THE ADVANTAGES OF DROPLET DIGITAL POLYMERASE CHAIN REACTION (ddPCR)

What is PCR?

Polymerase chain reaction (PCR) is a biochemical method enabling to produce millions to billions of copies of a specific DNA sequence from a sample that contains very small amounts of that DNA.[1] It basically allows to amplify a small DNA sample from biological materials, by means of an enzyme: polymerase. This technique is an enzymatic reaction based on the repetition of several temperature cycles, called PCR cycles.

What is a PCR cycle?

A PCR cycle is divided in 3 different steps: denaturation, annealing and elongation (Figure 1) that all occur in a reaction media, also called PCR mix. This PCR mix[2] includes different elements, such as:

-DNA: The DNA is extracted from a biological sample of interest and contains the DNA sequence to amplify.

-Two DNA primers: Primers are short DNA fragments capable of hybridizing specifically with one DNA strand, due to complementary bases. They are starting points for DNA synthesis.

-Taq polymerase: It is an enzyme able to synthesize a new strand of DNA from the template DNA strand after attaching itself to a primer.

-Deoxynucleotide triphosphates (dNTPs): dNTPs are monomeric units of DNA and refer to four nucleotides (dATP, dGTP, dTTP and dCTP). They are used by the Taq polymerase to synthesize the new DNA strand.

-Buffer and magnesium ions: They define a medium with optimal pH and salt concentration for the enzyme proper functioning.

The first step, namely DNA denaturation, consists in breaking hydrogen bonds between complementary bases at temperatures between 94 and 98°C and leads to 2 single-stranded DNA molecules. The second step, called annealing, is the hybridization of DNA primers on single-stranded DNA at temperatures between 50 and 68°C. Finally, in the elongation step, DNA strands are synthesized from primers by the polymerase enzyme at 72°C. By adding complementary nucleotides to the targeted sequence, polymerase synthesizes a new complementary strand.

By repeating this PCR cycle 20 to 30 times, millions to billions of copies of this specific DNA sequence can be produced (Figure 2).[3] Due to this exponential amplification, even a single DNA molecule can be detected, making PCR a very powerful technique getting rid of the amount of sampled DNA limitation.

PCR enables to amplify not only DNA but also RNA by Reverse Transcription Polymerase Chain Reaction (RT-PCR). In this technique, RNA is converted into complementary DNA (cDNA) using an enzyme called reverse transcriptase. The resulting cDNA is then amplified through PCR.

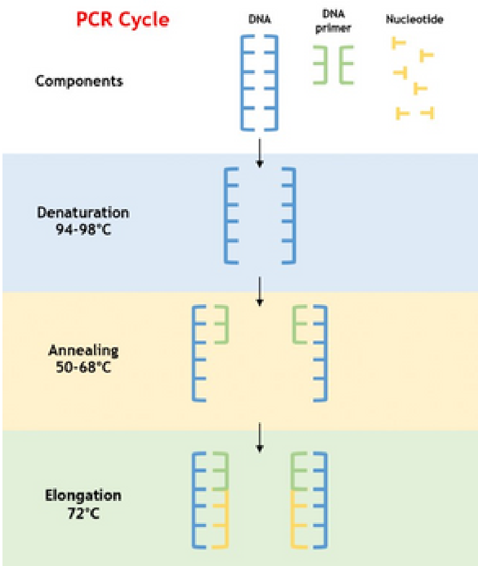


Figure 1: The different steps of a PCR cycle[2]

