Experiment Failure Troubleshooting Handbook

SLIM NAU -CHINA 2020

1 Making Reagents and Buffers

1.1 Calculate what you need

1.1.1 To determine the formula molecular weight

- Read the label carefully and be sure you are getting the formula weight of the substance you need. Be sure to look at the formula, not just the name;
- Salt or acid/base form. The major problem in using a salt vs the acid is in the difference in pH;
- Anhydrous or hydrated. Extra water in the material won't usually be a problem, as long as the water is calculated as part of the formula weight.

1.1.2 Dilution of Stock Buffers

- Do all manipulations as cleanly as possible. It is not necessary to flame the bottles, if they are not used for cell maintenance. Use sterile pipets, and replace caps immediately.
- Oftentimes, the stock solution is made wp in a solvent in which the substance could be dissolved but which is harmful for cells or for an enzymatic reaction. Dilution of the substance also serves to dilute the solvent to a non-harmful concentration.
- The carryover from high to low concentration tubes can erratically make the actual concentration higher than you have calculated it to be. This is not the time to conserve pipets.

1.2Weighing and mixing

- Some balances may not have an automatic tare feature. If not, you must tare the weighing vessel manually.
- The solution must be cooled to room temperature before your pH it or you may get an erroneous pH.

1.3 Measuring pH

- The pH meter should be calibrated with two buffers of known pH. From these calibrations are derived the pH determinations, so don't scrimp (and render your pH reading inaccurate) by standardizing only to one buffer of pH 7.
- pH is dependent on temperature. Be sure the buffer you are pH-ing is the same temperature as the buffer standards you use to calibrate the pH meter. And be sure the buffer and the standards are at the temperature at which the buffer will be used.

1.4 Sterilizing solutions

- Even if you require the buffer for a nonsterile application, it should still be sterilized because microbial growth can cause changes in pH and in the nature and function of the buffer.
- If a beat-labile or otherwise non-autoclavable ingredient must be added to an autoclavable buffer autoclave the buffer first. When the buffer has cooled to room temperature add the filter-sterilized ingredient.
- Autoclave glucose separately from amino acids/peptones or phosphate components.
- Autoclave phosphates separately from amino acids/peptones or other mineral salt components.
- Autoclave mineral salt components separately from agar.
- Avoid autoclaving media at a pH greater than 7.5. Autoclave at neutral pH and adjust to the desired pH with a sterile base solution after cooling.
- Avoid autoclaving agar solutions at less than pH 6.0.

2 Working without Contamination

2.1 When to use sterile technique

You must use sterile technique whenever you are working with living organisms, or with any media, buffers, or culture containers used for living organisms. For example: setting up a culture of E. coli for a transformation, making LB plates, splitting cells, filtering serum for media, opening and rehydrating a vial of lyophilized bacteria.

2.2 Mistakes that break sterility

- Pipetting up too far in the pipet. Discard the pipet and check the pipettor: You may need to change filters.
- Touching the tip of the pipet against a bottle, the ground, the outside of the pipet container, or anywhere solid. Discard the pipet.
- Dropping an opened container or tube to the ground. Discard it.
- Touching anything, including a gloved hand, to either the HEPA flter that prevents the suction of particulates through the vacuum system, or to the flter used in a flask vacuum system. This is a major source of contamination in hoods. Discard whatever touches the filter.
- Reusing pipets while working. Once a pipet has been wetted, it is much more likely to pick up airborne contaminants.

3 Bacteria culture

3.1 Making Tubes or Flasks of Liquid Medium

Make the medium, leaving out thermolabile substances such as antibiotics, growth factors, or vitamins. pH if required.

3.2 Making Plates of Solid Medium

- The surface of the plate must be smooth. If bubbles form on the surface of the plate, quickly apply the flame of the Bunsen burner to the surface to pop the bubbles. Be careful not to melt plastic petri dishes.
- If the antibiotic in the plates is light sensitive, wrap the plastic wrapped plates in aluminum foil or place them in a box. Tetracycline is light sensitive.

3.3 Reviving cultures

Usually, you should include antibiotics in the medium if your culture bears a plasmid for antibiotic resistance. However, strains carrying Tetr, Ampr, Kanr, or Camr should be grown in medium without antibiotic to allow expression of the antibiotic resistance before antibiotic selection is applied.

3.4 Antibiotics

Antibiotics are heat-labile, and are added to the medium after it has been sterilized and cooled.

3.5 Streaking with a Wire Loop over an Agar Plate: one Strain

To be sure the loop isn't too bot touch it lightly on a not-streaked area of the plate before you touch the bacterial streak. Designate this the cooling area of the plate, and don't streak over this bumpy surface.

