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How to Set Up an Experiment

AN EXPERIMENT IS A TEST made to examine the validity of a hypothesis. Experimentation is the reason you are where you are. If you don't conduct your experiments carefully and thoroughly from the beginning, you'll soon be mired in a bog of half-baked experiments and unclear results that can only be undone by leaving the lab.

There is one extremely important caveat to this advice on organization and clear thinking. DO AN EXPERIMENT DURING THE FIRST WEEK. Don't wait until you think you "know more." Planning experiments is rather like joining a union—you can't join until you have the experience, and you can't get the experience until you are in the union. You may have read every relevant paper, memorized the protocols, talked to everyone in the lab, but still do not really understand the new experiment you are getting ready to do. DO IT ANYWAY. After you have done the first experiment, *everything*—theory, techniques, implications—will become magically clearer. Do read and prepare yourself, but know that a full understanding of your project will not come until you have physically performed the experiments.

Not all experiments will be thrilling. It is terrific to think of doing only very exciting and crucial experiments, but it is more important to first be sure your experiments are reproducible, dependable, and have integrity.

PHILOSOPHICAL CONSIDERATIONS

Keep the importance of your experiments in mind. Chances are that once you start working, you won't have much chance to think consciously

about the universal relevance of your experiments. But as you set up your experiments, it is important to remember why you are doing what you are doing. Think about Science. Sometimes lab work can seem tedious, and remembering why you are repeating the same experiment for the tenth time can keep you going. Keeping the big picture in mind will also help you maintain integrity in your approach.

Remember that you must publish the results of your experiments. Publications are important—you can't survive without them. If you consider each experiment to be a separate and publishable entity, you are more likely to remember all the needed controls and variables. Thinking about each experiment as potentially publishable can keep you focused on your experiment. It is very easy to drift into other areas, and part of research is following new avenues. But exploration must be done rigorously, and not by default.

> *"...scientists often place at least as much weight on an experimentalist's general reputation for careful, painstaking work as on the technical details of the experiment in assessing whether the data constitute reliable evidence." (Reprinted by permission of American Scientist, magazine of Sigma Xi, The Scientific Research Society, from Woodward and Goodstein 1996.)*

Set yourself up as a careful and thorough investigator. You will make your reputation not only on your results, but also in the way you set up your experiments. Some people are known as good "bench" scientists, meaning that their experiments are well thought out and well executed. Set up your reputation early, and any unorthodox data you do produce is likely to be believed. If your labmates trust your data, the head of the lab is more likely to, and from this, the rest of the scientific community. Credibility is key.

Be a critical thinker. As for the rest of science, the theories behind experimentation are not carved in stone, and there are several ways to explain how and why experiments should be done.

Approaches to problem-solving and experimentation

Reductionist. Breaks a problem down into pieces, and solves each piece separately.
Deductive. Proposes a hypothesis and collects data to confirm or disprove the hypothesis.
Inductive. Examines a collection of evidence without prejudice and then proposes a theory to account for all the observed facts.
Falsification. Proposed by Karl Popper. If a prediction that stems from a hypothesis turns out to be false, the hypothesis from which it is deduced is said to be falsified and must be rejected.

Standing alone, every approach is inadequate. Incorporate all the approaches you can. Attack your data from all angles to look for flaws in your thinking or in the experimental approach. Try not to slant your results toward the conclusion you want or expect.

"I have often had cause to feel that my hands are cleverer than my head. That is a crude way of characterizing the dialectics of experimentation. When it is going well, it is like a quiet conversation with Nature. One asks a question and gets an answer; then one asks the next question, and gets the next answer. An experiment is a device to make Nature speak intelligibly. After that one has only to listen." (Reprinted, with permission, from George Wald 1968, © American Association for the Advancement of Science.)

PLANNING AN EXPERIMENT

1. **Define the question.** An experiment should address a hypothesis by answering one or two specific questions. Careful thought and reading, as well as discussion with other scientists, will help define the questions and set up the parameters of the experiment. Be sure not to pick questions that it is not possible to answer.
2. **Design the experiment.**
   - Experimental *variables.* At what do you want to look? Will you look, for example, at an effect over time, or of concentration, or both? What concentrations will you use, and what should the time points be?
   - *Controls.* Every experimental variable needs a control to show that the results obtained are the results of the treatment.
   - *Number of samples.* Should you run samples in duplicate? Triplicate? More? Duplicates are the minimum for any measure of biological activity.