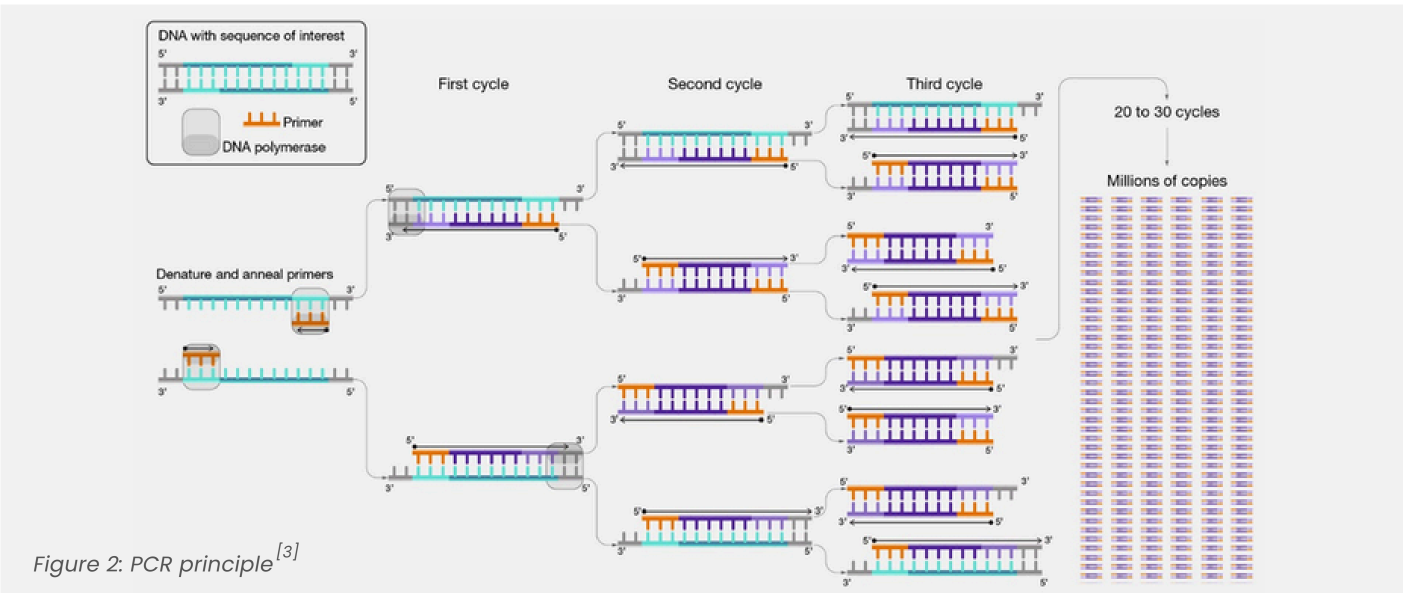
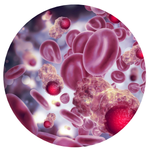


Figure 2: PCR principle[3]

What are the main applications of PCR?

PCR technology has become a cornerstone in various scientific fields, enabling significant advancements in diagnostics, forensics, agriculture, and biotechnology. The primary applications of PCR are the following:



Medical diagnostics
PCR is extensively utilized in clinical settings for the detection of infectious diseases and genetic disorders. It enables rapid identification of pathogens, including bacteria and viruses, which is crucial for early diagnosis and treatment. For instance, PCR tests have been pivotal during outbreaks such as COVID-19, where they detect the presence of SARS-CoV-2 in patient samples.[4], [5] Additionally, PCR is employed to identify genetic mutations associated with various cancers, allowing for personalized treatment plans based on an individual's genetic makeup. [6]

Forensic science
In forensic investigations, PCR plays a critical role in DNA profiling. It allows forensic scientists to amplify minute quantities of DNA extracted from crime scenes, facilitating the identification of suspects or victims through genetic comparison.[5] This application is particularly valuable in cold cases where evidence may be decades old, enabling re-examination with modern techniques. Furthermore, PCR is used in paternity testing and other legal contexts where genetic verification is required.[4]

Agriculture and food industry
PCR technology is also significant in agriculture, where it aids in the detection of genetically modified organisms (GMOs) and plant pathogens. It allows for the rapid screening of crops for resistance to diseases and pests, thereby enhancing food security.[4], [6] Additionally, PCR assists in plant breeding by enabling the genotyping of plants to select desirable traits more efficiently.

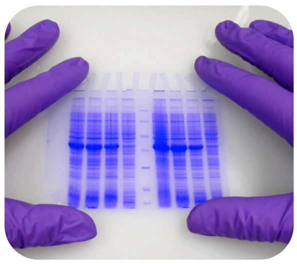
Genetic Research
In research laboratories, PCR is fundamental for cloning DNA sequences, studying gene expression, and conducting mutagenesis experiments. It facilitates the analysis of genetic variations, and the construction of phylogenetic trees based on DNA sequences.[5] Moreover, quantitative PCR (qPCR) provides insights into gene expression levels across different conditions or treatments.