3.6 Pollution

Yeast and bacteria should never be grown in the same incubator, and most labs maintain separate working quarters (hoods, even rooms) for each. This is more for the sake of the yeast, which more easily can get contaminated by bacteria than the other way around: Still, any sharing of equipment can lead to contamination of bacterial cultures.

4 DNA, RNA and Protein

4.1 DNA

4.1.1 Ethanol Precipitation of DNA

Caution is needed when handling large-molecular-weight DNA (over 30 kb). The DNA should never be vortexed but should be mixed by inversion or on a wheel. Instead of precipitating the DNA with ethanol, traces of chloroform should be removed by dialyzing the DNA solution against large volumes of cold TVE or by extraction with water-saturated ether.

4.1.2 Determining Nucleic Acid Concentration and Purity by UV Spectroscopy

Only quartz cuvettes, not glass or plastic, will allow you to take accurate readings in the UV range.

4.1.3 PCR

Keep separate pipets and other supplies for setting up PCR reactions. A positive displacement pipettor will prevent aerosols and reduce the chance of sample carryover. Pipettor tips with filters will also prevent carryover from sample to sample.

4.1.4 Introduce DNA into cells

With either method the bacteria must first be rendered competent; that is, able to take up DNA.

4.2 RNA

- Autoclave all plasticware and glassware that will touch anything that will touch the RNA.
- DEPC(diethylepyrocarbonate)-treat all water that will be used to make DNA buffers. Add DEPC to final 0.1%, leave overnight at room temperature, and autoclave for 15 minutes. Don't use DEPC for Tris buffers, as the DEPC will decompose into ethanol and carbon dioxide.
- Avoid alkaline buffers, as the hydroxy group in RNA makes the molecule very sensitive to alkali.
- Keep RNA buffers separate from other buffers, so they won't be accidentally used and contaminated with RNases.

4.3 Protein

4.3.1 Basic rules·

- Always have a bucket of ice handy when you are doing any protein work, and put tubes on ice immediately when removing them from freezers, centrifuges, etc.
- Spin cold unless otherwise noted. Centrifuges can get quite warm.
- During and after cell lysis, include the appropriate protease inhibitors in all buffers.

4.3.2 Determining Protein Concentration

- You cannot directly compare the results of one assay method with another. You must get used to working with the relative concentrations determined by one method.
- The nature of your protein sample will also suggest which assay to use. If you know you have a purified protein without tryptophan, you shouldn’t rely on absorption at 280 nm. And if you must have detergent in the protein sample, you must choose a method that is not particularly detergent sensitive, or you must remove the detergent.
- For all methods you must run your unknown samples against a standard curve, every time you perform the assay. Any purified protein can be chosen as a reference standard, if only relative protein concentrations are desired. Bovine serum albumin (BSA) and IgG are commonly used: Use BSA unless you are measuring antibodies.

5 Component failure checking

5.1 Set control groups

5.1.1 Why are the control groups set

If you check all of the above and other objective factors that may cause the experiment failure, but still do not get the desired results, you should consider the component itself.

Setting control groups is a common method for checking failed parts. Usually, we will set a negative control and a positive control, which are all based on the "expected result". The positive control is the group with the expected results, while in the negative control group the expected results will not occur.

5.1.2 Set several control groups

Consider all the factors that influence the results of the experiment. In theory, there should be a positive control and a negative control for each factor.

5.2 Determine the failed component according to the experimental results

How could you determine the failed component based on the results of experiments? Let's take a simple example:

In this device, we tested whether the gene G1 could express normally under the control of the induction promoter P1.

Obviously, two sets of experiments need to be designed first: testing the device with and without inducers, respectively.

Suppose that the experimental group with inducer added is denoted as Group A while the group without inducer is denoted as group B and gene 1 expression is denoted as 1 while gene 1 didn't express is denoted as 0, we will get four results:

	Group A	Group B
Result 1	0	0
Result 2	0	1
Result 3	1	0
Result 4	1	1

Except for result 3, the other results are not what we want. While if there happen the three other results, how can we find the wrong part of the device and adjust it to achieve the desired result 3?

Analyze the four possible outcomes:

If results 2 and 4 come up, gene 1 can express, it can be inferred that the promoter P1 has problems and we can replace the promoter P1. If the result 3 is obtained after the replacement, the device failure checking is completed.

If result 1 occurs, we cannot determine whether the promoter P1 or gene 1 is the problem. At this point, we can set up a positive control: replacing P1 by the promoter P2 which has been successfully verified. If gene 1 can express at this time, it means the promoter P1 is malfunctioning. If gene 1 cannot express. it indicates that the gene 1 is malfunctioning.