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# Real-Time Polymerase Chain Reaction (PCR) In The Microbiology Laboratory

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## ABSTRACT

Use of PCR techniques in the field of Microbiological, diagnostics and Forensic Science has increased to the point where it is now accepted as the standard method for detecting nucleic acids from a number of sample and microbial types.In the scientific laboratory, traditional PCR was already a necessary instrument, though. Due to its increased speed, sensitivity, and reproducibility as well as its reduced danger of carryover contamination, real-time PCR has facilitated the wider use of PCR. During real-time PCR, a growing variety of chemistries are employed to detect PCR products as they build within a closed reaction vessel. PCR is being used in two key areas in bioscience: high-throughput PCR systems and microfluidics-based PCR devices for point-of-care (POC) applications. We also address the commercialization of these techniques before concluding with an examination of their modifications and application in novel areas of biomedicine.. For example, real-time reverse transcription PCR is the gold standard for SARS-CoV-2 diagnoses. The gold standard for SARS-CoV-2 diagnosis is real-time reverse transcription PCR. The dynamic range of initial target molecule determination in real-time PCR is very large (at least five orders of magnitude). Real-time quantitative PCR is more accurate and requires less labor than traditional quantitative PCR procedures.

**Keywords**:Microbiological,Diagnostic,Forensic,RealtimePCR,contamination,poc,biomedicine,labor intensive, Transcription, Commercialization

## INTRODUCTION

Diagnostic microbiology is in the midst of a new era. Rapid nucleic acid amplification and detection technologies are quickly displacing the traditional assays based on pathogen phenotype rather than genotype. The polymerase chain reaction (PCR) [1, 2] has increasingly been described as the latest gold standard for

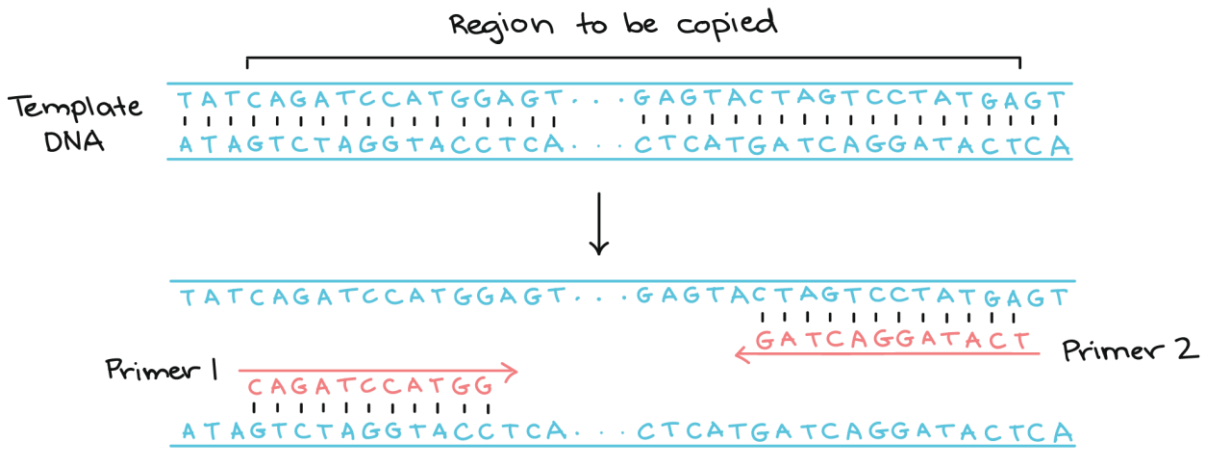
detecting some microbes, but such claims can only be taken seriously when each newly described assay is suitably compared to its characterised predecessors [3, 4, 5, 6, 7, 8, 9]. The PCR process can be divided into three cycles. First, double-stranded DNA (dsDNA) is separated at temperatures above 90 °C this process called Denaturation. Second, oligonucleotide primers generally annealing at 50–60 °C, and, finally, optimal primer extension occurs at 70–78 °C. The temperature at which the primer anneals is usually referred to as the $T_M$. This is the temperature at which 50% of the oligonucleotide target duplexes have formed.The oligonucleotide in real-time PCR could be a primer or a labeled probe.. The $T_M$ differs from the denaturation temperature ($T_D$), which refers to the $T_M$ as it applies to the melting of dsDNA. Third DNA synthesis to form the more copies of DNA.These can be limited by poor sensitivity, slow-growing or poorly viable organisms,narrowdetection,windows,complex,interpretation, immunosuppression, antimicrobial therapy, high levels of background and non-specific cross-reactions [11, 12]. Nonetheless, microbial culture produces valuable epidemiological data, revealing new, uncharacterised or atypical microbes and yielding intact or infectious organisms for further study [13].