DO SMALL EXPERIMENTS. It is very tempting to get as many data as possible from each experiment, as this will, theoretically, save money and time. Cells have to be grown fewer times, reagents are conserved, and time is maximized. **But the return/effort ratio is actually much higher for small experiments.** Carrying off a large experiment requires a great deal of expertise, as well as luck, and should never be attempted before several small experiments have already been performed.

The determination of what a small or large experiment is is completely subjective. But generally, **if there are so many tubes that you feel hassled or confused, your experiment is too big.** If you feel this happening, divide the experiment into two parts, and only do the first part.

In the end, you won't get rewarded because you managed to pull a huge experiment off, but you can be rewarded by a lovely figure in a published paper, and those are almost impossible to derive from a mind-boggling marathon. Don't get macho.

- *Statistics.* The statistical analysis that is needed will help dictate sample size and other aspects of experimental design. Find out if you will subject your data to statistical analysis—it is too late to do it at the end of the experiment.

It is a waste of time and reagents to not think carefully before setting up an experiment. Many a beginner (as well as seasoned experimenters) may mistake the busyness and sense of importance one feels when doing many experiments for actually doing something meaningful. Don't be fooled. THERE IS NO SUBSTITUTE FOR CAREFUL PLANNING!

3. **Set up the experiment.**
   - Obtain and prepare a *protocol.*
   - *Prepare reagents,* sign up for equipment, grow the cells, and be sure you have all the necessary components.
   - Do a *mental dry run,* to be sure you haven't forgotten anything.
   - If it is a tricky experiment, with precious reagents, do a *physical dry run* first, omitting the reagents. This will ensure that the physical manipulations you have planned are feasible.
   - *Observe* someone else do the experiment. If you will be using a new technique, ask someone who has performed a similar experiment if you could watch her do it next time, or if she could help you.

Don't talk while planning or doing a complicated experiment, and don't be afraid to ask people not to talk to you. Some people wear a certain hat, or put on a personal listening device (with or without a tape) to signal coworkers that they need to concentrate.

4. **Do the experiment.** Make sure you have set aside enough time to do it, and add another hour to your estimation. Write down results and observations as they happen.
5. **Collect and analyze the data.** Inspect all your data as soon as possible—before you do the next experiment!

   Don't let unread slides or ungraphed time points accumulate. This is a good time to consult with anoth-

Common mistakes made when setting up experiments

Not thinking about the necessity for the experiment.

Planning a huge and sloppy experiment intended to wrap up every major question in biology.

Forgetting to evaluate prior experiments.

Not thinking through every control to be sure the experiment will be interpretable.

Not checking to be sure all reagents are ready and available before starting the experiment.

er scientist in the lab or in the field about the results, whether they were "good" or "bad."

6. **Repeat the experiment.** A result must be repeatable or it is completely worthless. And ultimately, it must be repeated by someone else, so you must be sure your result is dependable.

Background Research

Many mistakes in setting up experiments can be avoided by knowing the field. Even if you have a protocol, you still need to understand the details and theories of the experiment. By checking the literature, you can find out what techniques are generally used, what results would be expected, what reagents are most likely to work, and at what concentration they have been used—plus a myriad of details that can help at unexpected times.

Start with a few papers, the classics on your topic. Look through the references used in these papers: it is likely that you should read most of them, certainly ones duplicated in other papers. Go to the library and read.

Do a keyword search on Medline or another database. Check not only current papers, but old ones, as an amazing number of good experiments are buried in obscure journals because their value wasn't appreciated. Papers are also found in obscure journals because they are obscure. Use your own judgment about the validity of the data in the papers. Try not to be swayed by reputations or journal names when evaluating data.

Keep up in your field by reading current literature. Most people have a few journals that they read regularly to stay posted on major developments. But experimental ideas and techniques are often found in specialty journals and in other journals not found in your library, so you should systematically search the current literature. Set aside a regular time to search Medline, using a search you define by keywords and authors. Or use one of the literature update programs that send a weekly disk with the latest journals, and search by keywords and authors.

Calling or writing one or two of the experts in a field can be a tremendous shortcut for you. Of course, you don't want to waste the other person's time by asking something you could (and should) have looked up yourself (For example, don't call up to ask what the sizes of mammalian ribosomal RNA are). But if you have a question about the specifics of the experiments, E-mail or call.

Controls

A control theoretically shows what would happen if you didn't do anything; thus, it shows you what your pretty results really mean. *There must be a control for every different variable in the experiment.* It is not uncommon to have more controls than

experimental samples in an experiment. Controls are not extra, they are not a waste of time, they are absolutely integral to the interpretation of every experiment.

