New generation of DNA sequencing technologies

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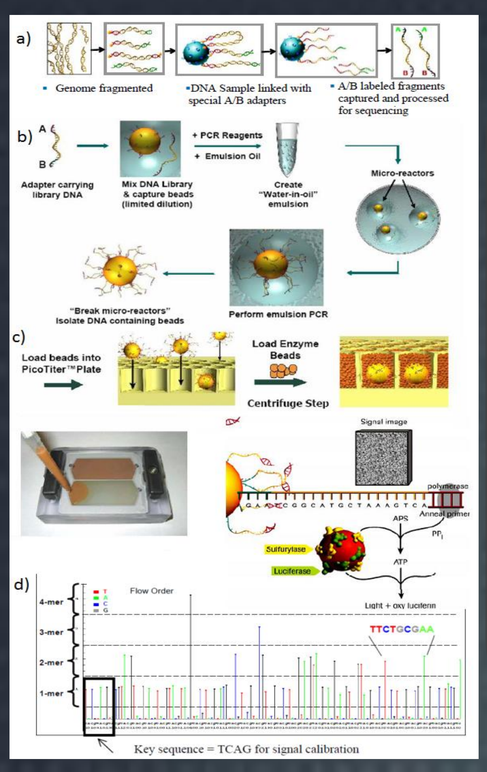
1. Introduction

Since the completion of the human genome project, substantive changes have occurred in the approach to genome sequencing. Several revolutionary approaches to DNA sequencing has been introduced on market, called next-generation sequencing (NGS). They are not only changing our genome sequencing approaches and the associated timelines and costs, they are also accelerating and altering a wide variety of types of biological inquiry that have historically used a sequencing-based readout. Main objective is explanation of 454, Illumina, Solid, Ion Torrent and Nanopore sequencers

2. 2nd Generation Sequencer:

2.1 454



First commercialized NGS on market. It was designed by 454 Life Sciences but now belongs to Roche. This sequencer use pyrosequencing technology. When one base is added, releases Ppi, we can see a light emission thanks coupled reaction and captured with CCD camera. Fragment amplification is done using emulsion PCR. Sequencing procedure is realized in plate with 1,6 millions of wells. Every well only can support one bead.

Fig 1: modified from http://my454.com/products/technology.asp. a) Library preparation and adapters addition. b) Beads addition and Emulsion PCR process. c) Amplified fragments with beads put inside plate with wells and beads with enzyme added in the reaction. Also coupled reactions and final emission of light can be seen. d) Flowgram with final result.

2.2. Solexa

Method uses sequencing by synthesis technology commercialized by Illumina. During sequencing, nucleotides compete binding to the DNA. Solexa adaptors are added when DNA library are prepared and bind to the surface. Amplification of fragments are done by using bridge PCR.

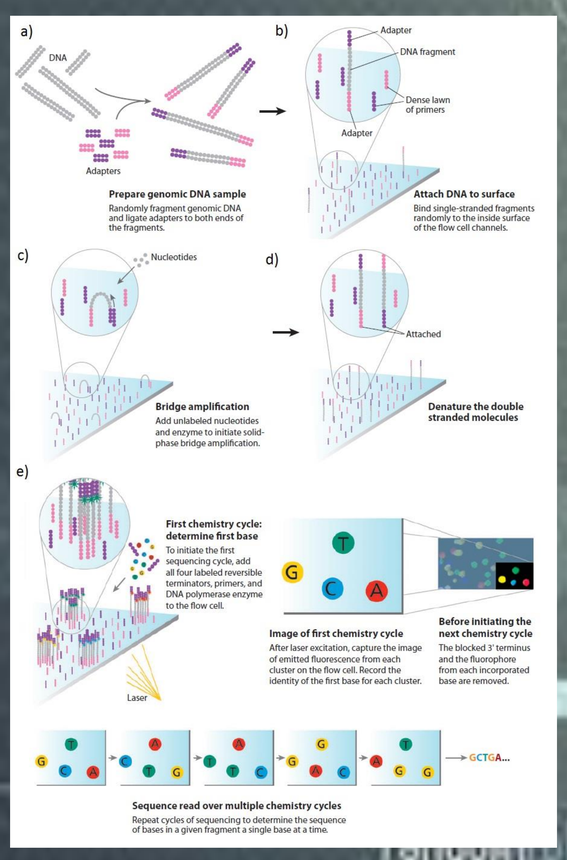
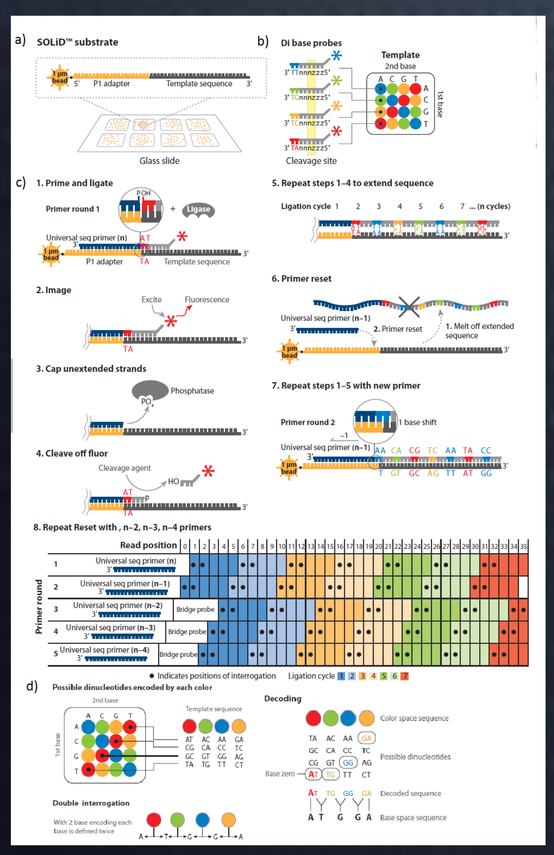


