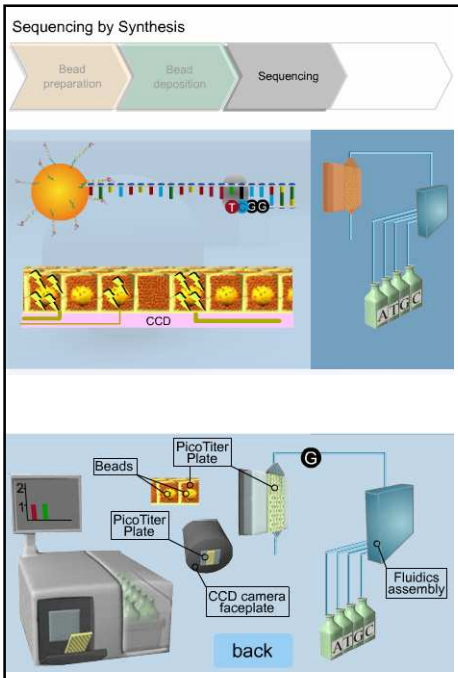


When a nucleotide is complementary to the next base of the template strand, it is **incorporated** into the growing DNA strand by the **polymerase** enzyme. The incorporation of a nucleotide releases **a pyrophosphate molecule**. The sulfurylase enzyme converts the pyrophosphate molecule into **ATP** using adenosine phosphosulfate.



The ATP is hydrolyzed by the luciferase enzyme using luciferin to produce oxy- luciferin and **light**.

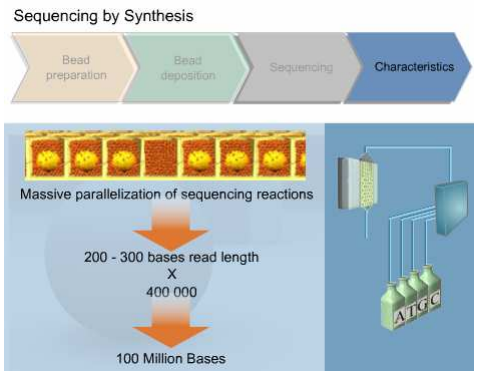
The light emission is detected by the CCD camera, which is coupled to the PicoTiterPlate device.



The intensity of light from a particular well indicates the incorporation of nucleotides.

In homopolymer regions, multiple nucleotides will be incorporated during a single nucleotide flow. In this case, the light intensity produced is increased and proportional to the number of nucleotides incorporated.

If no nucleotide is incorporated during a nucleotide flow, the complementary strand is not elongated and no light signal is generated.



The sequential flow of each of the four nucleotides is repeated for 100 cycles, yielding an average read length of two 200 to 300 bases. With a read length of over 200 to 300 bases and the parallel sequencing of over 400 000 reads, a single instrument run typically yields over 100 M high quality bases in less than eight hours.