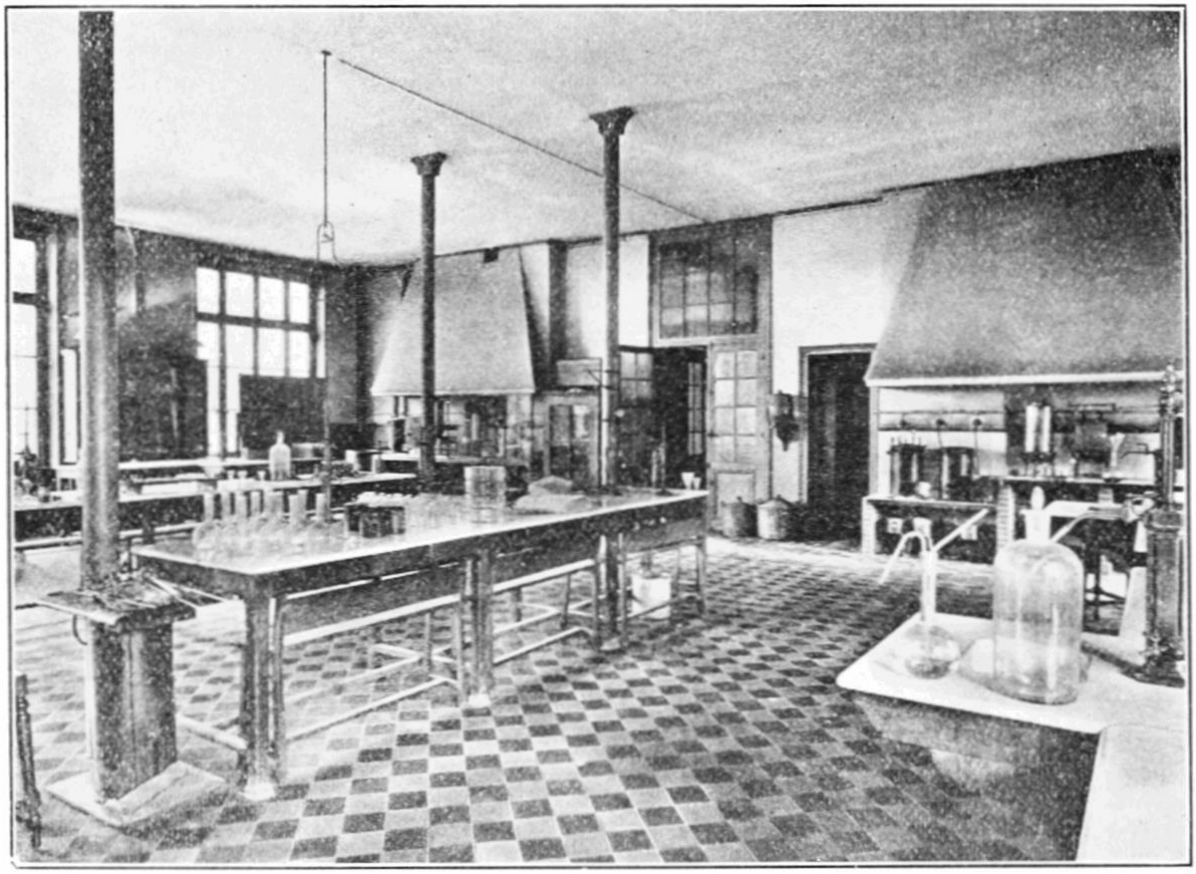
**Pasteur *vs* Possibility – the ever-changing face of the laboratory**

The Pasteur Museum in Paris has preserved the laboratory equipment of Louis Pasteur, one of microbiology's great founders. Antiquated but recognizable devices such as microscopes, Bunsen burners, and a variety of glassware adorned laboratories of the late 19$^{th}$ century. Isolation and identification of novel microbes was based on properties of growth, morphology and behavior rather than their DNA as we group them today.

The unique properties of electricity and its availability would add new devices for more advanced techniques such as centrifugation, chromatography, and electrophoresis. This allowed the separation of specific proteins and nucleic acids, birthing the fields of molecular biology and genetics.



