Second-generation nucleic-acid sequencing and bioarchaeology - Review

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Abstract

The nucleic-acid revolution started 31 years ago. The development of the second-generation sequencing (SGS) has allowed a higher throughput and lower price per sequenced base, opening the possibility to sequence ancient genomes, including epigenetics. The main SGS platforms are described in this review: i) Roche 454 Life Sciences, based on emulsion PCR (emPCR) and further pyrosequencing; ii) Illumina (bridge-amplification and subsequent reversible-terminator sequencing); iii) Life Technologies SOLiD (emPCR coupled with oligonucleotide ligation to interrogate DNA); and iv) Life Technologies Ion-Torrent-chip (emPCR, further using microchip pH-meters). Different ancient genomes (including viruses, microorganisms, plants and animals) have been sequenced. This has allowed to study the evolution of pathogens, domestication of microorganisms, plants and animals, paleodiets and paleoenvironments, including climate changes. Some hurdles and challenges must yet be overcome, but the steady technological advances in nucleic-acid isolation, sequencing and bioinformatics (together with higher computing power) promise a bright future for bioarchaeology in general, and paleogenomics in particular, allowing to analyze not just single genomes, but also to address ancient population genomics and evolution.

Key words (not in title or abstract, which are always indexed): ancient DNA (aDNA), high-throughput sequencing (HTS), paleoenvironmental DNA (PalEnDNA), paleogenomes, polymerase chain-reaction (PCR).

Resumen

La revolución de la secuenciación de ácidos nucleicos comenzó hace 31 años. El desarrollo de la secuenciación de segunda generación (SGS) ha permitido un mayor rendimiento y menor precio por base secuenciada, abriendo la posibilidad de secuenciar genomas antiguos, incluyendo epigenética. Esta revisión describe las principales plataformas de SGS: i) Roche 454 Life Sciences, basada en PCR en emulsión (emPCR) y posterior pirosecuenciación; ii) Illumina (amplificación por puente y secuenciación mediante terminadores reversibles); iii) Life Technologies SOLiD (emPCR y ligación de oligonucleótidos para interrogar ADN); y iv) Life Technologies Ion-Torrent-chip (emPCR y microchips pH-metros). Distintos genomas antiguos (incluyendo virus, microorganismos, plantas y animales) han sido secuenciados. Ello ha permitido estudiar la evolución de patógenos, domesticación de microorganismos, plantas y animales, paleodietas y paleoambientes, incluyendo cambios climáticos. Todavía quedan por superar obstáculos y desafíos, pero los avances tecnológicos continuos en aislamiento de ácidos nucleicos, secuenciación y bioinformática (junto con mayor potencia de computación) prometen un futuro brillante para la bioarqueología en general, y la paleogenómica en particular, permitiendo analizar no sólo genomas aislados, sino también abordar la genómica y evolución de poblaciones antiguas.

Palabras clave (no en título o resumen, que son siempre indexados): ADN antiguo (ADNa), secuenciación de alto rendimiento (SAR), ADN paleoambiental (ADNPalAmb), paleogenomas, reacción en cadena de la polimerasa (RCP).

Introduction

Sequencing of ancient DNA (aDNA) started more than three decades ago with the quagga (*Equus quagga*), a zebra-like species extinct in 1883 (Higuchi et al, 1984), using the first-generation sequencing (FGS) methodology. Later on, the second-generation sequencing (SGS) of DNA (sometimes described using the ambiguous "next-generation" sequencing terminology; NGS) revolutionized the life sciences and related disciplines, including archaeology (bioarchaeology). The main advantages over the previous first generation sequencing platforms were the significantly higher throughput and lower price per sequenced base.

Such high-throughput sequencing (HTS) technology allowed to sequence full genomes in an affordable way. Additionally, some second-generation nucleic-acid platforms have allowed what was previously considered an impossible task: to sequence full ancient genomes. Thus, most advances in aDNA sequencing have been carried out with such second-generation DNA-sequencing approach (Dorado et al, 2007-2014; Charman et al, 2015; Hagelberg et al, 2015; Orlando et al, 2015). The SGS also allows to study epigenetics, which has been recently applied to ancient DNA (Orlando et al, 2015; Pedersen et al, 2014; Smith et al, 2015; Seguin-Orlando et al, 2015).