## Taq polymerase

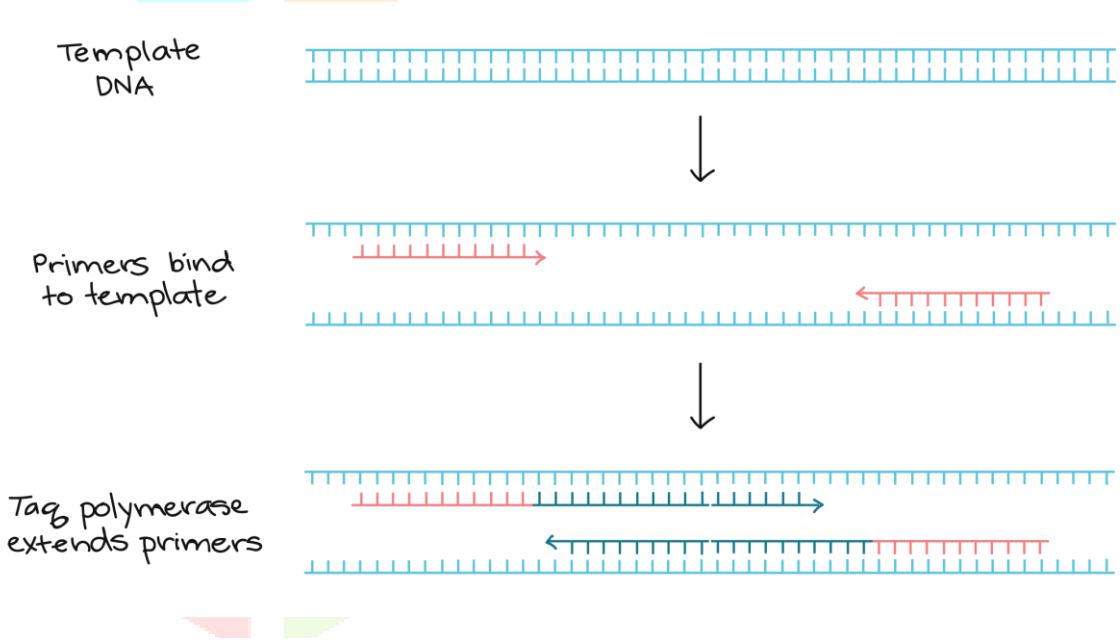
DNA replication in an organism, PCR requires a DNA polymerase enzyme that makes new strands of DNA, using existing strands as templates. The DNA polymerase typically used in PCR is called ***Taq* polymerase**, after the heat-tolerant bacterium from which it was isolated (***T**hermus **aq**uaticus*).*T.aquaticus* lives in hot springs and hydrothermal vents. Its DNA polymerase is very heat-stable and is most active around (a temperature at which a human or *E. coli* DNA polymerase would be nonfunctional). The Taq polymerase's thermal stability makes it perfect for PCR. As we'll see, PCR frequently uses high temperatures to denature, or separate the strands of, template DNA.