*The laboratory of Dr Émile Roux, protégé of Louis Pasteur, at the Pasteur Institute c. 1908.*

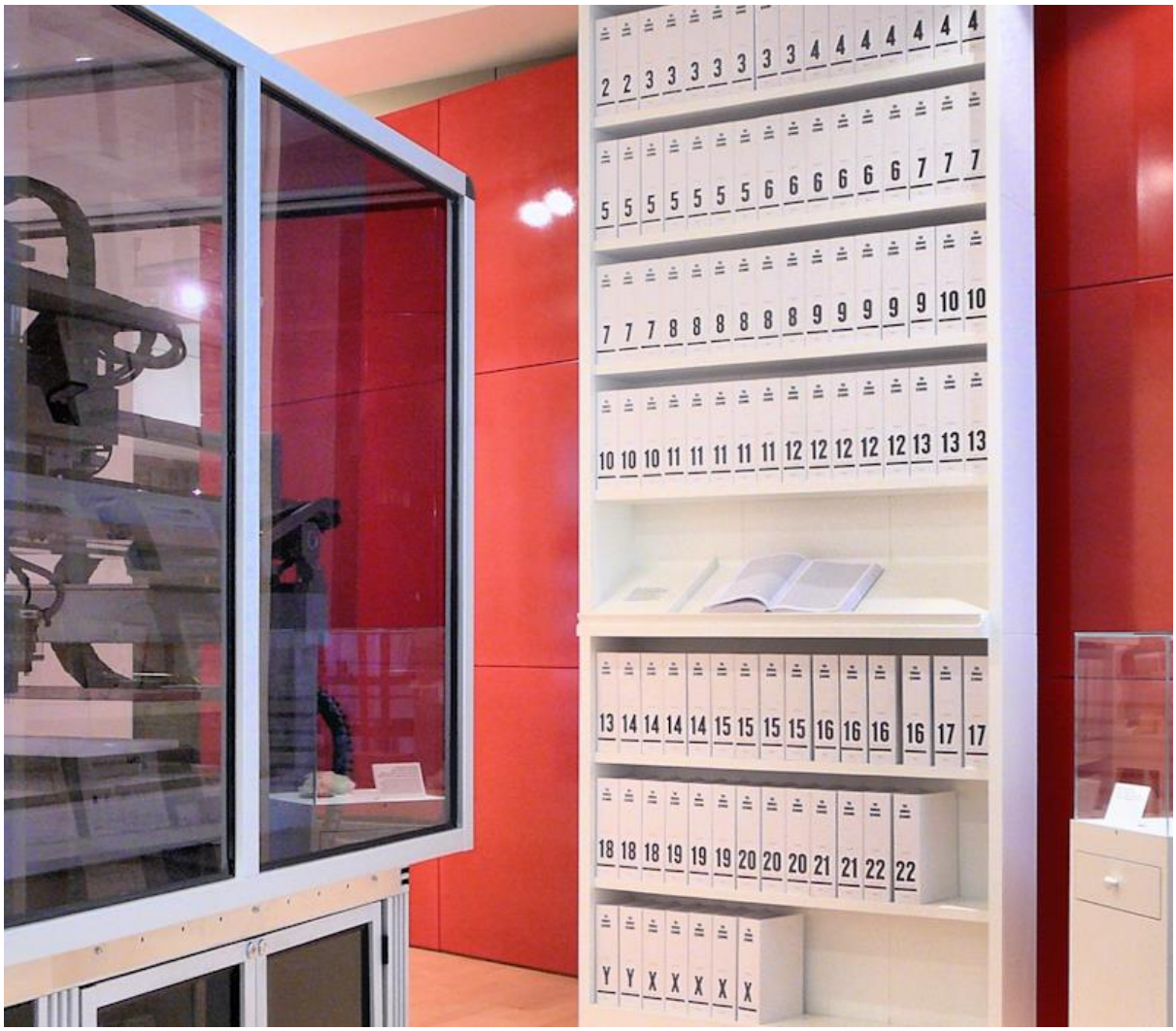
**Technology of yesterday**

The 1970s saw a burst of new technologies. Oligonucleotide synthesis by the phosphoramidite method was established, much as we still use it today. Fred Sanger developed the first reliable method to sequence hundreds of nucleobases, whereby radioactive nucleotides were detected after running through a gel matrix. The 1980s saw the invention of Polymerase Chain Reaction (PCR), a method to amplify DNA using pairs of short oligonucleotide primers bookending the sequence to amplify.

One hundred years on from Pasteur, another revolution was taking place in the lab – High throughput DNA sequencing. The structure and function of the DNA molecule had been elucidated some two decades prior. Isolating the individual nucleic acids and determining the order of their constituent bases proved incredibly difficult compared to isolating proteins and their amino acids. Sequencing technology was, however, advancing slowly.

The Sanger sequencing method adapted new approaches by using fluorescent nucleotides and capillary tubes, which helped automate and miniaturize the process. This made it more affordable and accessible as machines from Applied Biosystems became available. For the first time, whole genome sequences of model organisms like *Saccharomyces cerevisiae*, *Escherichia coli* and *Arabidopsis thaliana* were published and could be used to help us understand more about complex biology and disease.

These technical advances also led to the Human Genome Project (HGP). Completed at a cost of $3 billion dollars after 13 years, the HGP greatly advanced our knowledge of cancer and other genetic diseases, shedding light on the mechanisms behind genetic illnesses such as hemophilia and sickle cell anemia. As the HGP finished, another leap forward in the sequencing field was made: next generation sequencing.