A new third-generation sequencing (TGS) of nucleic acids promises to further revolutionize bioarchaeology in future years, sequencing single molecules (not requiring amplification). This has the potential to sequence not only unbiased aDNA, as demonstrated with horse (Orlando et al, 2011; Ginolhac et al, 2012) but also ancient RNA (aRNA), albeit it is now mostly in development. Thus, this review deals with the second-generation nucleic-acid sequencing in bioarchaeology.

Sequencing platforms

There are several second-generation nucleic-acid sequencing platforms. The most popular are described below.

a. Roche 454 Life Sciences sequencing

This technology revolutionized DNA sequencing, igniting the second-generation sequencing. It is based on emulsion polymerase chain-reaction (emPCR) in-vitro amplification, allowing a high multiplexing of parallel reactions in a water-in-oil (W/O) emulsion.

Further pyrosequencing reactions are used to read the DNA sequence in picowells. The reads are finally assembled using bioinformatics tools. It represents the second-generation platform with longer reads, albeit being also more expensive.

The power of this approach has been demonstrated through sequencing ancient genomes, like the one from Neanderthal (Green et al, 2006; Noonan et al, 2006).

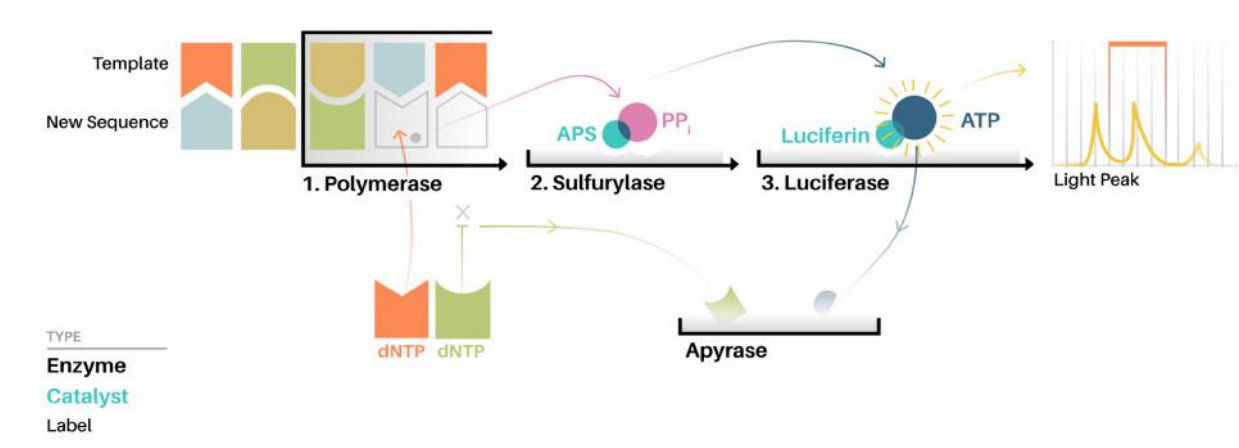

Figure 1. Roche 454 Life Sciences sequencing. The pyrosequencing reaction (coupled with DNA polymerization) generates light, allowing the DNA sequencing. Figure credit: How pyrosequencing works. © 2014 Jacopo Pompilii, Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

b. Illumina sequencing

This approach is based on the Polymerase Chain-Reaction (PCR) bridge-amplification technology, to generate DNA clusters on solid supports (PCR-free protocols are also available). Then, a reversible-terminator sequencing is carried out in a massively-parallel way, generating light in flow cells. Sophisticated software algorithms are then used to assemble the reads and generate the contigs, chromosomes and genomes.

This platform allows a high coverage of the sequenced genome. Although the original reads were short (which sometimes was a blocking problem, since the bioinformatics tools may not be capable of assembling short/repetitive sequences), the technology has since evolved to generate significantly longer reads.

