

CHAPTER 1

Introduction

Quantitative polymerase chain reaction (qPCR), has been adapted for use a rapid, laboratory-based method for enumerating fecal indicator bacteria (FIB) and suggested as an alternative to culture based methods in EPA's new Recreational Water Quality Criteria.

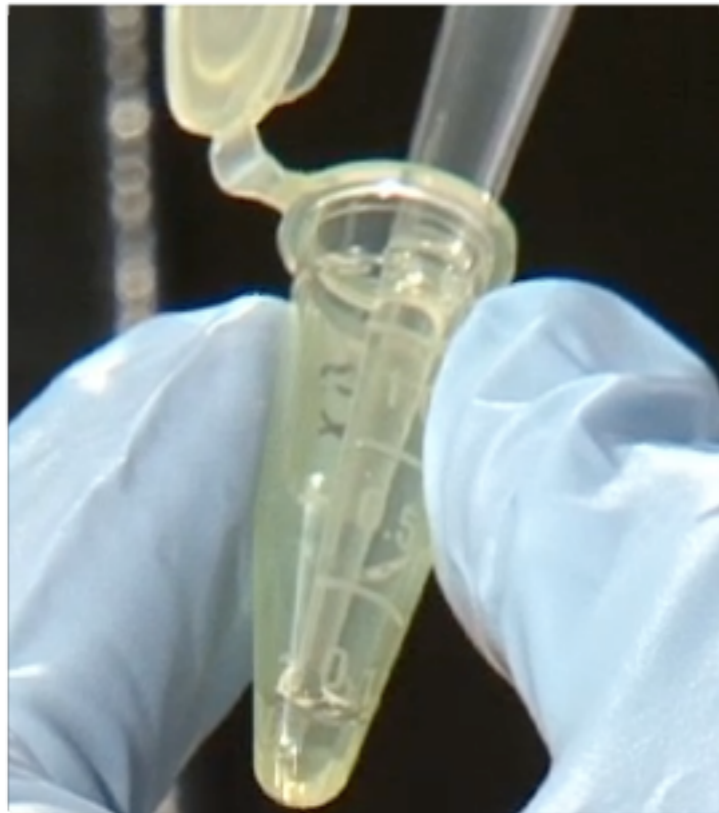
In this chapter, you will learn about the fundamentals of qPCR.



Fundamentals of qPCR

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- 1.1. What is polymerase chain reaction (PCR)?
- 1.2. What is quantitative PCR (qPCR)?
- 1.3. What does qPCR measure?



1.1. What is PCR?

The polymerase chain reaction (PCR) is one of the most powerful technologies in molecular biology. It is an enzymatic reaction that makes many copies of target DNA fragments from a template (e.g. *E. coli* isolated from surface water). Using PCR, specific sequences within a template (e.g. *E.coli* DNA) can be copied (amplified), to millions of copies.

With PCR; 2^n copies of DNA can be amplified from a template where “n” is the number of cycles. So, if there are 40 cycles of PCR, the yield would be 2^{40} copies of DNA.



For further information, please see:

<http://bit.ly/173g1HZ> and <http://bit.ly/15meyyn>

Components of PCR

The basic components of polymerase chain reaction include the following:

- Sample DNA (template DNA), containing target DNA sequence that will be amplified (copied)
- Heat stable DNA polymerase (i.e. Taq Polymerase)

