SCENARIO: PREPARING RNA SAMPLES FOR MICROARRAY ANALYSIS

Based on a prompt from Maddie, who is an analytical technician in a biotechnology company

BACKGROUND

You are working in a biotechnology laboratory, tasked with preparing RNA samples for microarray analysis. Microarray analysis is used to study gene expression[1] and it involves extracting RNA from cells and processing it for further analysis. However, before you can proceed to RNA fragmentation—the process of breaking the RNA into smaller pieces suitable for analysis—you must first ensure that all RNA samples contain the same concentration and amount of RNA. This is because RNA is extracted from different cell types and under various experimental conditions, leading to inconsistent RNA concentrations across samples. To proceed with accurate analysis, you need to **normalize the amount and concentration of RNA** in all samples, that is, make them the same. This requires some calculations.

You use a specialized device called a **Nanodrop** to measure RNA concentration in **nanograms per microliter (ng/µL)**. Your goal is to standardize each sample by diluting it to a target concentration of **0.46875 µg/µL** (micrograms per microliter), which is suitable for the fragmentation step.

However, there's an additional complexity: you need to have **12 µg** of RNA in each sample before diluting it to the target concentration.

Note that you will use micropipettes to measure out the RNA and water and these micropipettes cannot accurately measure volumes less than 1 µL. As you perform calculations, be sure that your strategy does not call for measuring volumes less than 1 µL.

Review of Key Terminology:

- **Gene Expression:** The cellular process in which the DNA inside the cell's nucleus is turned on so that the cell manufactures the protein for which that gene codes. RNA specific to that gene is produced by the cell as an intermediary in this process.
- **Nanodrop**: A small spectrophotometer used to measure the concentration of nucleic acids (RNA and DNA) by analyzing their absorbance of light.
- **Concentration (in units of ng/µL or µg/µL)**: A ratio that is the amount of something (in this case RNA) per a certain volume of liquid.
- **Fragmentation**: A process in molecular biology where long RNA strands are broken into smaller pieces to prepare them for analysis.
- **Microarray Analysis**: A technology used to study gene expression by analyzing RNA samples across many genes simultaneously.

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[1] When we say a gene is **expressed,** *we mean the gene is turned on and the protein for which it codes is produced by the cell.* If this idea seems unclear to you, consider that all your cells, with a few exceptions, have the same DNA, yet a muscle cell is obviously different from a skin cell. This is because different genes are expressed in skin, muscle, heart, nerve, and so on. RNA that is specific for a particular gene is produced as part of the process of gene expression. Therefore, scientists can study the RNA that has been isolated from cells to discover which genes were being expressed at a given time in those particular cells.

- **Normalization:** Normalization typically occurs immediately downstream of the extraction process of RNA and DNA. Normalization means adjusting sample volume, amount, and concentration to match the requirements of specific applications and to make all samples the same with regard to these characteristics.

A Specific Example

You've just completed a Nanodrop analysis on a batch of RNA samples and have the concentration results for each. Now, you need to make sure all samples are uniform in concentration before moving on to the next steps.

Let's assume your first RNA sample shows a concentration of **3372.4 ng/µL** (nanograms per microliter), as measured by the Nanodrop. How could you process this sample so that, in your resulting tube, there are **12 µg** of RNA and also that this RNA is present at a final concentration of **0.46875 µg/µL**?

ANSWER

Using the Nanodrop measurement for this specific sample, you will perform calculations in two steps:

- **Determine how many microliters (µL)** are required from this sample to obtain 12 µg of RNA.
- **Determine how much water to add** to dilute the RNA to a final concentration of **0.46875 µg/µL**.

The calculations will ensure that each RNA sample is consistent in terms of both **quantity** and **concentration**, which is crucial for the accuracy of the microarray results.

