Percent Q30 Scores per cycle for all lanes and both surfaces
Cluster Density

CONTMACXX Both Surfaces

Density (K/mm²)

Lane
c. Paired End Sequencing

Adapters containing attachment sequences (A1 & A2) and sequencing primer sites (SP1 & SP2) are ligated onto DNA fragments (e.g., genomic DNA). The resulting library of single molecules is attached to a flow cell. Each end of every template is read sequentially.

 Mate pair library preparation is designed to generate short fragments consisting of two segments that originally had a separation of several kilobases in the genome. Fragments of sample genomic DNA is end-biotinylated to tag the eventual mate pair segments. Self-circularization and refragmentation of these large fragments generates a population of small fragments, some of which contain both mate pair segments with no intervening sequence. These mate pair fragments are enriched using their biotin tag. Mate pairs are sequenced using a similar two-adaptor strategy as described for paired-end sequencing.

d. Mate Paired End Sequencing
1. Ligate
2. Image
3. Cap
4. Cleave
5. Repeat ligation cycle for n cycles
6. Primer Reset
7. Repeat Steps 1-6 for 5 Primer rounds

35bp/template
9 Billion bp/run
### SOLiD

#### 8. Repeat Reset with , n−2, n−3, n−4 primers

<table>
<thead>
<tr>
<th>Primer round</th>
<th>Read position</th>
<th>Primer 1</th>
<th>Primer 2</th>
<th>Primer 3</th>
<th>Primer 4</th>
<th>Primer 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Universal seq primer (n)</td>
<td>3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Universal seq primer (n-1)</td>
<td>3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Universal seq primer (n-2)</td>
<td>3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Universal seq primer (n-3)</td>
<td>3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Universal seq primer (n-4)</td>
<td>3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Indicates positions of interrogation
- Ligation cycle: 1 2 3 4 5 6 7

#### Decoding

**Possible dinucleotides encoded by each color**

- 2nd base
  - Template sequence
    - AT
    - AC
    - AA
    - GG
    - CA
    - CC
    - GC
    - GT
    - GG
    - TA
    - TG
    - TT
    - CT

- Double interrogation
  - With 2 base encoding each base is defined twice

**Color space sequence**
- TA
- AC
- AA
- GA
- GC
- CA
- CC
- TC
- CG
- GT
- GG
- AG
- AT
- TG
- TT
- CT

**Decoded sequence**
- AT
- TG
- GG
- GA

**Base space sequence**
- A
- T
- G
- A
454 LifeSciences

With Titanium Chemistry can generate up to 1000bp/template 700 Million bases/run
454

**Sequencing**

7.5 hours

- Well diameter: average of 44 μm
- 400,000 reads obtained in parallel
- A single cloned amplified ssDNA bead is deposited per well

Amplified ssDNA library beads → Quality filtered bases
Pacific Biosciences Technology

Complete Genomics Technology

R Drmanac et al. Science 2010;327:78-81
Next Next-Gen Sequencing (3G Seq)

Figure 1  Third-generation sequencing platforms. (a) Pacific Biosciences SMRT (single-molecule real-time) DNA sequencing method. The platform uses a DNA polymerase anchored to the bottom surface of a ZMW (pictured in cross section). Differentially labeled nucleotides enter the ZMW via diffusion and occupy the ‘detection volume’ (white translucent halo area) or microseconds. During an incorporation event, the labeled nucleotide is ‘held’ within the detection volume by the polymerase for tens of milliseconds. As each nucleotide is incorporated, the label, located on the terminal phosphate, is cleaved off and diffuses out of the ZMW. (b) Life Technologies FRET sequencing platform uses base fluorescent labeling technology, a DNA polymerase modified with a quantum dot and DNA template molecules immobilized onto a solid surface. During an incorporation event, energy is transferred from the quantum dot to an acceptor fluorescent moiety on each labeled base. Light emission can only emanate from labeled nucleotides as they are being incorporated. (c) The Oxford nanopore sequencing platform uses an exonuclease coupled to a modified α-hemolysin nanopore (purple, pictured in cross section) positioned within a lipid bilayer. As sequentially cleaved bases are directed through the nanopore, they are transiently bound by a cyclodextrin moiety (blue), disturbing current through the nanopore in a manner characteristic for each base. (d) The Ion Torrent sequencing platform uses a semiconductor-based high-density array of microwell reaction chambers positioned above an ion-sensitive layer and an ion sensor. Single nucleotides are added sequentially, and incorporation is recorded by measuring hydrogen ions released as a by-product of nucleotide chain elongation.
Sequencing DNA
Human Whole Genome Sequencing

- Initial Ref Sequence $300 million and took about a decade. (Draft reported in 2001)
- Craig Venter’s Genome for ~$10 million. (Pub Oct 2007).
- Yoruban from Nigeria in 8 weeks for $250,000. Approx. 30X coverage. (Pub Nov. 2008)
- Han Chinese in 8 weeks for ~$500,000 at approx. 36X coverage.
- Female patient with AML. Sequenced normal and tumor from same patient. 98 full runs on GAII for tumor DNA and 34 full runs for normal skin cell DNA. ~1.5 years to compete both genomes.
- As of January 2012 a human genome can be sequenced for about $5,000 at an average read depth of 30X in 10 days
DNA Sequencing with Next-Generation Technologies

A Draft Sequence of the Neandertal Genome

Richard E. Green,†‡ Johannes Krause,†§ Adrian W. Briggs,†§ Tomislav Maricic,†§ Udo Stenzel,†§ Martin Kircher,†§ Nick Patterson,†§ Heng Li,† Weiwei Zhai,†∥ Markus Hsi-Yang Fritz,∥ Nancy F. Hansen,∥ Eric Y. Durand,∥ Anna-Sapfo Malaspina,∥

Sequencing the nuclear genome of the extinct woolly mammoth

Webb Miller, Daniela I., Michael D. Packard, Fan Kerstin Lindblad-Toh, Eric Sharon Sheridan, Tom P.

Genetic history of an archaic hominin group from Denisova Cave in Siberia

David Reich, Richard E. Green, Martin Kircher, Johannes Krause, Nick Patterson, Eric Y. Durand, Bence Viola,

Insights into hominin evolution from the gorilla genome sequence

Applications

• Whole Genome Sequencing
• Exome Sequencing
• Targeted Genomic Sequencing
• Chromatin-IP-Sequencing
• DNase I Hypersensitivity Sequencing
• Methyl-Seq (RRBS, MeDIP, etc)
• Microbiome Sequencing
• Metagenomics