## PCR primers

DNA polymerases, *Taq* polymerase can only make DNA if it's given a **primer**, a short sequence of nucleotides that provides a starting point for DNA synthesis. The experimenter selects the section of DNA that will be amplified, or copied, by the primers used in a PCR reaction.PCR primers are brief, single-stranded DNA segments that typically have a length of several nucleotides. Each PCR reaction has two primers, which are positioned to flank the target region (the area that needs to be replicated). In other words, they are provided with sequences that, at the very borders of the region that needs to be duplicated, will cause them to attach to opposite strands of the template DNA. By matching bases that are complementary, the primers attach to the template.




Template DNA: 5' TATCAGATCCATGGAGT...GAGTACTAGTCCTATGAGT 3' 3' ATAGTCTAGGTACCTCA...CTCATGATCAGGATACTCA 5'Primer 1: 5' CAGATCCATG 3' Primer 2:When the primers are bound to the template, they can be extended by the polymerase, and the region that lies between them will get copied.



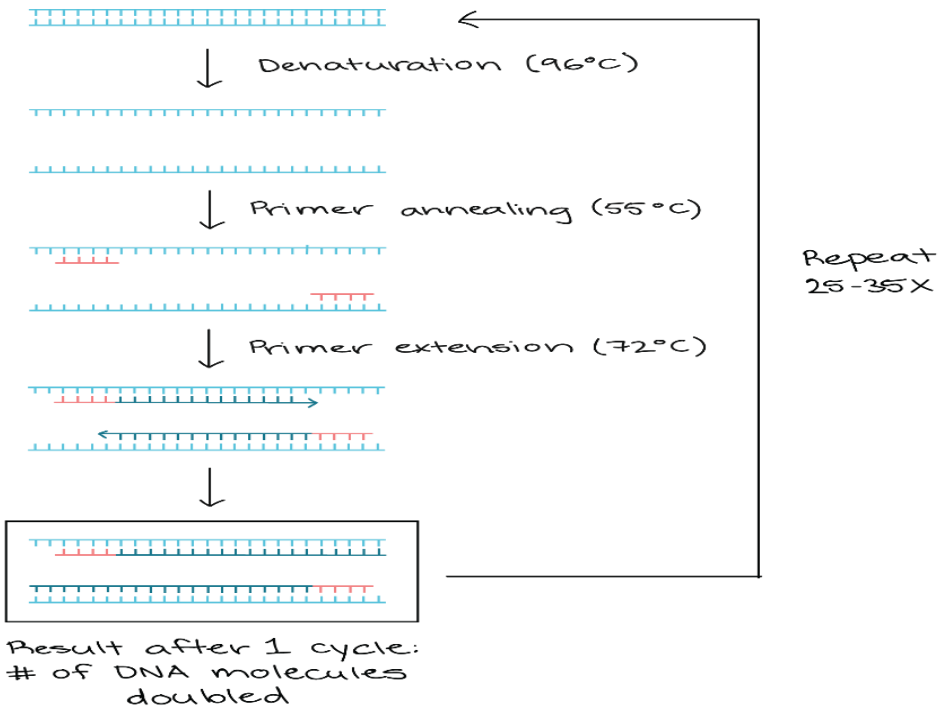

The diagram illustrates the polymerase chain reaction's three phases. The initial phase consists of two blue lines that are parallel to one another. Along the length of each line are small, uniformly spaced vertical lines that face one another to form a structure that resembles a ladder. Template DNA is the label on the picture.. The second image, where the label primers connect to the template, is shown by an arrow. The top blue line on the left has a red horizontal line with an arrow pointing to the right, and 10 small vertical lines that match up with 10 vertical lines from the top template strand. These lines divide the two parallel blue lines. An arrow pointing left and a red horizontal line can be seen on the bottom blue line on the right. Ten tiny vertical lines from the bottom template strand are aligned with ten vertical lines on the red line. The diagram's Taq polymerase extends primers image is indicated with an arrow pointing from the second to the third image. A gray horizontal line with fifteen vertical lines, an arrow pointing to the right, and a red horizontal line with ten vertical lines are united on the left side of the top template strand. The vertical lines on the top template strand and the red and gray line are in line with each other. A red horizontal line with ten vertical lines connected to

a gray horizontal line with fifteen vertical lines and a left-pointing arrow can be seen on the right side of the bottom template strand. The vertical lines on the bottom template strand and the red and gray line are in alignment with each other.