Step 1: Calculate the Volume of RNA Needed to Get 12 µg

Since the concentration is in ng/µL and you need 12 µg of RNA, you need to make the units consistent. For example:

12 µg = 12,000 ng

You want to know how many microliters of this RNA sample are needed to get 12,000 ng (which equals 12 µg). The reasoning of ratios and proportions can be applied:

$$\frac{3372.4\ ng}{1\ \mu L} = \frac{12{,}000\ ng}{?}$$

$$12{,}000\ ng\ (1\mu L) = \ ?\ (3372.4\ ng)$$

$$? \ = \frac{12{,}000\ \mu L}{3372.4}$$

? = **3.5582968 µL**

So, you need 3.5582968 µL to obtain the required 12 µg of RNA. In practice, depending on your micropipette, you will need to round this number, possibly to 3.6 µL. So, pipette out 3.6 µL and place into a small tube.

Step 2: Calculate the Volume of Water to Add for Dilution

Next you need to dilute the RNA sample to the final concentration of 0.46875 µg/ µL. To determine the total volume of solution required, you can again use the logic of ratios and proportions:

$$\frac{12\ \mu g}{?} = \frac{0.46875\ \mu g}{1\ \mu L}$$

$$12\ \mu g\ (1 \mu L) = \ ?\ (0.46875\ \mu g)$$

$$? \ = \frac{12\ \mu L}{0.46875}$$

? = 25.6 µL

This is the total volume of sample that you need. But recall that you already put 3.6 µL of sample into your tube. Therefore, you need to subtract this volume from the total required:

Volume of water to add now = 25.6 µL – 3.6 µL = **22.0 µL**

CONCLUSION

For this RNA sample:

- You will need to pipette out **3.5582968** µL of RNA sample to get 12 µg of RNA. However, using standard lab equipment, you will need to round this to 3.6 µL.
- You will then add 22.0 µL of water to dilute the sample to the final concentration of 0.46875 µg/µL.

Note that if you assumed your micropipette could be adjusted to 3.5582968 µL, then you will calculate that you need to add 22.042 µL of water to dilute the sample to the desired concentration.

Repeat this process for each sample using its nanodrop concentration reading. Use the same two calculation steps to ensure that every sample has the same final concentration and amount of RNA.

DISCUSSION

This scenario relates to an assay, microarray analysis. Assays are tests to determine some feature of a sample, in this case, which genes are expressed in cells under certain conditions. The information provided by various assays is of vital importance in almost any biotechnology setting. Assays must be performed properly, otherwise, they provide useless or misleading results. Many assays require calculations to determine amounts or concentrations of reagents, and it is essential to perform these calculations correctly.

This scenario introduces the concept of "normalization," which is important in many assays. In this case, the amount and concentration of RNA must be the same in all samples, that is, must be "normalized."

The actual calculations in this scenario require only elementary algebra and an understanding of ratios and proportions. Yet students might find these problems challenging because of the mix of mathematical concepts, scientific notation, and unfamiliar laboratory techniques. Here's a breakdown of some potential difficulties:

1. Unit Conversions

- **Nanograms and Micrograms**: The problem involves converting between units like nanograms (ng) and micrograms (µg), which can confuse students who are not comfortable with metric unit prefixes (nano, micro, milli, etc.). Understanding that **1 µg = 1,000 ng** is a critical concept that some students may overlook.
- **Understanding the significance of small quantities**: The measurements are tiny, and students may struggle with visualizing or grasping how significant small differences are when dealing with such minute quantities. Similarly, while students might be familiar with metric prefixes for larger quantities, such as "kilo," "micro" and "nano" are likely to be new to them.

2. Proportional Reasoning

- **Setting up the equation**: The equations used here require understanding proportions. Students might not immediately recognize how to set up the problem or may struggle with solving for the unknown variable (indicated above as a question mark), particularly if they haven't had much practice with algebraic manipulation. Note, however, that the vast majority of students do have an intuitive understanding of proportions. For example, if you tell them that they will earn $20/hour and ask how much they would earn in three hours, they can readily tell you the answer. It is important to show students how they can extend this understanding to the problems encountered in the workplace.
- **Cross-multiplying and solving**: The step of cross-multiplying to solve for the volume (?) is simple algebra, but students with weak algebra skills might find this process confusing.

3. Multi-Step Problem Solving

- **Handling multiple steps**: This is a major issue in real world problems that can make them different from many textbook problems. This scenario requires students to perform several calculations in a row (determine RNA volume, then calculate total solution volume, and finally

find water volume). Beginning students often struggle with multi-step problems because they may lose track of where they are in the process or make mistakes by mixing up steps.

