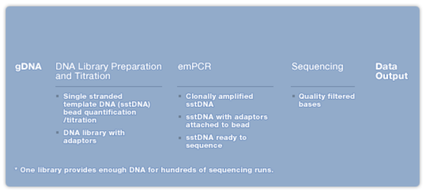
Second generation sequencing technologies

454

Read length: ~300 bases
Total bases per run: 100 MBase

Sequence more than 20 million bases with the specially designed PicoTiterPlate device. Choose from two different plate sizes to meet your specific sequencing needs.

* Multiple optical fibers are fused to form an optical array
* Proprietary etching method produces wells that serve as picoliter reaction vessels
* Each well is only able to accept a single DNA bead
* Reactions in the wells are measured by the CCD camera

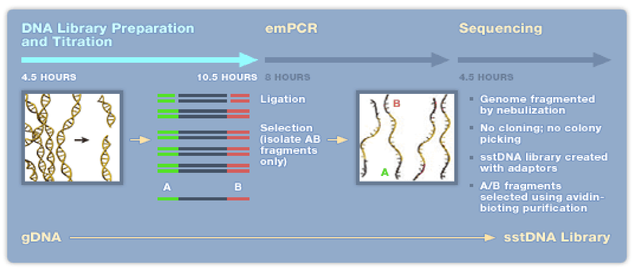


DNA Library Preparation

Preparation of the DNA library consists of a few simple steps (Figure 7). Genomic DNA (gDNA) is fractionated into smaller fragments (300-500 base pairs) that are subsequently polished (blunted).

Short Adaptors (A and B) are then ligated onto the ends of the fragments. These adaptors provide priming sequences for both amplification and sequencing of the sample-library fragments. Adaptor B contains a 5'-biotin tag that enables immobilization of the library onto streptavidin coated beads. After nick repair, the non-biotinylated strand is released and used as a single-stranded template DNA (sstDNA) library. The sstDNA library is assessed for its quality and the optimal amount (DNA copies per bead) needed for emPCR™ is determined by titration.

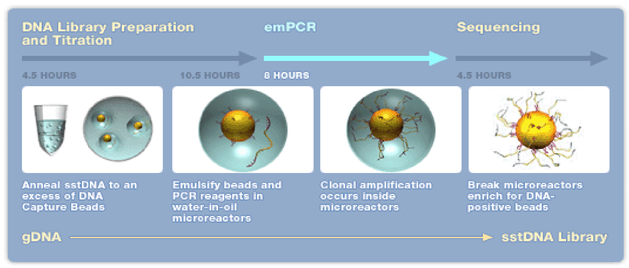
FIGURE 7



emPCR™

The sstDNA library is immobilized onto beads. The beads containing a library fragment carry a single sstDNA molecule. The bead-bound library is emulsified with the amplification reagents in a water-in-oil mixture. Each bead is captured within its own microreactor where PCR amplification occurs. This results in bead-immobilized, clonally amplified DNA fragments.

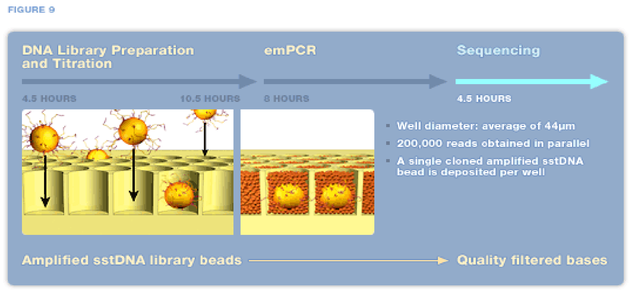
FIGURE 8



Sequencing

sstDNA library beads are added to the DNA Bead Incubation Mix (containing DNA polymerase) and are layered with Enzyme Beads (containing sulfurylase and luciferase) onto the PicoTiterPlate™ device. The device is centrifuged to deposit the beads into the wells. The layer of Enzyme Beads ensures that the DNA beads remain positioned in the wells during the sequencing reaction. The bead-deposition process maximizes the number of wells that contain a single amplified library bead (avoiding more than one sstDNA library bead per well).

FIGURE 9



The loaded PicoTiterPlate device is placed into the Genome Sequencer 20™ Instrument. The fluidics sub-system flows sequencing reagents (containing buffers and nucleotides) across the wells of the plate. Nucleotides are flowed sequentially in a fixed order across the PicoTiterPlate device during a sequencing run. During the nucleotide flow, each of the hundreds of thousands of beads with millions of copies of DNA is sequenced in parallel. If a nucleotide complementary to the template strand is flowed into a well, the polymerase extends the existing DNA strand by adding nucleotide(s). Addition of one (or more) nucleotide(s) results in a reaction that generates a light signal that is recorded by the CCD camera in the Instrument. The signal strength is proportional to the number of nucleotides, for example, homopolymer stretches, incorporated in a single nucleotide flow.