## STEPS OF PCR:

Taq polymerase, primers, template DNA, and nucleotides (DNA building blocks) are the essential components of a PCR reaction. DNA is manufactured by assembling the components in a tube with cofactors required by the enzyme and subjecting them to repeated cycles of heating and cooling.The basic steps are:

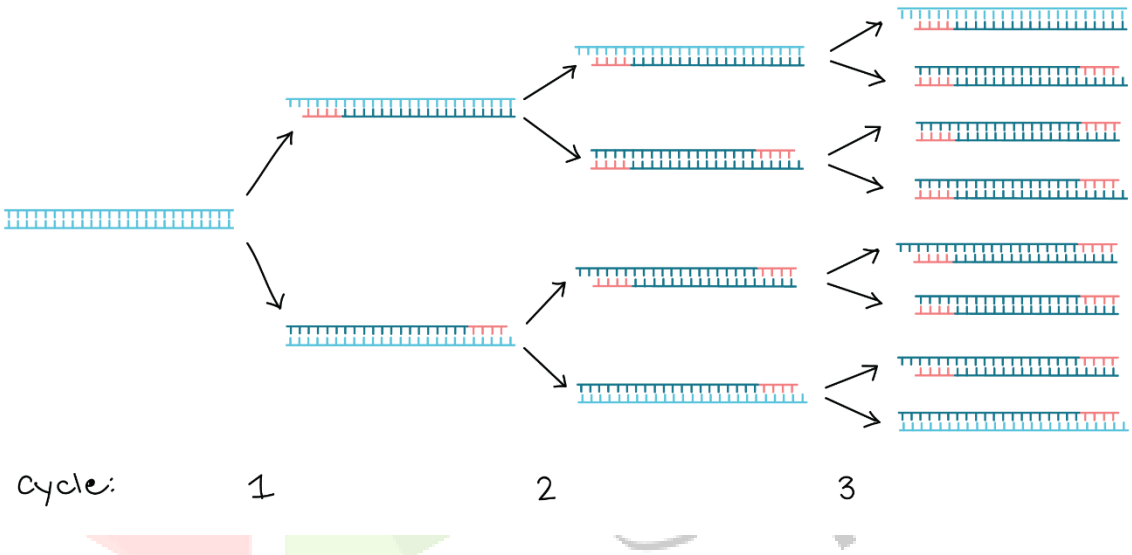
1. **Denaturation** : Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.(94-98°C)
2. **Annealing** : Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.( 45-60°C)
3. **Extension** : Raise the reaction temperatures so *Taq* polymerase extends the primers, synthesizing new strands of DNA.( 72°C)




The five steps of the polymerase chain reaction are depicted in a diagram. The first phase involves showing two blue parallel lines at the top of the diagram, joined by tiny vertical lines that branch off of each horizontal line. An arrow labelled "denaturation," at 96 degrees Celsius, indicates the location of the two blue lines that are parallel to one another and indicate the second stage. The two parallel blue lines in the third phase are indicated by an arrow that reads "primer annealing," 55 degrees Celsius, with a small red line connected to each line. Two red lines are joined to the blue lines: one on the left side of the top line and one on the right side of the bottom line. The two parallel blue lines with a short red line connected to each and a larger gray line extending from the red line are indicated by an arrow labeled "primer extension, 72 degrees Celsius" in the fourth phase. There are red and gray lines with an arrow going from the gray line to the right on the top

blue line and a red and gray line pointing to the left on the bottom blue line..An arrow from this image leads to a box with two ladder-shaped constructions in the fifth step. The top structure features a blue line connected to a small red and gray line, while the bottom structure features a gray line, small red line, and blue line connected to a gray line on top and a blue line on bottom. The structures are located in a box labeled "Result after 1 cycle: doubled number of DNA molecules." An arrow with the description "Repeat 25 to 35 times" points upward and backward from the box to the original blue ladder construction in the first step.