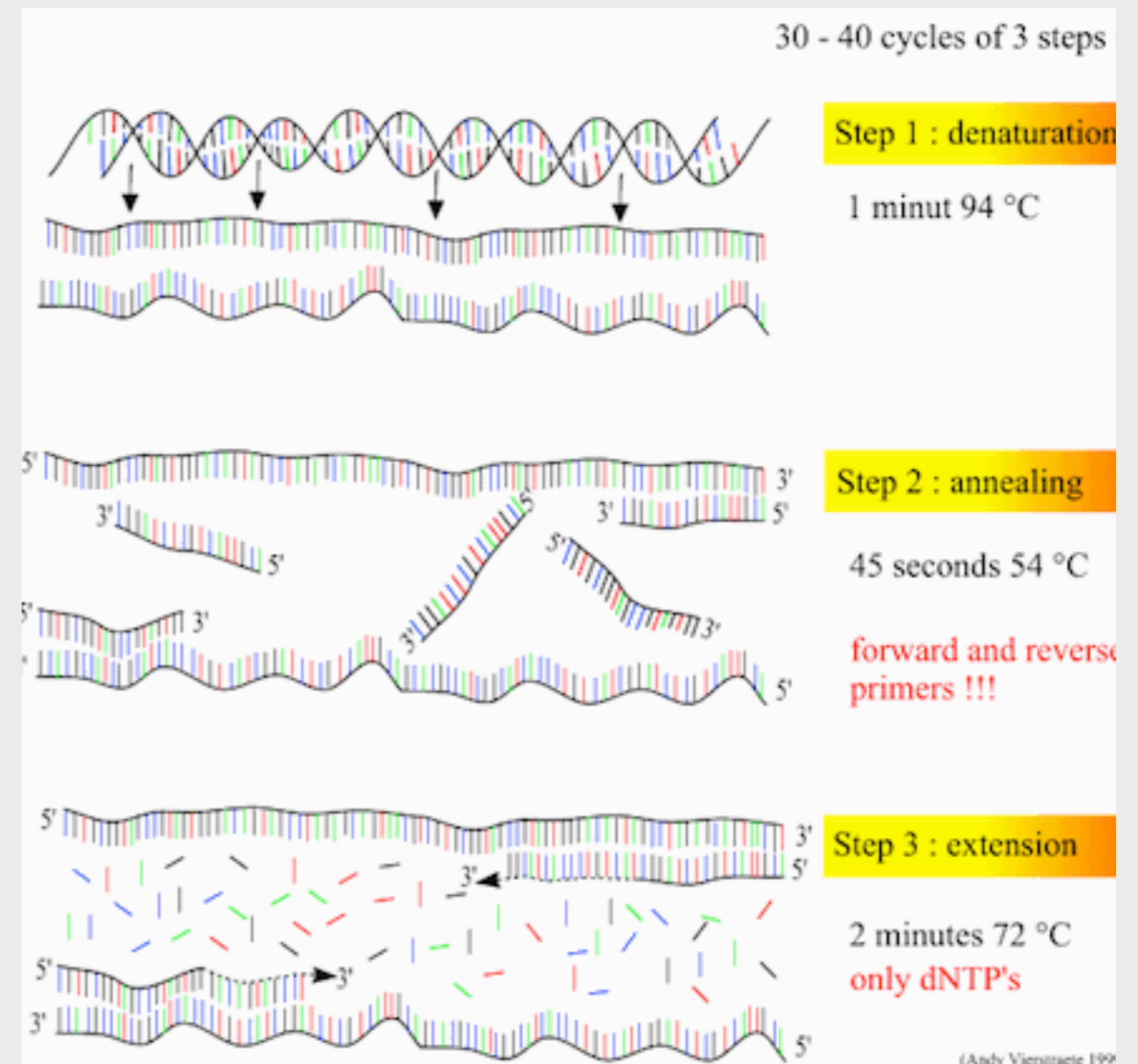
- Forward and reverse primers (short nucleotide sequences complimentary to target DNA)
- Deoxynucleoside triphosphates (dNTPs; the building blocks of DNA, used to synthesize the new strand)
- Buffer solution that provides optimal pH conditions for the reaction to proceed
- Ions (i.e. Mg^{+2})

PCR Steps

A single PCR cycle consists of three basic steps that occur at different temperatures (Figure 1.1). These steps are:

