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## DNA diagnostics in the fifty-year retrospect

The journey from the double helix and complementarity principle to DNA chips, PNA technology, DNA nanotechnology, real-time PCR and MEMS-based systems

'Welcome to the wild and wonderful world of DNA technology.'  
IE Alcamo *DNA Technology: The Awesome Skill* (2000)

'The history of DNA technology, as we know, is full of remarkable discoveries.'  
MD Frank-Kamenetskii *Unraveling DNA: The Most Important Molecule of Life* (1997)

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Due to their relative simplicity, fair rapidity, solid reliability, universal workability and remarkable sensitivity, DNA diagnostics hold the distinct position in the area of molecular *in vitro* diagnostics. Altogether, they are the most rapidly developing branch in this field, with an annual growth rate of the global market being in the range of 13 to 25%, depending on different estimates [1,2]. As a result, in the next 2 years, DNA probes are expected to account for 12–15% of a multibillion *in vitro* diagnostics market worldwide. Dozens of biotech companies are now involved in the DNA diagnostic business and their number has also increased remarkably during the past few years.

Such a promising trend is well represented in the *Expert Review Molecular Diagnostics* journal, which regularly reviews various aspects of DNA diagnostics ranging from PCR-based techniques [3–5], DNA microarrays and microfluidics [5–7] to fluorescent *in situ* hybridization (FISH) [8], peptide nucleic acid (PNA) technology [9–11], SNP detection [11], DNA nanotechnology [12], molecular beacons [13], invasive cleavage assays [14], rolling circle amplification (RCA) [15] and many others. All these approaches are milestones in the development of DNA diagnostics (TABLE 1) and, according to expert opinion, they will play an important role in

the medical practice, public health, pharmaceutical industry, forensics and biodefense in the 21st century [2].

As an intrinsic part of DNA technology, DNA diagnostics are rooted in the April 1953 discovery of the intertwined double-stranded structure of DNA biopolymers, five decades of which we are now celebrating. This breakthrough was well prepared by earlier key developments in molecular biology, biochemistry, genetics and related fields (TABLE 2). Yet, it was the genius of Watson and Crick, who ultimately recognized all the previous data as a whole and, based on this understanding, finally realized that DNA is a double helix and who also comprehended the deep biological impact of the new insight. Namely this elegant structure and the complementarity rules directing its formation ushered in the advent of DNA diagnostics (TABLE 1).

One can see that DNA technology in general, and DNA diagnostics in particular, embody a set of notable technological and methodological advances. Starting from the DNA double helix discovery 50 years ago, these developments revolutionized the entire field of bioanalysis and biodiagnostics. Indeed, DNA testing is now possible on a single molecule and thousands of diagnostic reactions can be performed at once, thus

allowing a range of characteristics to be rapidly and simultaneously determined. Moreover, current DNA tests are conducted not only in the laboratory or in the clinic: some can also be run at home using portable disposable devices, such as the DNA paternity 'do-it-yourself' kits.

At present, millions of samples from blood, semen, hair and tissues are analyzed annually worldwide with a variety of DNA diagnostics to identify pathogens, diseases or individuals. Currently, DNA testing can acquit the falsely accused (dozens lives of innocent people in the death row were saved by an unambiguous DNA trial), make us familiar with the sex life of live and late presidents (recent cases of Bill Clinton and Thomas Jefferson), specify the identity of Ground Zero samples, answer the questions on how our ancestors spread over the globe from Africa in the past and where American Indians came from long ago, or be used to decide who should pay child support.

Impressive advances of DNA testing let the experts look forward to near instantaneous detecting of pathogens from clinical material for early infection diagnosis. Under the sign of DNA, physicians hope to assess the risks of disease, determine likely responses to medication and ultimately choose the best courses of therapy (personalized medicine). To be more realistic, many of these expectations are still based on promises, though they are quite optimistic ones [16]. Thus, some of new uses in this field could be a decade away and several challenges yet remain in their realization [16–19].

Along with other prospective technological improvements, a wider employment of microelectromechanical systems (MEMS) and MEMS-based microfluidics (lab-on-a-chip) for DNA analysis has to facilitate the fulfillment of these promises [20]. To this end, the large-scale initiatives such as MEMS Project and Advanced Technology Program (ATP), which focus in part on the development of innovative tools for DNA diagnostics and are sponsored by the US National Institute of Standards and Technology (NIST), are very welcomed. All these should significantly stimulate further progression of DNA diagnostics to eventually make them an ordinary implement of our everyday life.