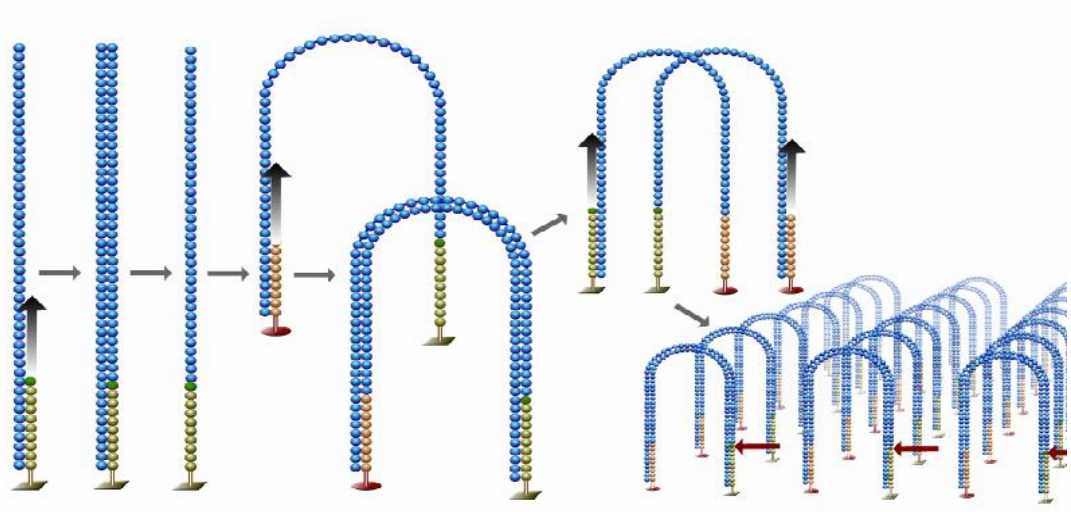

Figure 2. Illumina sequencing. The bridge amplification allows a high-throughput sequencing. Figure credit: Bridge amplification. © 2009 Abizar, Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

c. Life Technologies SOLiD sequencing

This methodology uses emPCR in a solid support, coupled with oligonucleotide ligation of different universal primers and labeled probes to interrogate DNA. The fluorescence generated by the probe cleavage allows to read the DNA in the sequencing reaction. Then, bioinformatics tools are used to assemble the reads.