Fig 2: extracted from [1] a) Library preparation. B) Bind to the surface when adapters are added. c) Bridge PCR. d) When fragment amplification are done, double strand molecules are denaturalized. Alternative cycles of addition of nucleotides and denaturalization are done creating clusters e) Extension of the cluster strand by one nucleotide. Fluorescent molecule is eliminated when image is captured .

2.3. SOLiD



NGS design by Applied Biosystems. Can do two types of library, conventional and Mate-Pair fragments. Adapters are added and allow binding to beads. For amplifying it uses emulsion PCR. Beads with amplified fragments binds to the glass slide. The main difference of this method is the sequencing by synthesis using DNA ligase. It sequences using two nucleotides every time. 4 possible nucleotides of 16 possibilities are synthetized. Using 4 different primers changing the reading patron allows sequencing by fluorescence light emission.

Fig 3: Extracted from [2] a) Interaction of beads with glass slide. b) Colour classification of dibases. c) Workflow of sequencing. d) Decoding process

2.4. Ion Torrent

Method commercialized by Life Technologies. Uses standard sequencing chemistry but using a novel detection method, a semiconductor. When one nucleotide is added into a strand of DNA, and hydrogen ion is released, its charge changes the solution pH and its detected by the semiconductor. These method also use adaptors and beads. It is amplified by emulsion PCR.

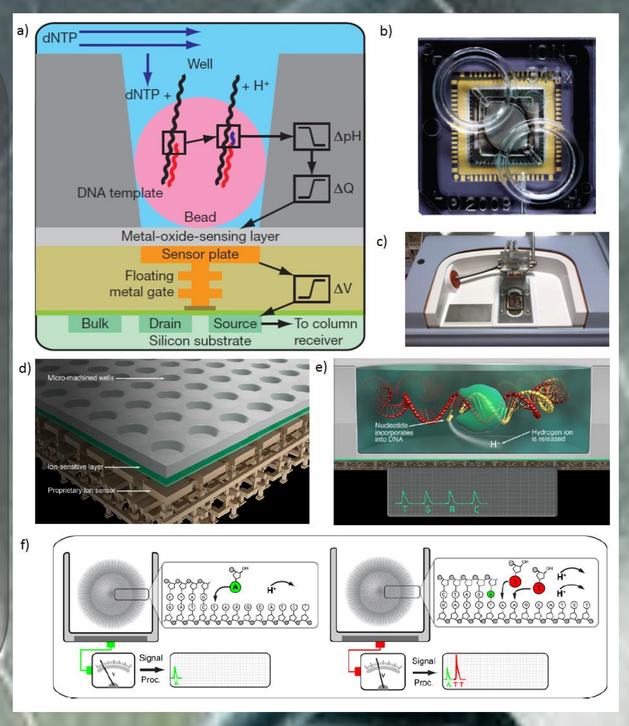
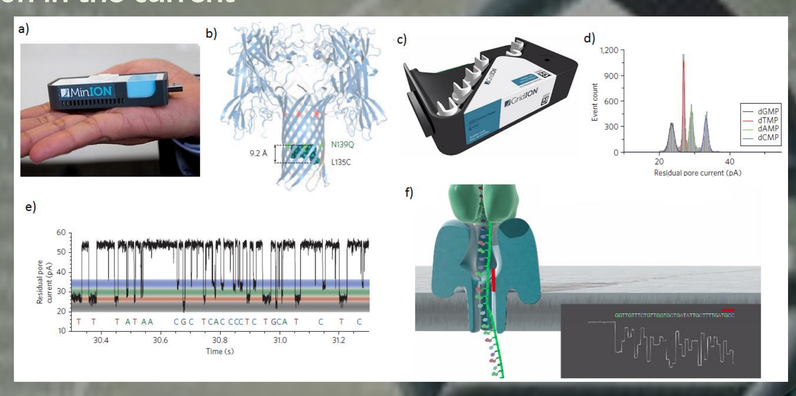