Environmental Monitoring
PCR is increasingly used for environmental applications such as monitoring biodiversity and detecting pathogens in water sources. This capability is vital for assessing ecosystem health and managing resources effectively.[6], [7]

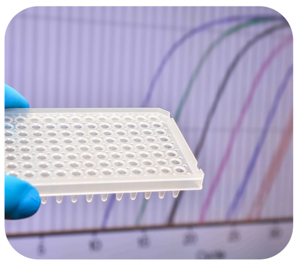
These applications illustrate the broad impact of PCR technology across multiple disciplines, enhancing our ability to diagnose diseases, ensure food safety, and advance scientific research.

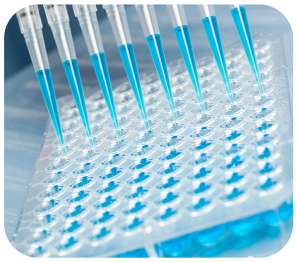
A wide variety of PCR methods

There are many different PCR methods, each with its own advantages and limitations. The most basic PCR method is **standard or conventional PCR**. This method is based on a post-PCR step for detection and visualization of amplified DNA and provides qualitative or semi-quantitative results. This visualization can be done through agarose gel electrophoresis or polyacrylamide gel electrophoresis (PAGE). Even though it is easily accessible in term of research facilities and pretty low cost, this technique is also limited by its low sensitivity, its time cost and its non-quantitative results.



Another common method is **real-time PCR or quantitative PCR (qPCR)**. Compared to conventional PCR, it has multiple advantages including quantitative results. By comparing samples to a standard curve of known amounts of DNA, starting quantities of a targeted sequence can be determined. Another benefit of this technique is its specificity, due to the use of specific DNA probes and/or melt curve analysis after PCR reaction. Moreover, this method enables the real time detection of the PCR product and so does not require an agarose gel for the visualization of DNA after PCR. However, it has several limitations like its cost, the necessity of specialized thermocyclers and a limited precision in the quantification of starting amounts of the targeted sequences. Real-time PCR is suitable for routine quantification applications, but for applications requiring higher performances, droplet digital polymerase chain reaction (**dPCR**) stands out as the method for absolute quantification of targeted sequences.[8]





In dPCR, the sample is divided into thousands of separate partitions, each partition acting like an individual microreactor for PCR reaction and where amplified sequences are detected by means of fluorescence.[9] Depending on the presence or absence of the sequence of interest, each partition produces a positive or negative signal. The concentration of the targeted DNA sequence can be determined without calibration based on the proportion of PCR-positive partitions. dPCR provides precise and accurate results with high sensitivity and absolute quantification. Due to smaller sample requirement, droplet digital PCR differs from others dPCR techniques, allowing cost reduction and preservation of valuable samples.

What is droplet digital PCR ?

Droplet digital PCR, or **ddPCR**, is a digital PCR method implying a water-oil emulsion, where the partitions are tiny aqueous droplets dispersed in oil. The sample is divided into thousands to millions nanoliter-sized droplets and PCR reaction occurs inside each droplet exactly like in test tubes or microwells. Key aspects of this technique lie in the fact that the sample undergoes a massive partitioning, and in the very small size of these partitions. **ddPCR** method involves 4 different and consecutive steps described in Figure 3: [10], [11]

1. Droplet generation: Once the DNA (or RNA) sample has been mixed with the PCR reagents, including primers and fluorescent probes, the mixture is divided into thousands to millions nanoliter-sized water-in-oil droplets using microfluidics and specialized surfactants.
2. PCR amplification: Each droplet undergoes PCR amplification independently, essentially becoming a mini test tube.
3. Droplet reading: After amplification, droplets are analyzed one-by-one using a droplet reader. Fluorescence in each droplet is measured to determine if target DNA is present (positive) or absent (negative).
4. Data analysis: The ratio of positive to negative droplets is used to calculate the absolute quantity of target DNA in the original sample using Poisson statistics.

This partitioning of the sample allows for high precision and sensitivity, allowing the highly accurate quantification of biomarkers of interest[4] and making **ddPCR** ideal for applications such as rare mutation detection, gene expression analysis, and copy number variation studies.