- **Logical flow of the problem**: Students may not understand why they are performing each step. For instance, they might not grasp why calculating RNA volume comes before adding water, or why the water volume is found by subtracting the RNA volume from the total solution volume.

4. Lab Terminology and Context

- **Technical context**: Students who are new to molecular biology may be unfamiliar with terms like **Nanodrop**, **RNA fragmentation**, **microarray analysis**, and **concentration (ng/µL)**. This unfamiliarity can make it harder to grasp the problem itself because they may not understand why they are doing what they're doing or how the math relates to the lab procedure. Most importantly, the technical context makes it difficult for beginners to "find" the required math.
- **Distinguishing between "amount" and "concentration."** Amount is how much of something exists while concentration is a ratio, usually amount/volume. Beginners often confuse the two, and to perform calculations it is essential to know whether one is talking about an amount or a concentration. This scenario provides an explicit introduction to these terms because both the amount and concentration of RNA is normalized.
- **Connecting math to science**: Some students may struggle to see the connection between math and its application in the lab. For instance, they might view math and science as separate subjects and may not easily transfer their math skills to a biological context.

5. Precision in Measurement

- **Exact volume measurements**: The scenario specifies that micropipettes have limitations. Understanding the limitations of lab equipment can be difficult for students who are not yet accustomed to thinking about precision in measurement.
- **Rounding**: This scenario provides a good example of how real-world lab equipment impacts the precision that one can achieve. Beginning students (and more senior researchers) may find it tricky to decide when to round the results of calculations. Also, students need to understand that in science, while precision is important, tools like pipettes have limits. Grasping this balance can be a challenge for students who may think math problems always have exact answers.
- **Real-world accuracy**: Students may also not realize that in lab work, being slightly off with measurements can have significant impacts, which might not be the case in more abstract math problems they've seen before. This makes accuracy crucial, but it also adds pressure and complexity to the problem. Again, the issue of lab equipment limitations plays a role here.

6. Application of Abstract Math

- **Abstract thinking**: For beginning students, applying abstract math skills to a practical, real-world problem can be hard. They may be comfortable solving equations in isolation but struggle when they need to apply these equations in a context where the math is just one part of a larger process (in this case, RNA sample preparation).

Beginning to Help Students Overcome These Difficulties:

1. **Review Unit Conversions**: Spend time practicing unit conversions, especially between metric units.
2. **Teach Scientific Notation**: Ensure students understand how to work with large and small numbers.
3. **Break Down Multi-Step Problems**: Guide students step-by-step through the problem, clearly explaining why each step is necessary.
4. **Clarify Terminology**: Provide clear definitions and explanations for lab equipment and procedures.
5. **Connect Math to Lab Work**: Help students see how the math they are doing is directly related to the real-world work in the lab, emphasizing the importance of precision and accuracy.

By addressing these challenges and focusing on these strategies, students can become more comfortable and confident in solving these types of problems.

A FEW MORE EXAMPLES OF THE SAME SITUATION WITH ANSWERS

Example 2: Nanodrop Reading: **2894.7 ng/µL**

Step 1: You need **≈ 4.1455 µL** of RNA to get 12 µg.

Step 2: The total volume of water required is again 25.6 µL but now subtract the volume already added to the tube. In this case you might use 4.1 µL, so the answer is **21.5 µL**.

Example 3: Nanodrop Reading: **4150.2 ng/µL**

Step 1: You need **≈ 2.8914 µL** of RNA to get 12 µg.

Step 2: The total volume of water required is again 25.6 µL but now subtract the volume already added to the tube. In this case you might use 2.9 µL, so the answer is **22.7 µL**.

Example 4: Nanodrop Reading: **1845.3 ng/µL**

Step 1: You need **≈ 6.5030 µL** of RNA to get 12 µg.

Step 2: The total volume of water required is again 25.6 µL but now subtract the volume already added to the tube. In this case you might use 6.5 µL, so the answer is **19.1 µL**.