Fig 4: extracted from http://www.invitrogen.com/ and [3]. a) Drawing of a well b) ION 314x chip c) Frontal part of sequencer. d) Array image. e) and h) Signal processing

3. 3rdGeneration sequencers: Nanopore

Method developed by Oxford Nanopore Technologies. Its sequencing is based on nanopores that can measure single molecules directly, without the need for nucleic acid amplification. This sequencer uses bespoke pore-forming proteins to create holes in membranes formed from lipid bilayers. Multiple nanopore measurements are made in parallel using the company's arrayed sensor chip. Voltage is applied across the membrane in which the pore is set, and the resulting ionic current through the pore is measured. When an analyte of interest passes through the pore or near its aperture, it creates a characteristic disruption in the current

Fig 4: extracted from http://nanoporetech.com and [4] a) MinION device. b) Structure of pore (haemolysin) c) GridION cartrige d) Residual current of histogram of nucleotide binding events. e) Nucleotide distribution an alteration of the field. f) Image simulation of sequencing.



4. Valoration

These techniques reduce time effort and sequencing cost but not enough to achieve a new level on research. Instead of each sequencer has its benefits, other techniques are now developing. These methods will be more effective and will cause a revolution in genomic camp. Each platform has distinct advantages and limitations and the choice depends on several variables; for example, research or clinical intent, cost and other parameters.

[5]	Template preparation	Sequencing method	Accuracy (Raw error rate)	Reads (time/run) [length]	Advantages	Disvantages	System cost (cost per run) [Cost per Mb]
454	Fragment , Mate Pair (MP), Emulsion PCR	Pyrophosfate released at time of base incorporation	99,9% (0,4-1,5 %)	700 Mb (23 h) [400 pb]	1-5 µg DNA needed Read length Fast	Price Long time preparation	500.000 $ (8439 $) [84,39 $]
Solexa	Fragment, MP, Bridge PCR	Fluorescent-labeled nucleotides added simultaneously driven by DNA polymerase	98% (0,5-2 %)	600 Mb (11 days) [2 x 150 pb]	< 1 µg DNA needed High throughput	Read lengths More false positives Time proceeding High information data	400.000 $ (8950 $) [0,03 $]
SOLiD	Fragment, MP, Emulsion PCR	Fluorescent-labeled nucleotides added simultaneously driven DNA ligase instead of DNA polymerase	99,94% (0,2%)	200 Mb (7 days) [35 x 75 pb]	2-20 µg DNA needed Accuracy	Read lengths High information data More gaps	525.000 $ (17.447 $) [0,04 $]
Ion Torrent	Fragment, Emulsion PCR	Non-optical DNA sequencing; massively parallel semiconductor senses ions produced as nucleotides are incorporated by DNA polymerae-based synthesis	99,99% (1-3%)	1 Gb (2 h) [200 pb]	100 ng -1 ug needed High accuracy Short run time (fast) Cheaper	Error rate Limitations in homopolymer repeats	50.000$ (<500 $) [5,00 $]
Heliscope	Fragment, MP, Single molecule	Imaging of dye-labeled nucleotides incorporated during DNA synthesis by single DNA polymerase molecule	99% (3-5%)	35 Gb (8 days) [35 bp]	< 2 µg DNA needed Direct RNA-sequencing application	Read lengths	750.000 $ (5.000 $) [<0,0005 $]
SMRT	Fragment only, Single molecule	Imaging of dye labeled incorporated during DNA synthesis by single DNA polymerase molecule.	99,999% (13-15%)	100 Mb (1 h) [4500 bp]	Long reads Short run time High accuracy High sensitivity	High error rates Low total reads/run High cost/Mb	695.000 $ (1000 $)
Nanopore	Fragment only, Single molecule	Electric current change when DNA strands pass, all using nanopore technology.	96% (4% error rates)	Unlimited (3Tb/day) Run until	Whole genome scan 15 min Run until method Very low cost	Low accuracy	Commercially unavailable [0,00000003 $]

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