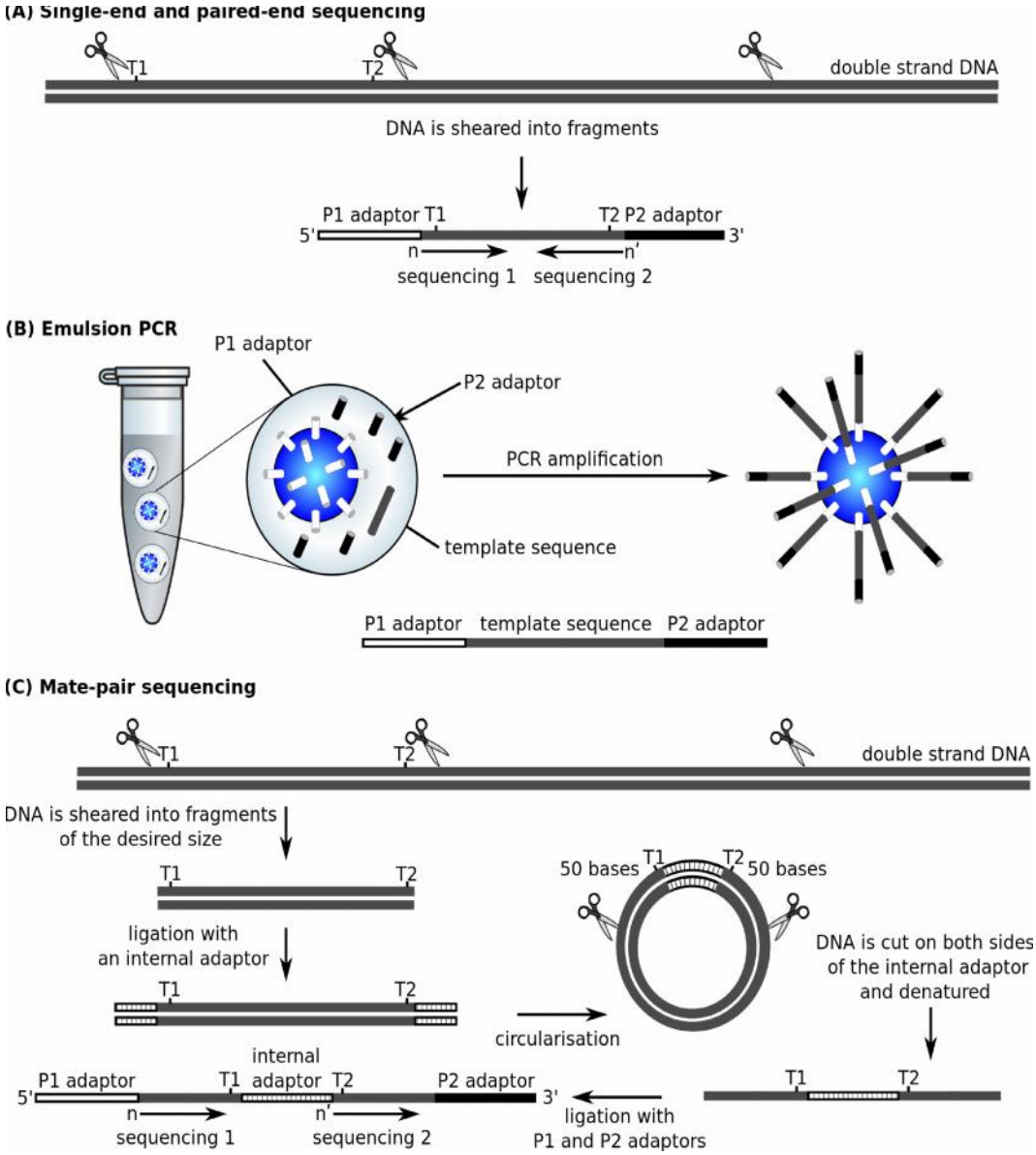

Figure 3. Life Technologies SOLiD sequencing. The sheared DNA is subjected to emPCR and further sequencing. Figure credit: Library preparation for the SOLiD platform. © 2012 Philippe Hupé, Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

d. Life Technologies Ion-Torrent-chip sequencing

This approach generates a library of DNA fragments ligated to adapters with biotin, which are captured with streptavidin-coated beads. The DNA is

denatured and the non-biotinylated fragments are captured by primer-coated beads. The DNA is amplified by emPCR using biotinylated primers, allowing to isolate the extension products attached to the beads. They are captured in picowells and the sequencing reactions are carried out in femtowells that detect the generated protons (H^+) in the DNA polymerization, thus effectively working as microchip pH-meters. The reads are finally assembled using bioinformatics applications.