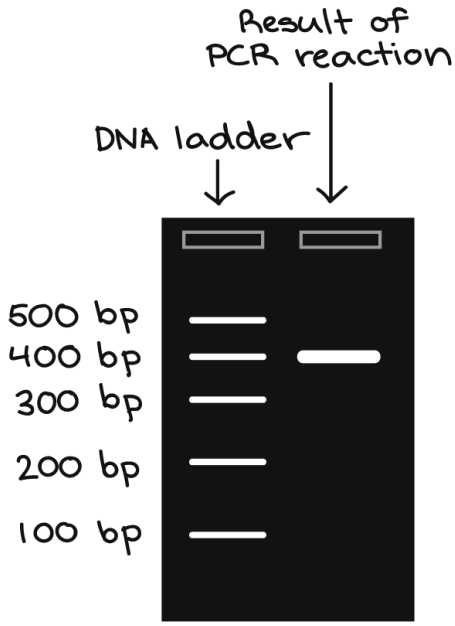
In a standard PCR reaction, which typically takes two to four hours, depending on the length of the DNA area being copied, this cycle is repeated twenty-five times. The target region can grow from one or a few copies to billions if the reaction is effective.That's because the original DNA isn't always employed as a template. Instead, the new DNA created in one round can be used as a template in the next round of DNA synthesis. Because there are numerous copies of the primers and Taq polymerase molecules floating around in the process, the quantity of DNA molecules can roughly double with each round of cycling. The picture below illustrates this exponential growth trend.




The figure depicts the three-cycle process of DNA amplification using polymerase chain reaction. Cycle 1 is an illustration of a ladder with two arrows heading to two new buildings. In cycle 2, both ladders in cycle 1 have two arrows leading to two new ladder images, for a total of four ladder images identified. Each cycle 2 ladder features two arrows heading to two fresh ladder pictures, all labeled cycle 3.After cycle 3, there are eight ladders in all.

## RESULT

The results of a PCR reaction are usually visualized (made visible) using gel electrophoresis. Gel electrophoresis is a technique that separates DNA fragments based on size by pulling DNA fragments through a gel matrix with an electric current. A standard, or DNA ladder, is usually provided to determine the size of the fragments in the PCR sample.DNA fragments of the same length form a "band" on the gel, which can be seen by eye if the gel is stained with a DNA-binding dye. For example, a PCR reaction producing a base pair (bp) fragment would look like this on a gel:

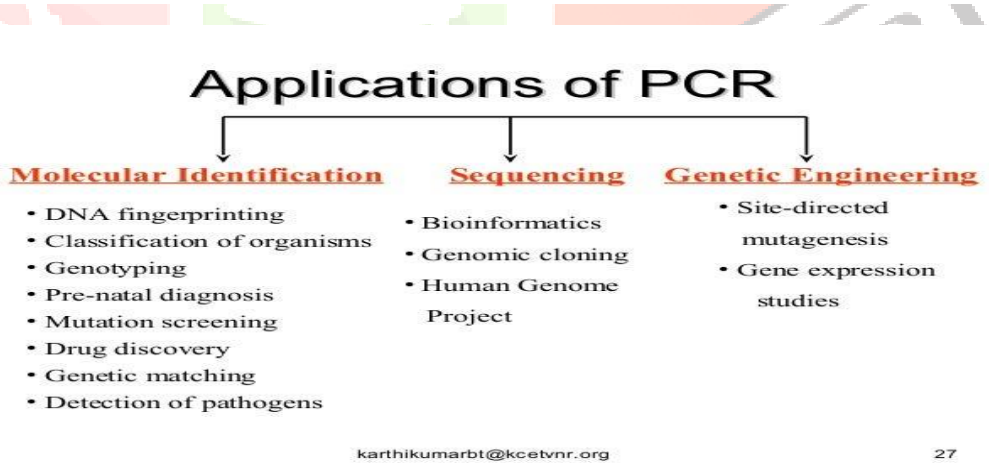



DNA ladder with 100, 200, 300, 400, 500 bp bands.:Left Lane

Result of PCR reaction, a band at 400 bp:Right lane

The target DNA region is present in a DNA band in many, many copies rather than one or a small number of copies. Since DNA is so small, it needs to be present in large quantities in order for us to perceive it with the naked eye. The fact that PCR generates enough copies of a DNA sequence for us to examine or work with that particular DNA region is a major factor in its usefulness.

