

Polymerase Chain Reaction (PCR)

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PROBLEM STATEMENT

This scenario is about a step in the polymerase chain reaction (PCR) where it is critical for a technician to accurately calculate how much of a component (reagent) is required.

SCENARIO DESCRIPTION AND SPECIFIC EXAMPLE

To understand this scenario, it is helpful to know a bit about PCR.

PCR is an example of an analytical method, that is, a method that is used to answer a question about a sample. Quality control analysts in all manufacturing settings routinely use analytical methods to ensure that processes proceed properly, and products are correctly made. Analytical methods are also essential as they provide data for scientific research.

PCR is familiar to us because it is the gold standard test for diagnosing COVID-19. The test for COVID-19 analyzes a specimen from the upper respiratory system to detect the presence of the SARS-CoV-2 virus that causes COVID-19. PCR works by making millions of copies of a piece of DNA, much as a photocopy machine might be used over and over again to make many copies of a document. By specifically amplifying SARS-CoV-2 genetic material, if it is present, it is possible to detect miniscule amounts of viral genetic material that might be present in a sample containing vastly more host genetic material together with billions of other molecules.

Beside diagnosing COVID-19, there are thousands of other applications for PCR. For example, biomanufacturing involves the use of living cells to make products, such as insulin. Before biomanufacturing begins, the cells must be tested to see if they are contaminated with pathogens. PCR can be used by quality control analysts to amplify, and thus detect, the DNA of pathogens that might be lurking in minute quantities in the cells.

ISSUES TO BE ADDRESSED IN THE LESSON

A quality control analyst testing cells for pathogens will follow an established procedure to perform PCR. This procedure will tell the analyst to pipette out and combine various aqueous reagents into a single PCR test tube, see figure 1. If the wrong amounts of any of the reagents are pipetted out and added to the PCR test tube, then the process will not work properly, and it will not be possible to tell if the cells are

contaminated or not. In the worst case, it might erroneously appear that the cells are not contaminated by a pathogen, when, in fact, they are contaminated.

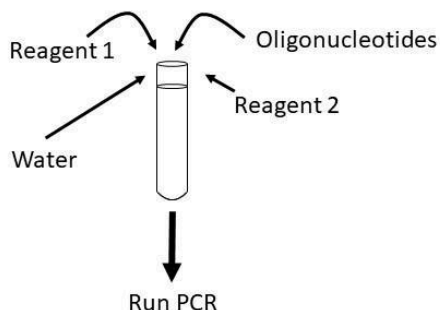


Figure 1. PCR requires a mixture of reagents that are dissolved in water. This combination is called a “reaction mixture.” One of these reagents is called “oligonucleotides.” The correct amounts of each reagent must be added to the mixture to ensure a correct result. (Table 1 indicates what these reagents typically are.)

You can see in Table 1 seven reagents that are typically added to a test tube in order to perform PCR. In the table you can also see that the *concentration* of each component is noted but the table does not tell the analyst the *amount* of every reagent to add to the tube. So, for each reagent, the technician or analyst needs to determine the amount required.

TABLE 1. THE MAIN COMPONENTS OF A PCR REACTION MIXTURE		
COMPONENT	PURPOSE	CONCENTRATION, OR AMOUNT, IN REACTION MIXTURE
PCR reaction buffer	Controls the pH	1 X
Magnesium, usually as 2MgCl (may be combined in the reaction buffer)	A required cofactor for the polymerase enzyme	1.5 – 2.0 mM
DNA template	The DNA containing the target that will be amplified	0.001 – 1.0 µg (forensic analysis may use as little as 0.5 to 10 ng of DNA)
Forward primer	Oligonucleotide that recognizes the target sequence on one strand and initiates replication	0.05 – 2.0 µM
Reverse primer	Oligonucleotide that recognizes the target sequence on the other strand and initiates replication	0.05 – 2.0 µM
Four dNTPs (dATP, dGTP, dCTP, dTTP)	The subunits of DNA that are incorporated into the new DNA copies	200 µM each is typical
Thermostable DNA Polymerase	Enzyme that adds nucleotides to the new DNA strands	0.01 units/µL – 0.05 units/µL (0.025 units/µL is common)

Oligonucleotides (short molecules of DNA referred to as primers in Table 1) are one of the reagents required for PCR. For the purpose of this scenario, it is not important to know the function of oligonucleotides, just know that the right amount of oligonucleotides must be pipetted into the PCR test tube.