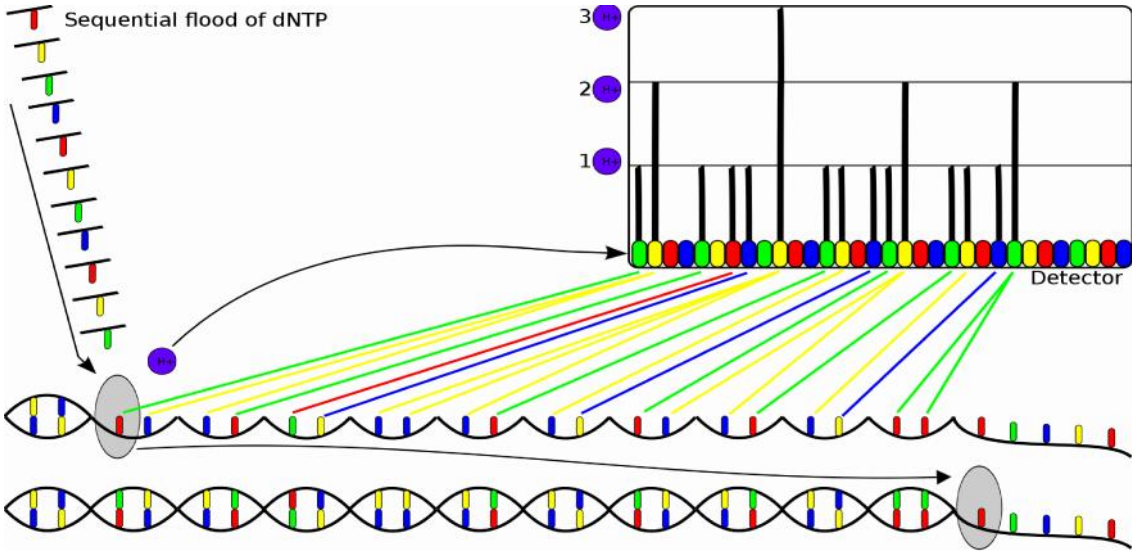

Figure 4. Life Technologies Ion-Torrent-chip sequencing. Detection of protons during DNA polymerization. Figure credit: dNTP incorporation - hydrogen magnitude. © 2011 David Tack, Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

Sequencing ancient genomes

To date, different ancient genomes (paleogenomes) have been sequenced. This was initially allowed by the development of the PCR in vitro amplification. The methodology has also improved in recent years, including the isolation of higher quantity/quality DNA, as well as reading longer DNA lengths at lower prices. Thus, paleogenomics allows the sequencing of partial or complete ancient genomes, including viruses (Ng et al, 2014) and microorganisms like cyanobacteria (MartínezDeLaEscalera et al, 2014; Pal et al, 2015). Likewise, organelles like chloroplasts and mitochondria (Haak et al, 2010; Bon et al, 2012; Fu et al, 2012; Hung et al, 2013; Paijmans et al, 2013; Fernández et al, 2014; Meyer et al, 2014; Orlando, 2014; Shapiro and Ho, 2014; Sheng et al, 2014; Hervella et al, 2015; Immel et al, 2015; Marsolier-Kergoat et al, 2015) and nuclear genomes of plants such as cotton (Palmer et al, 2012a,b; Brown et al, 2015) and wild animals like mammoth (Poinar et al, 2006; Miller et al, 2008), bear (Barnes et al, 2002; Noonan et al, 2005), cave hyena (Bon et al, 2012), horse (Orlando et al, 2013) and bison (Marsolier-Kergoat et al, 2015), as well as livestock like sheep (Teasdale et al, 2015) and hominins such as Neanderthals (Paabo, 2015) and Denisovans (Meyer et al, 2012; Brown and Barnes, 2015) from the Middle Pleistocene; up to about one million years ago (Orlando, 2014). The new sequencing technologies also allow to address ancient population genomics and evolution, as recently described

for the Adelie penguin (*Pygoscelis adeliae*) (Parks et al, 2015) and humans (Allentoft et al, 2015).

Paleogenomics is also being used to study the evolution of pathogens, including the ones causing infectious diseases like the potato blight (*Phytophthora infestans*), plague (*Yersinia pestis*), leprosy (*Mycobacterium leprae*) and tuberculosis (*Mycobacterium tuberculosis*) (Donoghue et al, 2015). Likewise, the domestication of microorganisms (eg., fermentations), plants and animals and paleodiets. Interestingly, a massive sequencing of 101 ancient human genomes of the Bronze Age in Eurasia (3000 to 1000 BC) has revealed the surprising fact that the lactose tolerance during this period was only 10% in Europe (Allentoft et al, 2015), showing that its main onset of positive selection arose more recently (1000 BC or later) than previously thought (5500 BC) (Itan et al, 2009). On the other hand, sequencing extinct plants and animals may shed new light on climate change, migrations, adaptations and the evolutionary history of the species (Haak et al, 2010; Fu et al, 2012; Fernández et al, 2014; Brandao et al, 2015; Cooper et al, 2015; Hervella et al, 2015). This way, the gene flows can also be determined, demonstrating ancient genetic admixtures in which modern humans inbred with Denisovans and Neanderthals (which also interbred between themselves) (Prufer et al, 2014; DerSarkissian et al, 2015; Ermini et al, 2015; Hofreiter et al, 2015; Knapp et al, 2015; Paabo, 2015; Perry and Orlando, 2015; Vernot and Akey, 2015). This has demonstrated that they are subspecies of the same species.