Controls are not done once—they must be repeated with every experiment. This includes standard curves for enzymatic assays and molecular weight standards for gel electrophoresis. It is absolutely not valid to go back to an earlier experiment and use the data from those controls, assuming that the controls would have been the same.

Types of controls

Experimental controls. These controls tell you if the basic experimental procedures are working correctly.

Examples of experimental controls are the molecular weight standards used for DNA, RNA, and protein gels (as well as being used to measure molecular weights). From the molecular weight standards, one can tell if the gel was the presumed concentration of agarose or acrylamide, if transfer of the gel to a filter was fairly complete, and if electrophoresis was effective.

Treatment controls. Treatment controls are positive and negative, and show you whether the experimental handling of the cells has elicited an effect. If you have samples that have been treated with multiple factors, the effects of the individual factors must be controlled for independently. Thus, if you are looking at the effect of incubation with factors X and Y, you must have a control incubated with X alone, and one incubated with Y alone.

Positive controls. A positive control is usually an experimental control, which shows you how the data would look if a treatment had an effect.

Examples of positive control are the addition of a cell line expressing receptor X when performing immunofluorescence on cells transfected with receptor X; and inclusion of cells known to respond to factor X by stimulated growth when testing for the effect of factor X on cells never tested.

Negative controls. The negative control shows you what the effect of non-treatment is on your readout.

Examples of negative controls are the addition of a cell line known not to express receptor X when performing immunofluorescence on cells transfected with receptor X; and inclusion of cells known to not respond to factor X by stimulated growth when testing for the effect of factor X on cells never tested.

The negative control is the one most often forgotten, and the one whose omission won't hurt until it counts. It is especially important to distinguish between a positive result and high background when first setting up a system or a set of experiments. Many hopes have been dashed when an initial positive result was found, and the experiment repeated with the same happy results—and then, a negative control showed that absolutely everything, appropriate and inappropriate, tested positively.

Time points. There must be a control for every variation in the time of the experiment. If you are looking at the half-life of mRNA at 0, 5, 15, and 30 minutes after treatment with factor X, you must have a control for incubation of your cells without factor X at 0, 5, 15, and 30 minutes. Don't shortcut by trying to have a control for only time 0 and 30 minutes; every treatment needs a control.

Set up the time points so that the harvesting of one sample is completed before the harvesting of the next sample. If a sample jam does happen, find a "resting place" partially through the harvesting—for example, cells could be held on ice until the end of the experiment. But what you do to one sample must be repeated on the rest: If you hold one set of samples on ice for 5 minutes, you must do the same to all the samples.

Zero time control. You must collect a sample as immediately after treatment as possible. Yes, in many cases zero actually means a lapse of seconds or minutes, but you must get a sample (and the requisite control) as close to Now as possible.

To do a zero time point, it may make the most sense to do it AFTER the other very early time points, only if the "age" of the samples doesn't matter. If you want to collect cells 0, 5, 10, and 30 minutes after the addition of factor X, add factor X to the 5-, 10-, and 30-minute samples and put them aside to incubate. Then add factor X to the 0 time sample and immediately collect the sample or stop the reaction.

What controls should you eliminate if you don't have enough samples?

Despite the best planning, things happen: Some samples may be dropped or become contaminated, there may not be enough treatment factors, you may get called away from the lab and be unable to harvest all the time points, but still, the samples are valuable and you need to get as much data as possible.

The control you leave out will probably turn out to be the most important one, but leave out, in order

1. Procedural controls.
2. Duplicate experimental controls.

If you must leave out more controls than this, it isn't worth doing the experiment. There is no valid experiment without controls!

Statistics

Will your data need to be analyzed statistically in order to be convincing? In biological research, this isn't a straightforward question. Some areas of study apply statistics religiously, but there are entire fields of study which insist that the effects are so big and the results so obviously significant that playing with numbers isn't necessary. Generally, your lab either does or doesn't do statistical analysis on the data it generates.

You will have to decide yourself whether you will analyze your data. It may be an obvious decision, especially if you are trained in statistics. Look at other papers in the lab as well as in the field, and see how the data have been handled. Bring those papers and your experimental plan and talk to a statistician at the institution, perhaps in the computer center. Call one of the many companies that sells statistics software packages, or visit an on-line site and ask. Post a question at a relevant newsgroup.

Be aware that formulas are sometimes different for populations than for samples. This is why you can't simply open a book and pull a formula, unless you understand what you are doing. Better no statistics than bad statistics.