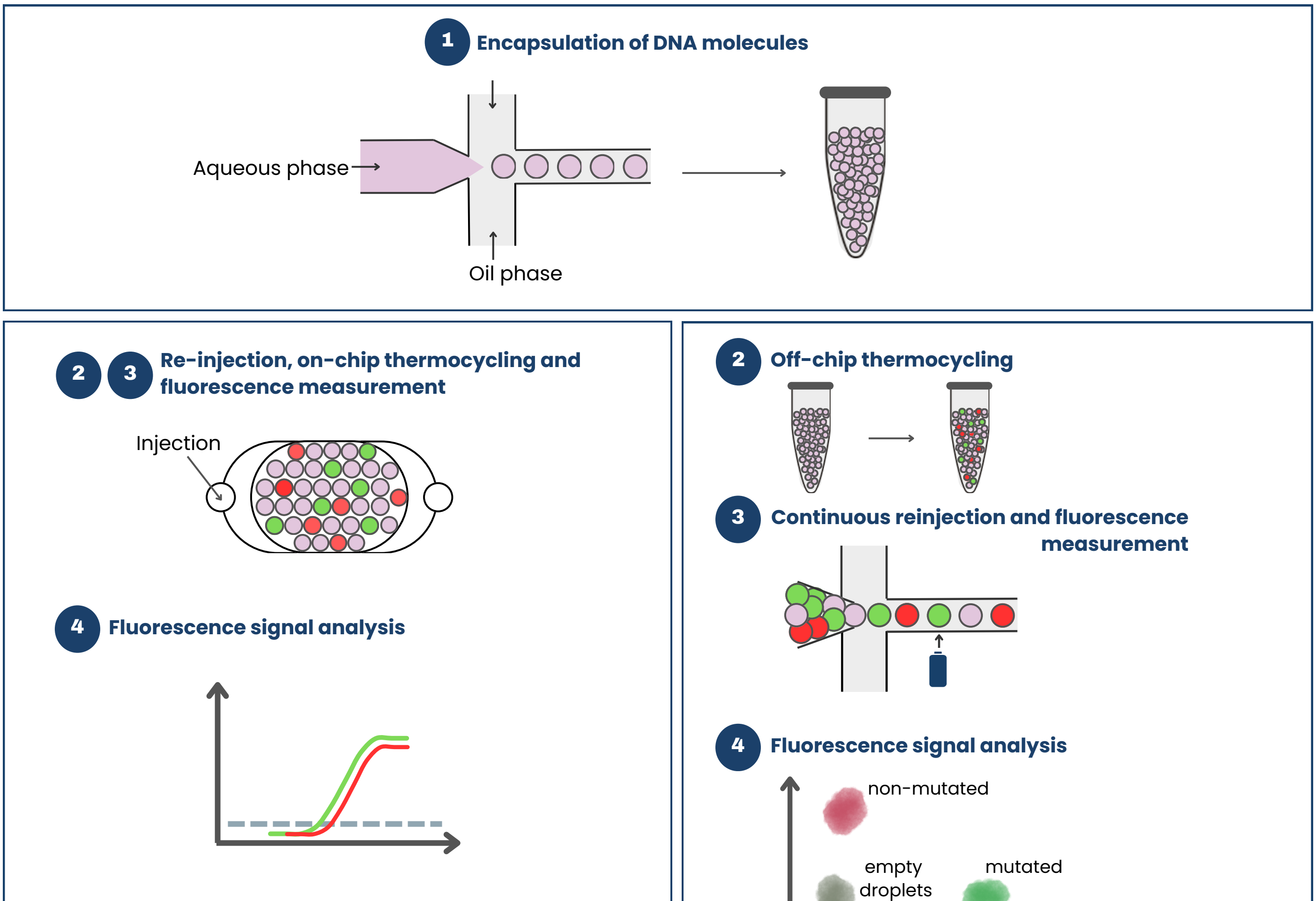
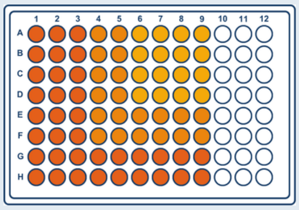
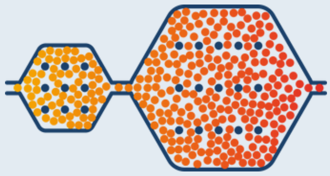


Figure 3: ddPCR workflow [12]