On the other hand, environmental DNA (eDNA), and in particular paleoenvironmental DNA (PalEnDNA), includes deposits like sediments and soils, and remains such as coprolites and gut contents (Clack et al, 2012; MartínezDeLaEscalera et al, 2014; Ng et al, 2014; Pawlowski et al, 2014; Rawlence et al, 2014; Pal et al, 2015; Pedersen et al, 2015; Thomsen and Willerslev, 2015). Thus, the sequencing of extinct species may be used to reconstruct ancient ecosystems, even in the absence of fossils visible to the naked eye or the microscope.

But different challenges must be overcome to successfully sequence aDNA (Kircher, 2012; Shapiro and Hofreiter, 2012), including its recovery with enough quantity/quality, with the required chemical and physical integrity (Overballe-Petersen et al, 2012; Parks and Lamber, 2015), not being cross-contaminated with modern DNA. Besides physical fragmentation, possible chemical DNA alterations include tautomerization, deamination, base loss (mainly depurination; mostly at guanosines), oxidation and hydrolysis (mostly at purine bases). The sequencing artifacts may also be platform-specific (Seguin-Orlando et al, 2013). Thus, a palindromic-sequence artifact has been recently identified (Star et al, 2014). Indeed, it has been found that although the age of the sample may be obviously relevant, other aspects like the taphonomic history of the remains may be more important to determine both the quantity and quality of recoverable DNA from the archaeological remains. Thus, coldness (eg., permafrost in the Arctic and Antarctic regions) and dryness (eg., desertic and saline environments) usually yield the best-preserved DNA samples.

Bioinformatics applications have been developed to ascertain aDNA damage (Jonsson et al, 2013). It has been recently advised that samples with limited DNA fragmentation and deamination should be used to avoid biased results in epigenomic studies (Seguin-Orlando et al, 2015). Some enzymatic-repair approaches to decrease aDNA damages and increase its quantity have been evaluated (Mouttham et al, 2015). Furthermore, some sample treatments, like enzymatic digestions in the presence of ethylenediaminetetraacetic acid (EDTA) may significantly increase the recovered aDNA (Damgaard et al, 2015). Additionally, and not surprisingly, there may also be a differential quantity/quality of DNA isolated from different parts of samples, like teeth and bones (Damgaard et al, 2015; Pinhasi et al, 2015). The enrichment of aDNA fragments by probe-hybridization capture, or probe-free approaches such as affinity of methylated binding-domains (MBD) for methylated CpG dinucleotides (mCpG), as well as the isolation of single-stranded DNA (ssDNA) from ancient samples, have represented milestones in aDNA sequencing progress in recent years (Carpenter et al, 2013; Gansauge and Meyer, 2013; Enk et al, 2014; Avila-Arcos et al, 2015; Brown and Barnes, 2015; Hofreiter et al, 2015).

Additionally, the huge amount of data generated with the second-generation sequencing demand new bioinformatics approaches for both hardware and software development. Thus, parallel processing using many-core microprocessors are being used together with bioinformatics tools to assemble short-reads. Consequently, these developments are also fueling a conceptual shift towards properly addressing the experimental challenges, analyzing and interpreting the results (Schubert et al, 2012; Rawlence et al, 2014; Hofreiter et al, 2015).

Future prospects and concluding remarks

The future looks promising for aDNA in general and paleogenomics in particular, mostly due to the development of new sequencing platforms that allow to quickly sequence ancient genomes using less starting material, with much longer reads, higher accuracy and throughput, at a lower cost. Thus, population studies on paleogenomics will be more cost-effective with the third-generation sequencing of nucleic acids, which are capable of directly reading single nucleic-acid sequences without previous in vivo (eg., molecular cloning inside bacteria) or in vitro (eg., PCR) amplifications. Besides, such new platforms could also allow to sequence aRNA. But to reach such goals, further developments and refinements in computing processing power (mostly from parallel executions on many-core chips) and bioinformatics algorithms will also be required to handle the growing complexity of the generated data sets. Of course, special care should be taken to maintain the nucleic-acid integrity, increase yield and avoid cross-contaminations, as learned from the aDNA research experience in the past 31 years.

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