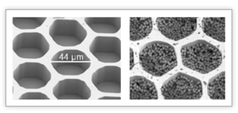
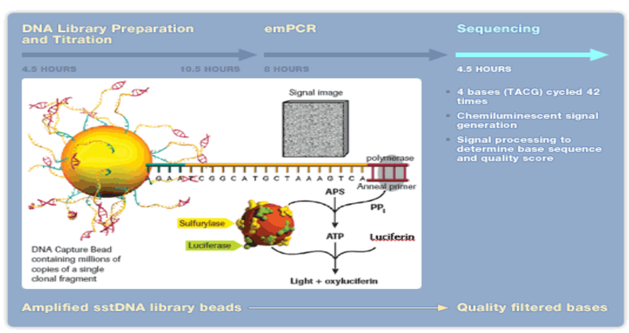


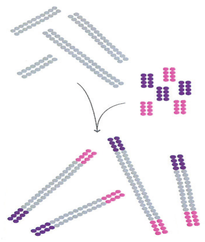
FIGURE 10



Solexa

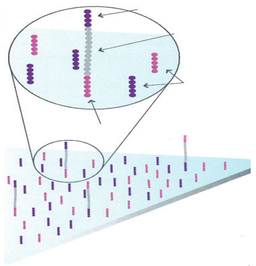
Read length: 30-36 bases
Total bases per run: 1000 MBase

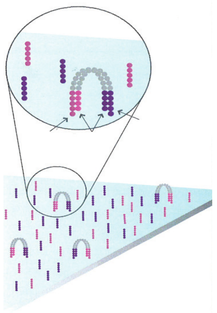
Generating one billion bases of high quality DNA sequence per run at less than 1% of the cost of capillary-based methods. The Solexa sequencing approach is built around a very large number of short sequence reads. Deep sampling of more than ten-fold even coverage is required to generate a consensus and thus ensure high confidence in determination of genetic differences. Such differences are identified by comparison of sequence reads to a reference. Deep sampling allows the use of weighted "majority voting"



1. PREPARE GENOMIC DNA SAMPLE
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

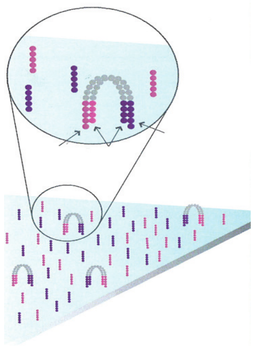
2. ATTACH DNA TO SURFACE
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

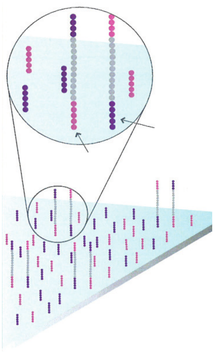




3. BRIDGE AMPLIFICATION
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification

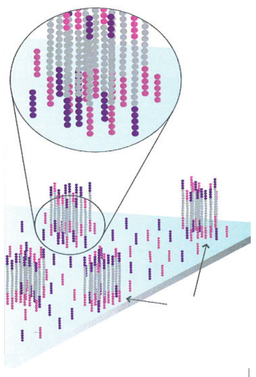
4. FRAGMENTS BECOME DOUBLE STRANDED
The enzyme incorporates nucleotides to build double-stranded bridges on the solidphase substrate.

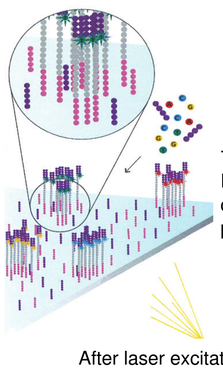




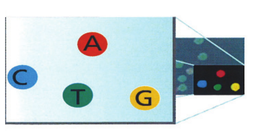
5. DENATURE THE DOUBLE-STRANDED MOLECULES
Denaturation leaves single-stranded tempates anchored to the substrate.

6. COMPLETE AMPLIFICATION
Several million dense clusters of double stranded DNA are generated in each channel of the flow cell.

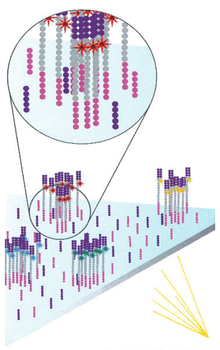




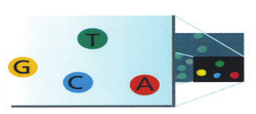
7. DETERMINE FIRST BASE
First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.



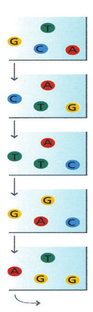
8. IMAGE FIRST BASE
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.



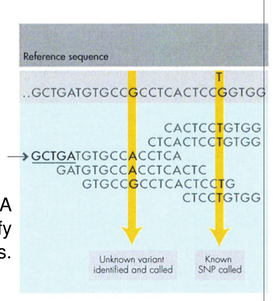
9. DETERMINE SECOND BASE
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.



10. IMAGE SECOND CHEMISTRY CYCLE
After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.



11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES
Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at at time.



12. ALIGN DATA
Align data, compare to a reference, and identify sequence differences.

ABI

Read length: 20-25 bases
Total bases per run: 500 MBase