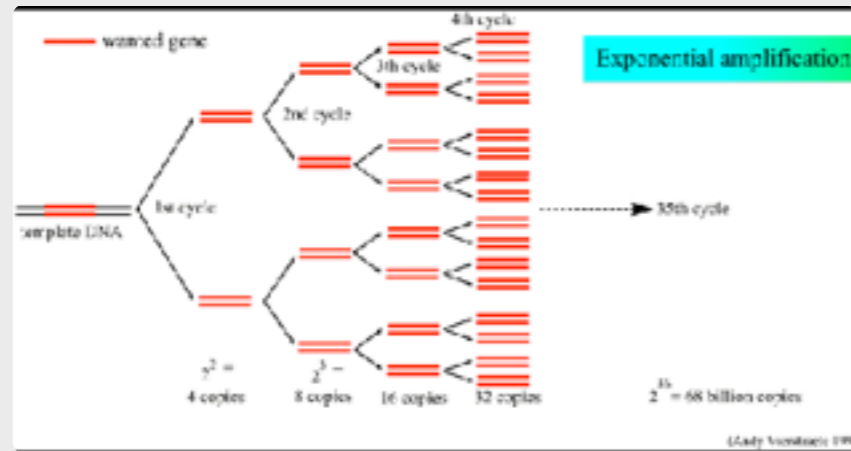
1. Denaturation (92-98°C): This step occurs at a higher temperature causing a disruption of hydrogen bonding between the template DNA strands and separation of double-stranded DNA into single-stranded DNA.
2. Annealing (50-65°C): Primers anneal (or bind) to the target DNA sequence, followed by annealing of Taq Polymerase to the target DNA sequence.
3. Elongation (72-80°C): Taq Polymerase synthesizes a new complimentary strand.

Figure 1.1. The PCR Steps



Source: Andy Vierstraete,
<http://users.ugent.be/~avierstr/index.html>

Figure 1.2. PCR Cycles



Source: Andy Vierstraete,
<http://users.ugent.be/~avierstr/index.html>

PCR cycles are repeated 20–45 times yielding millions of target sequence copies (Figure 1.2).

1.2. What is qPCR?

Quantitative Polymerase Chain Reaction (qPCR) combines the working principle of PCR with the use of a fluorescent-

labeled probe, which is an oligonucleotide complementary to the target DNA sequence.

There are several qPCR chemistries available for detection and quantification of DNA targets (TaqMan, Syber Green, Scorpion, etc.).

In this manual, we will focus on TaqMan chemistry, which is the chemistry presented by EPA in Method 1611.