Benefits of ddPCR Technology

ddPCR stands at the forefront of molecular quantification technologies, offering a suite of compelling advantages that set it apart from earlier methods. The comparative analysis below highlights the progression from conventional PCR to the cutting-edge ddPCR.[13], [14], [15]

Conventional PCR	qPCR	dPCR
• Semi-quantitative	• Relatively quantitative • Need for standard curves or references	• Absolute quantification • No need for standard curves or references • High sensitivity due to sample partitioning • High precision

Other dPCR technologies	ddPCR
	
• Not scalable • Serial dilution of the sample / experimental error risk	• Superior partitioning and analysis accuracy • High precision and sensitivity • Rapid detection • Reduced quantity of reagents, cost and time

This comparison clearly demonstrates the superiority of **ddPCR** in terms of precision and sensitivity. By offering absolute quantification without the need for standard curves and providing reduced analysis cost and time, **ddPCR** emerges as the most advanced and reliable method for nucleic acid analysis across a wide range of applications.

Emulseo products for ddPCR

Emulseo is a French company offering cutting-edge products that revolutionize **ddPCR** experiments. Our flagship product line, FluoSurf™, includes high-performance fluorinated surfactants specifically designed to stabilize aqueous droplets in fluorinated oils, crucial components of **ddPCR** technology.[16]
FluoSurf surfactants, including FluoSurf-C™, FluoSurf-O™, and FluoSurf-S™, are biocompatible and optimized for droplet-based microfluidic experiments like **ddPCR**. Our surfactants are specifically compatible with the fluorinated oils in our range (Fluo-Oil). In particular, we have integrated Syensqo's Galden® PFPE into our Fluo-Oil range to enhance the performance and reliability of our solutions. These surfactant/oil formulations excel in several key areas like:

Unparalleled droplet stability
FluoSurf-based products enable the stabilization of droplets ranging from 1 to 300 µm, maintaining their integrity even during high-frequency generation and heating cycles. This exceptional stability is crucial for accurate and reliable **ddPCR** results, especially during thermocycling processes. FluoSurf-S™ is especially efficient in stabilizing droplets under extreme thermocycling conditions (40 heating cycles for example).

Rigorous quality control and purity
At Emulseo, we are committed to delivering products of the highest quality. Each batch of FluoSurf undergoes strict quality control specifications, ensuring structural integrity and performance consistency. Our well-established post-processing techniques remove impurities, resulting in surfactants of exceptional purity.

Enhanced experimental control and reproducibility
FluoSurf-based formulations enable minimizing leakage-related issues. This control is vital for precise quantification in **ddPCR** experiments, particularly when dealing with rare DNA target copies or gene expression analysis. The reproducibility of our surfactants ensures consistent results across experiments, a critical factor in research and diagnostic applications.

High resolution results
FluoSurf-O™, a key product in Emulseo's surfactant line, offers exceptional performance for **ddPCR** applications due to its ultra-low autofluorescence properties. This unique characteristic makes FluoSurf-O™ particularly efficient for detecting fluorescent dyes, even at low concentrations. The reduced background noise significantly enhances the signal-to-noise ratio, leading to improved resolution and sensitivity in **ddPCR** experiments. By minimizing interference from autofluorescence, FluoSurf-O™ enables clearer distinction between positive and negative droplets, thereby enhancing the overall quality and reliability of **ddPCR** results.

Versatility and biocompatibility
Our products have been tested with various biological entities, including plankton, yeast, E. coli, and mammalian cells, demonstrating their broad applicability in life science research. This versatility makes FluoSurf-based formulations an ideal choice for a wide range of **ddPCR** applications, from cancer diagnostics to viral and bacterial studies.

By choosing Emulseo's products for your ddPCR experiments, you benefit from unparalleled droplet stability, exceptional reproducibility and superior experimental control. These advantages translate into more reliable results, increased sensitivity in detecting rare DNA targets, and improved accuracy in gene expression analysis. For scientists seeking to push the boundaries of digital PCR technology, Emulseo's formulations are an indispensable tool in achieving groundbreaking research outcomes.

If you are interested in our products for your experiments or testing, please feel free to reach out to our experts at sales@emulseo.com. At Emulseo, we pride ourselves on offering solutions tailored to your projects. We are here to support you: discover how we can help you achieve your goals.

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