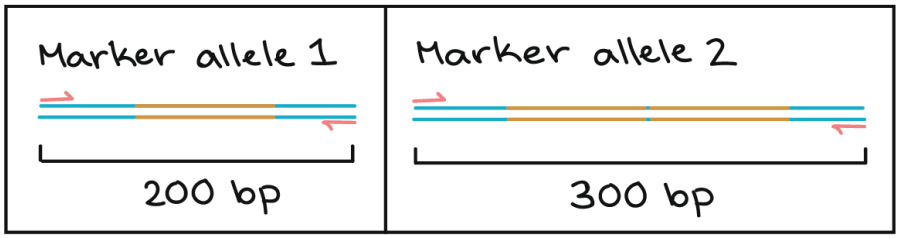
## Applications of PCR :



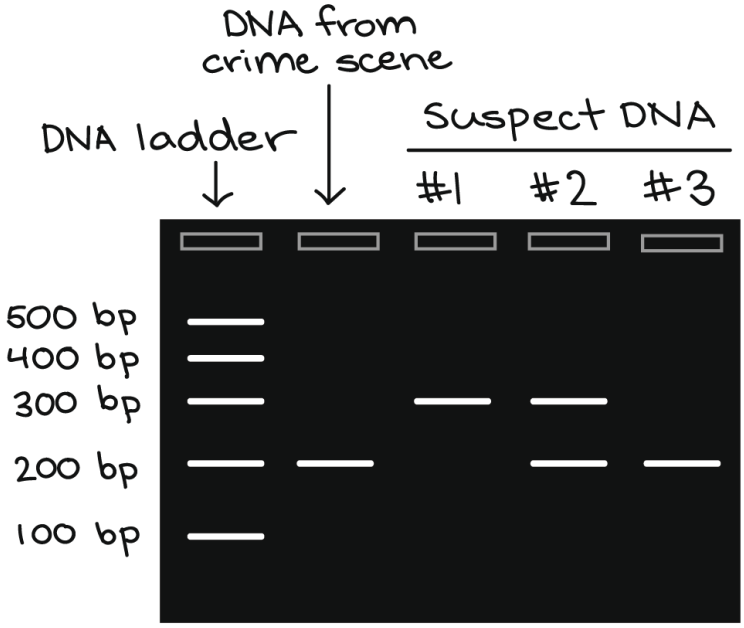

# PCR in forensics: Sample problem

Let's say you are employed at a forensics lab. Three potential suspects' samples of DNA and a hair sample from the crime scene have just been delivered to you.. It is our responsibility to look at a certain genetic marker and determine which of the three suspects has hair DNA that matches this marker.There are two alleles, or variants, of the marker. The brown area below represents the single repeat in one, and the two copies of the

repeat in the other. In a PCR reaction with primers that flank the repeat region, the first allele produces a  DNA fragment, while the second produces  DNA fragment:




Marker allele 1: A 200 bp DNA fragment is amplified using primers surrounding the repetition region.Marker allele 2: primers flanking repeat region amplify a 300 bp fragment of DNAYou perform PCR on the four DNA samples and visualize the results by gel electrophoresis.Shows