Most of what you will probably be doing is trying to establish whether your data are due to experimental manipulation or chance, and whether the data are consistent with a hypothesis. You may want to predict the characteristics of a sample based on information about the population, or you may want to predict the characteristics of a population based on a sample.

Examples of the uses of statistics in the biomedical laboratory

- To estimate the characteristics of a population on the basis of information about a sample: The Students's *t*-test enables you to establish a confidence interval for the mean for a small, normally distributed population. You can then figure the probability from a table.
- To predict the characteristics of a sample on the basis of information about the population: Find the probability, using a normal distribution curve.
- To see whether the differences between the means of two sets of observations, as for before and after treatment, can be explained by chance: Use difference scores for large sample sizes (over 30), and the *t*-test for smaller sample sizes.

The P value, or probability, tests whether the observed deviations from the hypothesis could occur by chance. If the P value is very small, the hypothesis can be said to be true.

Standard deviation is the measure of the deviation of an individual measurement from the mean of many measurements. The **standard error** (also known as the standard deviation of the mean) measures the mean of all the data observed from the mean of a hypothetical database and is a measure of how close the average is to the "true" mean value (Koch 1994). Standard deviation and the standard error are sometimes (and wrongly) used interchangeably, with the standard error being chosen because it gives a smaller value and apparently minimizes data spread.

Use standard deviation to show how reproducible a particular data point is, based on multiple samples.

- To determine the probability that any given sample was drawn from a population within a given population: Use chi square, a sample distribution. You can also use ANOVA or the Student's *t*-test.
- To predict the effect of one related measure on another: Use linear regression. If the two measures have a linear relationship, you can calculate a correlation coefficient. Calculating doubling times is the most common use of linear regression in the lab.
- To consider data from several samples at the same time and distinguish systematic differences between groups from the chance variations found in each group: Do an analysis of variance (ANOVA). If you want to look at the effect of two experimental treatments on a measure, do a two-way ANOVA.
- To look at the difference between two independent samples, as when Factor X has been added to one group, Factor Y to another: Use the null hypothesis and determine the probability. Use the z-test for large sample numbers, the Student's *t*-test for small sample numbers.

Be sure that the data are linear. There are other models, such as quadratic or exponential, that can be used for calculations. Assuming linearity when there is none is a common mistake that gives inaccurate numbers.

Many statistical tests (for example, ANOVA and the t-test) should not be used if you expect a strong effect to skew the population. Most tests presume a normal distribution of the unknown population.

Most computer spreadsheet programs can perform the above statistics (Koosis 1997). Statistics programs can do this as well and will also help you decide what statistics are appropriate.

The null hypothesis is the assumption that experimental results are due to chance. If the probability of obtaining that sample number is less than a predetermined small percentage, the results will be significant, and the null hypothesis is rejected.

Using a Protocol

1. **Obtain a protocol from**
   - *Another investigator.* The best place to get a protocol is from another investigator in the lab, especially for your first experiments. The protocol will be tailored to the resources and expertise of the lab, and may contain important

Every experiment, no matter how experimental, must have a protocol. *Every* time. Even if you are working a technique out for the first time, or repeating a commonly done procedure, you should do it according to a written protocol. If you have done the experiment 50 times, you should still have a protocol or protocol reference (such as "Protocol for extraction from p 3 followed exactly") for your lab book.

details on the tubes to use in the lab's centrifuge or tricks to get the standards into solution. And you will have an expert on hand.

- *A book of protocols.* There are many laboratory manuals commercially available, with simple and clear protocols given for many fields of biology. Laboratory, course, and departmental lab manuals are also a good source of simple protocols. The disadvantage of using a commercial manual is that you will have to fine-tune the protocol yourself.

- *"Methods" section in published papers.* This is the least reliable place to find a protocol. Methods sections are notorious for the important details that may be left out due to space or other considerations.

If you have any questions about a protocol in a paper, do not hesitate to call the author of the paper! The address, phone number, and E-mail address of the person to whom correspondence should be addressed are on the first or last page of the article. E-mail with a followup phone call if there is no electronic response is the preferred sequence of communication.

2. **Read the protocol to see if it makes sense to you.** Pretend you are doing the experiment, and look for obviously missing steps in logic (yours or the source's) or function. Many assumptions are made in most protocols, but this may not be obvious to you. For example, "phenol extraction" often means one extraction with buffer-saturated and pH-ed phenol, followed by two extractions with phenol:chloroform:isoamyl alcohol.

