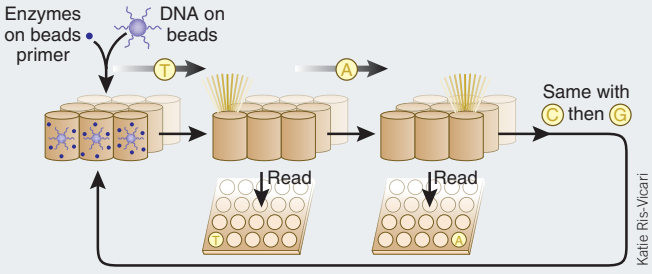
Primer: Sequencing—the next generation

Different sequencing technologies, at a glance.

454 technology

Sample preparation. Fragments of DNA are ligated to adapters that facilitate their capture on beads (one fragment per bead). A water-in-oil emulsion containing PCR reagents and one bead per droplet is created to amplify each fragment individually in its droplet. After amplification, the emulsion is broken, DNA is denatured and the beads, containing one amplified DNA fragment each, are distributed into the wells of a fiber-optic slide.

Pyrosequencing. The wells are loaded with sequencing enzymes and primer (complementary to the adapter on the fragment ends), then exposed to a flow of one unlabeled nucleotide at a time, allowing synthesis of the complementary strand of DNA to proceed. When a nucleotide is incorporated, pyrophosphate is released and converted to ATP, which fuels the luciferase-driven conversion of luciferin to oxyluciferin and light. As a result, the well lights up. The read length is between 100 and 150 nucleotides.



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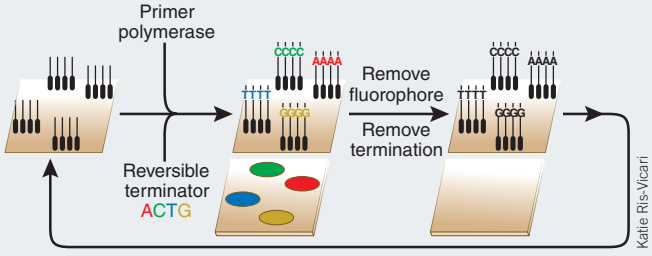
Solexa technology

Sample preparation. Fragments of DNA are ligated to end adapters, denatured and bound at one end to a solid surface already coated with a dense layer of the adapters. Each single-stranded fragment is immobilized at one end, while its free end 'bends over' and hybridizes to a complementary adapter on the surface, which initiates the synthesis of the complementary strand in the presence of amplification reagents. Multiple cycles of this solid-phase amplification followed by denaturation create clusters of ~1,000 copies of single-stranded DNA molecules distributed randomly on the surface.



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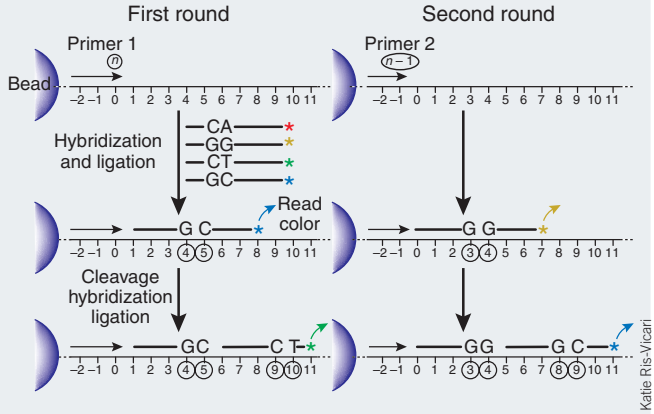
Sequencing with reversible terminators. Synthesis reagents, added to the flow cell, consist of primers, DNA polymerase and four differently labeled, reversible terminator nucleotides. After incorporation of a nucleotide, which is identified by its color, the 3′ terminator on the base and the fluorophore are removed, and the cycle is repeated for a read length of 30–35 nucleotides.

SOLiD technology

Sample preparation. Fragments of DNA are ligated to adapters and amplified on beads by emulsion PCR. The DNA is denatured and the beads deposited onto a glass slide.

Sequencing by ligation. A sequencing primer is hybridized to the adapter and its 5' end is available for ligation to an oligonucleotide hybridizing to the adjacent sequence. A mixture of octamer oligonucleotides compete for ligation to the primer (the bases in fourth and fifth position on these oligos are encoded by one of four color labels). After its color has been recorded, the ligated oligonucleotide is cleaved between position 5 and 6, which removes the label, and the cycle of ligation-cleavage is repeated. In the first round, the process determines possible identities of bases in positions 4, 5, 9, 10, 14, 15, etc. The entire process is repeated, offset by one base by using a shorter sequencing primer, to determine positions 3, 4, 8, 9, 13, 14, etc., until the first base in the sequencing primer (position 0) is reached. Since the identity of this base is known, the color is used to decode its neighboring base at position 1, which in turn decodes the base at position 2, etc., until all sequence pairs are identified. The current read length is between 30 and 35 nucleotides.



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