**Table 1. DNA diagnostics timeline: principal developments in the field.**

Date	Development
1953	Discovery of duplex DNA structure and complementarity rules
1957	Phosphonate synthesis of short oligodeoxynucleotides Triple-stranded nucleic acid complexes are found
1958–1959	Discovery and isolation of DNA polymerases
1960s	Early hybridization techniques and inception of electrochemical DNA detection
1965	Solid-phase oligodeoxynucleotide synthesis Enzymatic synthesis of short RNAs
1969	<i>In situ</i> hybridization
1970	Discovery of restriction enzymes and reverse transcriptase
1975	Southern blotting
1977	Methods for DNA sequencing First genome (bacteriophage $\phi$ X174) is sequenced
1983–1985	First syntheses of modified oligonucleotides
1985	Restriction fragment length polymorphism analysis
1985–1986	Invention and first steps of PCR; thermophilic DNA polymerases
1986	Automated DNA sequencing is pioneered Development of FISH method
1987–1988	First triplex DNA probes
1988–1991	Origination of first DNA chip conceptions
1990s	Emergence of microelectromechanical systems and DNA nanotechnology
1991	Design of first DNA/RNA mimics: peptide nucleic acid probes/PNA openers Ligase chain reaction; thermophilic DNA ligases
1992	First methods for whole genome amplification Strand-displacement amplification
1992–1993	Branched DNA (oligonucleotide dendrimers) Real-time PCR
1993	Discovery of structure-specific endonucleases for invasive-cleavage assays
1994	DNA topological labeling (padlock probes)
1995	First bacteria ( <i>Haemophilus influenzae</i> ) is sequenced
1995–1996	Invention of rolling amplification of circular probes
1996	Molecular beacon probes First eukaryote (yeast) is sequenced First applications of DNA chips
1998	Lab-on-a-chip (microfluidics) for DNA analysis
2000	Genomic sequence of fruit fly ( <i>Drosophila melanogaster</i> )
2000–2001	First versions of human genome sequence

**Table 2. Prehistory of the DNA double helix: some scientific landmarks leading to the Watson–Crick discovery.**

Date	Landmark
1865	Crossbreeding of peas (Mendel): the laws of heredity are discovered
1865–1866	Design of early 3D molecular models: 'croquet ball' (Hofmann), 'brass strip' (Dewar) and 'ball-and-stick' (Kekulé) constructs
1868–1872	Discovery of nucleoprotein complex, 'nuclein', and identification of nucleic acids (Miescher)
1889	Preparation of protein-free nucleic acids (Altmann)
1900	Rediscovery of the laws of heredity (Correns, Tschermak and de Vries)
1890s–1940s	Thorough characterization of DNA/RNA chemical composition (Jones, Levene, Hammarsten <i>et al.</i> ) Advanced design of refined 3D models for complex organic molecules <sup>§</sup>
1909	The term 'gene' for the unit of heredity is adopted by geneticists
1912	X-ray crystallography is invented (von Laue and Braggs)
1915	Chromosomal localization of genes (Morgan)
1915–1917	Discovery of bacteriophages (Twort and d'Herelle)
1920	The term 'genome' is coined for the haploid chromosome set to specify the inherited material basis of the species
1930s	First x-ray patterns of biological molecules, including nucleic acid fibres
1930s–1940s	Polymeric nature of genes: giant hereditary molecules (Koltsov) and 1D aperiodic genetic crystal (Schrödinger) <sup>§</sup> . General concept of biomolecular steric complementarity/specificity and notion of bipartite, self-complementary genes as templates for their offspring replicas (Pauling) <sup>§</sup>
1940s	Models of bacteriophage replication (Luria and Delbrück) <sup>§</sup>
1944	DNA is shown to be the hereditary factor (Avery) <sup>§</sup>
1950	The discovery of $\alpha$ -helix in proteins (Pauling and Corey) The Chargaff's rules: $P_u = P_y$ , $A = T$ , $G = C$ <sup>§</sup>
1951–1952	Refined x-ray patterns of DNA fibers (Franklin and Wilkins) <sup>§</sup> Simulated x-ray patterns of a helix (Stokes, Cochran and Crick) <sup>§</sup>
1952	Further proof that genes are made of DNA (Hershey and Chase) <sup>§</sup>

<sup>§</sup>The prior advances, which are most linked to the Watson–Crick discovery.

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