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DNA – From Discovery to Today's Applications

As scientific research advances, there is an increased requirement to reliably gain access to regions of DNA with more complex sequences. It is as important to address this high complexity as it is to address the variation between different PCR primers. Eppendorf has developed the Mastercycler® X50 family with innovative 2D-Gradient technology that combines the optimization of annealing and denaturation temperature – all in a single run. This is the next milestone in PCR optimization.

DNA – fascinating from the start

For nearly 150 years, DNA has fascinated humankind from its very first observation as a component of the cell nucleus to today's advanced understanding of genetics and how it plays a role in every characteristic. The idea of something inherited from generation to generation has been in existence for a long time. Charles Darwin put forward the theory of evolution occurring by the process of natural selection in his book "The Origin of Species" which was published in 1859. Gregor Mendel, the "father of genetics", shed light on the way in which characteristics are passed from generation to generation by interpreting pea breeding experiments. His ideas of inheriting factors were so far ahead of time that it took more than 30 years for his research to be accepted by the scientific community.

It was in the early 20th century when the three plant researchers Hugo De Vries, Carl Erich Correns, and Erich von Tschermark independently verified Mendel's theory. At the same time, Sir Archibald Edward Garrod was the first, who proved the generality of these plant breeding-based findings by associating them with a human disease. It became clear that the applications derived from the understanding of inheritance were going to reach far and wide.

By the 1940's the knowledge of genetics was refined: Mendel's inheritance factors were named "Genes".

In the meantime, it had become clear that genes are the blueprint of proteins. However, nobody had confirmed what this wonderful molecule looked like. Rosalind Franklin produced two photographs of DNA fibers using X-ray diffraction in 1952. Less than a year later, Watson and Crick discovered the double helix structure of DNA and were awarded the prestigious Noble Prize in 1962.

Kary Mullis invented the technique "PCR" (Polymerase Chain Reaction) in 1983 to allow the amplification of specific DNA sequences. PCR became a central technique in years to come. For the PCR breakthrough, Mullis was awarded the Noble Prize in 1993.

PCR: an indispensable tool of molecular biology

PCR facilitates scientists by amplifying specific regions of DNA for subsequent processing, such as sequencing or cloning. Besides the quality of template molecules, the reaction is highly dependent on the parameters of the reaction. There is no universal recipe: Each PCR needs to be optimized for the target DNA to be amplified and the specific primers used in the reaction. This is to ensure the primers not only bind to the DNA – resulting in amplification, but bind in the right position – ensuring "specific amplification". Gradient technology addresses the need to optimize the reaction when using different primers or multiple primers. It allows the empirical determination of the optimum annealing temperature, often in a single run. The gradient technology developed by Eppendorf for PCR optimization has been used by a number of cycler manufacturers. It was developed to address the difficulties with manual optimization of the annealing temperature, usually carried out by multiple PCR runs with varying conditions (see also Fig. 1).

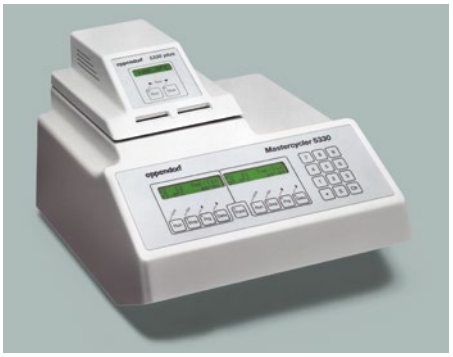

Fig. 1: Mastercycler 5330, one of the first Eppendorf thermal cyclers with gradient technology (1997)

Eppendorf is seeing this trend emerge amongst scientists once again, but this time in relation to the complexities within the template DNA.

How things have changed

In the 1980's, the first use of DNA in a criminal case was noted – at this point the status quo of knowledge and technology only allowed to differentiate very basic and specific repeat sequences. The translated characteristics were unimportant for this purpose, differentiation was the focus.

Fig. 2: Mastercycler X50 with 2D-Gradient technology: the next stage of PCR optimization

In contrast, medical research, plant science, screening of diseases, food science, and other recent scientific focus areas have advanced greatly. There is now a need to understand the impact of the differences that originate within the sequence of DNA. This is a paradigm shift in the requirements of the scientists. It is now vitally important to ensure access to the target region of DNA, irrespective of complexities within the sequence, to enable specific amplification resulting in high yield for subsequent applications, such as Next Generation Sequencing.

Fig. 3: Eppendorf's new 2D-Gradient allows optimization of the annealing and the denaturation temperature in a single run – taking less time than ever before.

As our knowledge of DNA has advanced, so have the complexities of the target regions we are interested in. The consequences of not addressing complex structures can result in little to no amplification. While GC-rich is the simplest of complexities, many more complexities have been identified. These may require more energy, meaning an increased denaturation temperature, to ensure access to the target region. However, consideration needs to be given to the enzymes, because an increased denaturation temperature addressing the DNA also results in an increased rate of polymerase denaturation. After all, it's in the same reaction.

Today, scientists optimize the denaturation temperature in a familiar manual way: by carrying out multiple experiments under varying conditions and comparing the results to find the best parameters. This can now be addressed in a single PCR run. Scientists can today optimize the denaturation and annealing temperature in a single reaction, using the new 2D-Gradient functionality on the Eppendorf Mastercycler X50 family (Fig. 2 and 3). For the first time, scientists don't need to choose between yield or specificity, they can have both – in a single run.

Tip: Please also read the Application Note 3–4 "Ultimate PCR Optimization with the Eppendorf Mastercycler® X50 2D-Gradient" in this issue.

More info at
www.eppendorf.com/mastercycler

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News

New: Innova® S44i

Innova S44i biological shaker from Eppendorf is designed for laboratories that will settle for nothing but the best. This innovative new shaker is equipped with maximal platform capacity at a small footprint. Sample capacity can be further increased without sacrificing lab space by stacking up to three shakers.

The proprietary technology of Eppendorf X-Drive allows reproducible shaking of both uneven and heavy loads without compromising performance or longevity of the shaker.

The slide-out platform of the Innova S44i enables easy access to all samples on the platform. Comprehensive multi-step programming is available for automated control of the shaker to save time. Built-in user management allows controlled access, operation, and traceability required in regulated laboratories. This new shaker can be integrated into a central monitoring and data management software using the VisioNize® system from Eppendorf (see page 7). The versatile Innova S44i comes in six different formats and is suitable for culturing microbial and phototrophic organisms.

More information at
www.eppendorf.com/InnovaS44i

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