*A printout of the human genome as a series of books on display at the 'Medicine Now' room at the Wellcome Collection, London. Image courtesy of Russ London, CC BY-SA 3.0.*

**Technology of today**

Next generation sequencing (NGS) combines the best techniques of the previous three decades. Where Sanger sequencing evolved into a miniaturized process, NGS took a leap forward. Nucleobases can now be detected in real time as DNA is amplified, vastly speeding up the process compared to the Sanger sequencing method and making the technology more affordable and accessible to research

groups. NGS has transformed the face of laboratories, having a major impact in clinical diagnostics and medical research.

Research and diagnostic labs have also been transformed in just a few decades with newfound reliance on PCR and Real-Time PCR instruments for detection and quantitative analysis of biological samples. In diagnostics especially, DNA amplification and overnight sequencing now plays a huge role in testing clinical samples, identifying bacterial infection, and performing non-invasive tests such as prenatal screening. DNA sequencing and amplification have had a tremendous impact on research and medicine.

**Today's limitations**

Today another transformation is required: a transformation in DNA synthesis. The high cost of equipment and expertise required to produce DNA has led most labs to outsource their DNA synthesis needs.

Unlike our ability to sequence DNA, DNA synthesis has not made the same leaps and bounds forward. The phosphoramidite cycle remains largely unchanged since its first development, remaining a lengthy, error-prone and costly process that most labs need to outsource. A major challenge when synthesizing DNA is preventing the accumulation of random errors: the phosphoramidite method of oligo synthesis has a one in two-hundred error rate. Worse still, these errors cannot be identified until after the synthesis, making it impossible to generate long, accurate DNA sequences.

However, with the advent of synthetic biology, and the increasing demand for high-quality DNA at its core, we need to make that leap in technological advancement.

*A modern molecular biology lab: Research Institute of Molecular Pathology, Vienna. CC BY-SA 4.0.*

**The world of tomorrow**

The realization of any technological advancement boils down to overcoming bottlenecks in the required processes. Sanger sequencing developed slowly through miniaturization and today remains

a staple technology. NGS took a great leap forward in technology, meeting the increasing demand for rapid delivery and affordability. Researchers can even equip and manage NGS devices themselves. The bottleneck remains in DNA synthesis. Like the miniaturization of the Sanger method, the phosphoramidite method has seen marginal improvements, but is still fundamentally unchanged. The next giant leap will come from print-it-yourself DNA synthesis technology.

Evonetix is developing a radically different approach to DNA synthesis that may provide this leap forward – a highly parallel desktop DNA writer to synthesise DNA at high accuracy, scale and speed with proprietary consumable silicon chips, combining CMOS technology (think computer chip) with a micro-electromechanical system. The chips comprise thousands of islands of heat within a continuous flowing liquid acting as "virtual wells" or reaction sites, each with independent temperature control for parallel DNA synthesis.

Thousands of DNA strands can be built within each virtual well under thermal control using proprietary chemistry. Following synthesis, these thousands of DNA strand variants from across the chip can be selectively released from the "virtual wells" and assembled on-chip to produce longer double-stranded DNA molecules. Because the assembly of single-stranded DNA into longer double-stranded DNA is temperature-sensitive, Evonetix's assembly process can eliminate potential errors during assembly. This is achieved by allowing complementary strands to anneal to each other only at the annealing temperature predicted for perfect sequence alignment. Those strands not bound at a specific temperature fail the check and are removed. In this way, thousands of different DNA strands can be automatically assembled in parallel into long accurate double-stranded DNA molecules at unprecedented speed and scale.

This technology has the potential to both miniaturize DNA synthesis and simplify the sequence verification process, with the added benefit of bringing it straight to the lab bench. Ordering primers, codon optimization genes, longer custom DNA constructs and bespoke DNA pool synthesis may soon be as easy as hitting "Print".

**Big Impact**

Genetics is rapidly advancing into everyday use and its biggest impact will be felt in the world of medicine. Personalized medicine is already a reality and will continue to advance. Patients with genetic illnesses and cancer are finding hope with gene and CAR T cell therapy. The medicines of the future are shaping up to be bespoke seamless "living medicines" that work with our bodies against disease. Future clinicians and drug developers will have genetic tools in their arsenal of treatments and will increasingly rely on accurate DNA to supply effective treatments to patients worldwide.

Louis Pasteur may not recognize the lab of today, much less the lab of tomorrow. Maybe it's time to look around our own labs today and see where we can clear a little space on the bench for Evonetix's DNA writer. If yesterday's lab has taught us anything, it's that technology changes fast. We need to be ready for it.