Suppose that, in our scenario, the PCR procedure specifies that the analyst must add **400 ng** of oligonucleotides to the PCR tube. In this scenario, the analyst removes a vial containing oligonucleotides from the freezer, see Figure 2. (This tube contains a mixture of the “forward and reverse primers” that are noted in Table 1.)

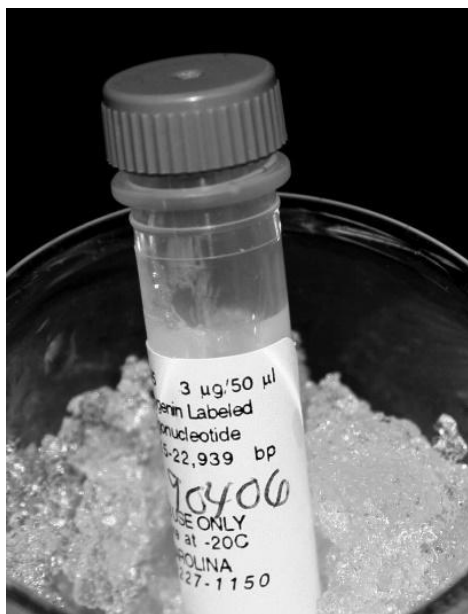


Figure 2. The vial containing oligonucleotides. Observe that the vial is labeled with a concentration, that is, $3\ \mu\text{g}/50\ \mu\text{l}$, at the top of the label.

Based on this label’s information, how much volume should the analyst remove (pipette out) to obtain 400 ng?

SUPPORTING MATERIALS

QUESTIONS TO ADDRESS AND EXPLANATION OF CALCULATIONS

The label on the vial says that the concentration of oligonucleotides in the vial is $3\ \mu\text{g}/50\ \mu\text{L}$. Remember that *concentration* is a ratio. Note also that 400 ng is an *amount*. The words “amount” and “concentration” are not synonyms because an “amount” is not a ratio.

To solve this problem, first make sure the units match; the procedure being followed by the analyst specifies that 400 ng are required but the label on the vial uses μg :

$$400 \text{ ng} = 0.400 \mu\text{g}$$

Next, a proportion equation can be used to calculate how much volume is needed to get 400 ng of oligonucleotides:

$$\frac{3 \mu\text{g}}{50 \mu\text{L}} = \frac{0.400 \mu\text{g}}{?}$$

? is approximately equal to 6.7 μL

So, the analyst must pipette out 6.7 μL from the vial to get the correct *amount* of oligonucleotides.

There is an alternative strategy that some people prefer to solve this problem. Some analysts would use an equation, like this:

$$\left(\frac{400 \text{ ng}}{1} \right) \left(\frac{1 \mu\text{g}}{1,000 \text{ ng}} \right) \left(\frac{50 \mu\text{L}}{3 \mu\text{g}} \right) \approx 6.7 \mu\text{L}$$

Thus, there is more than one way to think about solving this particular math problem. Either way is correct. Not everyone thinks about math problems in the same way and more than one pathway can take you to the right destination.

FURTHER NOTES

While this is a relatively straightforward calculation problem, there are complexities in the real world and these issues can make the problem hard for students.

It is important that students clearly understand the concept of concentration. Concentration is a ratio. Before solving calculation problems, such as the one shown here, it is essential that students can distinguish between an amount (such as a gram, liter, or mole) and a concentration (such as grams/liter or moles/liter).

It is important that students are comfortable with what might initially be unfamiliar metric prefixes. Biotechnologists commonly work with nanograms, nanoliters, micrograms and microliters, which might be new to students.

To extend the conversation, an instructor might ask the students if they can accurately measure out 6.7 μL . There are micropipettes that display readings to the nearest tenth of a microliter, but students might

want to consider whether it is possible to accurately pipette exactly 6.7 μL . A discussion of the accuracy of micropipettes is outside the scope of this scenario, but a technical instructor who teaches the use of micropipettes might want to probe into this question. Pipette manufacturers provide technical specifications that can inform a discussion of this topic.

An instructor might also want to ask students what they think might happen if the reagents are not accurately pipetted into the PCR tube. Analyzing this question in depth requires more understanding about PCR than is provided here. However, the concept of false positives and false negatives can be introduced in a general way. In this scenario, a false positive occurs when a sample appears to be contaminated, but, in reality, is not. A false negative is where a sample does not appear to be contaminated, but really is. This concept is applicable to any analytical assay that gives either a “yes” or “no” answer. It is obvious that a false negative would be disastrous in a biomanufacturing situation because contaminated cells would be used in manufacturing, resulting in a contaminated product. It is worth noting that a false positive could result in costly delays and expensive loss of healthy cells.