TaqMan Chemistry

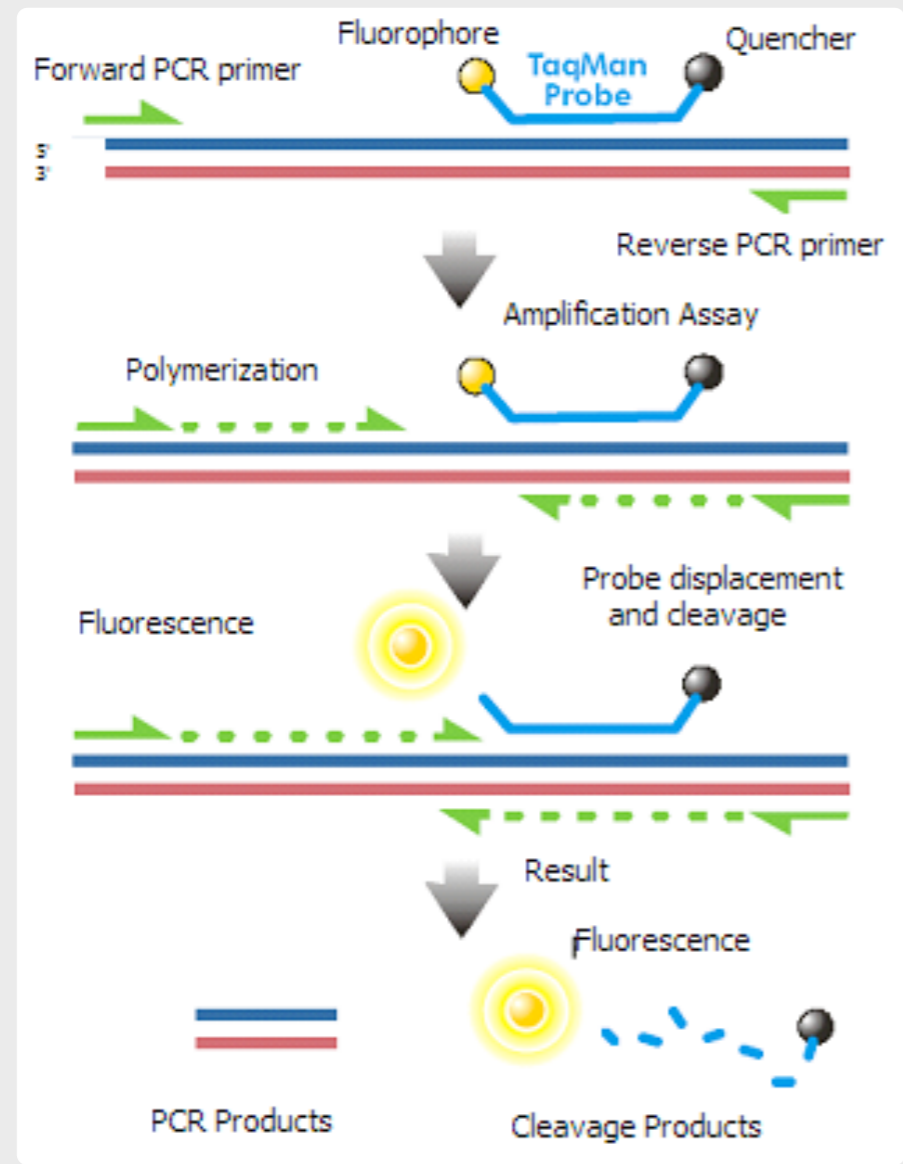
A TaqMan based qPCR method typically consists of two PCR primers (forward and reverse) and a TaqMan Probe. Before PCR begins, the TaqMan probe is intact and the reporter (a fluorescent dye used to monitor product accumulation) and quencher (a molecule that absorbs the emission of fluorescent reporter) are close together. During PCR, the primers and probe anneal to the target DNA sequence and the DNA polymerase extends the primer upstream of the probe. If the probe is bound to the correct target sequence, the polymerase's 5' end cleaves the probe, releasing a fragment containing the reporter dye.



For further information, please see:

<http://bit.ly/XRhFMi>

Figure 1.3. TaqMan® chemistry



Source: <http://bit.ly/XGvVoP>

With each qPCR cycle, new double stranded DNA (amplicon) is assembled causing the reporter dye and the quencher to be-

come further and further apart. When the distance between the two is sufficient, the dye fluoresces (Figure 1.3).

The intensity of the fluorescence is measured by a thermocycler and is proportional to the concentration target DNA in the sample. At the end of the reaction, the amount/intensity of fluorescence is plotted against the number of cycles which represents the accumulation of DNA product over the duration of the entire PCR reaction.

1.3. What does qPCR measure?

qPCR measures all DNA, including the DNA from live cells, viable but not culturable cells, dead cells, and the DNA that is found outside of a cell and in the environment (free DNA). qPCR can be used to detect organisms that are difficult to culture in vitro or that cannot be cultured.



qPCR is different from culture-based methods, because the culture based methods target only the living cells whereas qPCR targets DNA.

qPCR is a rapid tool and has advantages such as:

- The reaction occurs in less than 3 hours, enabling rapid public notification
- Accurate and precise quantification over wide range of target concentrations

However, because the target is different (total DNA) in qPCR versus culture-based methods (living cells) and has different sensitivity levels, laboratories have to go through a decision stage in which they compare these two methods and find out whether a switch to qPCR would be beneficial in their respective water environment. Therefore, careful consideration should be given before the qPCR method can be approved for use.

Conducting feasibility and method validation studies will ensure accurate result reporting and also provide information on implementation considerations at the individual laboratory level including, but not limited to: physical requirements, appropriate choice of instrument platform, approved product sourcing, costs, staff training needs, turn-around-time, and the ability of the method to accurately characterize water quality (Kinzelman and Anan'eva, in review).

The next chapter will discuss how to conduct a feasibility study and actions to be taken before investing in a qPCR laboratory.