**MICROBIAL GENOTYPING**

Although nucleotide sequencing is still the gold standard for characterising unknown nucleic acids, it is a relatively lengthy process. This shortcoming has been somewhat overcome by the advent of real-time PCR, which offers a technique for routinely detecting characterized mutations, insertions, or deletions.The majority of fluorescent chemicals employed in real-time PCR do not produce a signal by a destructive process. Thus, after the PCR is finished, they might be able to carry out a genotyping function.HyBeacons, double-stranded, and light-up oligoprobes, as well as SYBR green and HybProbe, are the most often used chemistries for these kinds of investigations. In order to distinguish between the wild-type and modified sequences, alternative chemistries, such as the TaqMan and Eclipse oligoprobes and hairpin oligonucleotides, use two sets of oligoprobes to discriminate these nucleotide alterations.While this is a perfectly legitimate and practical real-time PCR genotyping approach, the added fluorogenic oligonucleotides increase the overall cost of the assay. Furthermore, the number of diverse microorganisms that may be recognized using multiplex real-time PCR is reduced since two fluorophores need to be allocated to each germ. Less destabilization of the duplex occurs when an equivalent mismatch between the target and a linear oligoprobe is introduced than when a hairpin

oligonucleotide and its target mismatch. This is so because an extremely stable alternate configuration is offered by the hairpin structure. As a result, hairpin oligonucleotides are superior to the more widely used linear oligoprobes in terms of specificity, which makes them perfect for SNP detection..

Since genotyping data are acquired after to PCR completion, they signify an endpoint analysis. To promote the formation of fluorophore and target strand complexes, the amplicon is denatured and rapidly chilled. The temperature is then gradually raised, and the fluorescence from each vessel is continuously recorded. The destabilization brought on by the alteration is necessary for the identification of sequence variation using fluorescent chemicals . Fluorogenic molecules must separate from the dsDNA in order for the non-specific chemistries to reflect these changes over the course of the whole dsDNA amplicon, which can only happen when the duplex melts. The sequence modifications affect the distinct fluorogenic chemistries differently, changing the anticipated TM in a way that corresponds to the specific nucleotide alteration. With software that can compute the negative derivative of the fluorescence change with temperature, the ensuing sharp decline in fluorescence using either method can be represented as a "melt peak." (Fig. 3).

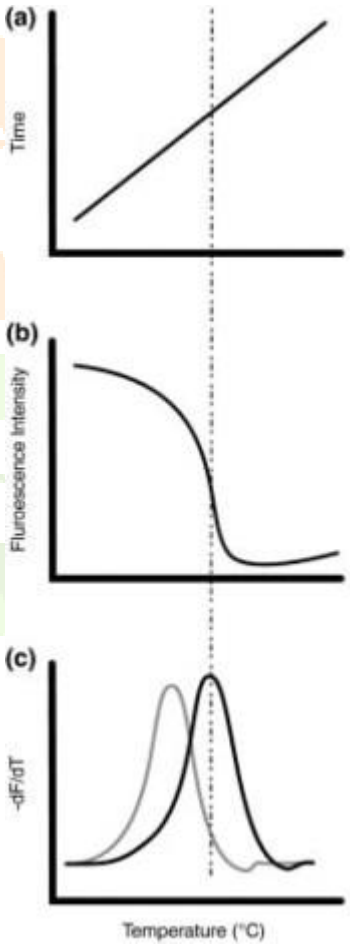



Fig. 3.Examination of the fluorescence melting curve. After a fluorogenic chemistry-based real-time PCR is finished, the reaction can be cooled to a temperature lower than the oligoprobes' predicted TM and then heated to a temperature higher than 90°C at a rate of less than one degree per second (a). The reporter or acceptor fluorophore's emissions can be continuously recorded during heating (b). Software determines the negative derivative of the fluorescence with temperature, resulting in a distinct melt peak that represents the TD of melting dsDNA or the TM of the oligoprobe-target melting transition (black peak; c). When one or more nucleotide changes are present, the $T_M$ or $T_D$ is shifted

(grey peak). This shift can be utilized to genotype microbial templates for diagnostic purposes and is repeatable.Crucially, distinct nucleotide modifications cause varying degrees of hybridization instability, and this can be taken into account when designing genotyping tests to provide the greatest possible discrimination across melt peaks.Mismatches with G (G:T, G:A, and G:G) are the least unstable, while those with C (C:C, C:A, and C:T) are the most unstable.

## Competing Interests:

Auther state that there is no conflict of interest in the publishing of this work.

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