3. **Change the protocol as you require and rewrite it.** This only refers to making steps more understandable to you, or changing specific equipment as needed. If a protocol said a sample was centrifuged for 13,000*g*, you must find the appropriate centrifuge to use and record that on the protocol.

4. **Prepare all reagents listed on the protocol and be sure you have everything you need.** Everything! Nothing is too obvious. It could be a drag to race to the centrifuge with your tubes, only to find that someone else had just started an hour run. Have a backup lined up, or sign up for a centrifuge. Sign-ups are risky on first experiments, as it is hard to define the time you will need, so it is probably safer to have a backup plan. Be particularly sure you have the radioisotope you require. This may need to be ordered several weeks before the experiment.

Should you use a kit? The reagents are assembled, the directions are clear, and in most cases, yes, you should, if the price isn't prohibitive. But there is an important caveat: ***Don't let a kit substitute for thinking.*** *Know every component of the kit, and be sure you understand just what the kit is doing.*

5. **Follow the protocol exactly the first time you do the experiment.** Why? If someone gives you a protocol and you do not follow it exactly, that person cannot

(and might not want to) help you interpret the data. You must be able to reproduce the usual result before you can start to vary conditions. You must know that you are measuring the effect of a variable, not of your technique. You must try not to be a variable in your own experiment!

Don't worry if you don't have immediate success. Everyone who has been around a lab knows this: A new experiment doesn't work the first time. You repeat it, carefully. It still doesn't work. You repeat it again, and maybe, again. It still doesn't work. But on the next try, although you are sure you are doing exactly as you had been doing, the experiment works. And it always works, from then on.

6. **Modify the protocol based on your experience with it.** As you proceed through the experiment, note down any improvements to the protocol that would make sense. When you are evaluating your data, rethink the alterations and check with someone in the lab to see if they make sense. Rewrite the protocol for the next go. Usually, you have a printed protocol on which you make changes over several experiments. It is worth modifying, scanning, or even typing new protocols into your own computer files, and the sooner you computerize your protocols, the better. Then as the protocol continues to evolve, you can add changes and still have a legible protocol.

Examples of Protocols

6-12-92

ELECTROPORATION OF M. SMEGMATIS
B.Jacobs,Meth.Enzymol:204,527,1991

Make sure 10% glycerol is cold — PM 5:30 → 9:30 AM

1. Inoculate 1 liter M-ADC-TW broth and incubate approximately 48 h until A_{600} reached ~~0.5~~ .89 1.0.

2. Harvest in 250 ml bottles (disposs. Conical) by spinning for 10 minutes at ~~10,000~~ 3200 rpm at 4°C. In RC3C

3. Resuspend each pellet in 250 ml of 10% glycerol and spin as above. at 2800 10' 4°C

4. Resuspend each pellet in 10 ml cold 10% glycerol, pool into 2 50 ml polypropylene conical tubes, and raise volume to 50 ml with 10% glycerol. Centrifuge for 10 min at ~~3000~~ 2000 at 4°C.

5. Repeat the wash in step 4. [1800 rpm].

6. Resuspend each pellet to a final volume of 1 ml in 10% glycerol.* — 10 tubes ~ 250 each 200

7. Place DNA (5 pg-5 ug in 5 ul maximum volume; be sure DNA has been washed with 70% ethanol) and 50 ul of cells in an Eppendorf tube and mix by pipetting up and down. Hold on ice 1 min. (100 ml) — By push raise to 2.5 — 200 at 1mg/ml 1ul — comp. 155

8. Set the voltage of the pulser (Bio-Rad) to 2500 V, 25 uF, and the pulse controller to 1000 ohms. turn on in back. place cuvette in white Rack (4°C) + put on machine

9. Transfer the solution containing the cells and DNA into a cuvette with a 0.2 cm electrode gap. Tap the cuvette against the bench several times to get cells to the bottom and remove bubbles.

10. Place the cuvette in the pulser and expose to one pulse (time constants are usually between 15.0 and 25.0 msec). Turn machine immediately on-off

11. Add 1 ml of M-ADC-TW broth, resuspend the cells, and transfer to a round bottomed 15 ml polypropylene tube. Incubate at 37°C for 3 h. water bath, not shaking, but gently tap every 30' or so.

12. Plate 300 ul cells on selective medium. 1 plate 7H9 (plate 7H9/kan)

*The bugs can be aliquoted into Eppendorf tubes, frozen quickly in dry ice-ethanol, and stored in liquid nitrogen or -70°C. When ready to use, thaw slowly at room temperature. Wash cells in 10% glcyerol twice.

PROTOCOL 1.

A copy of the protocol can be used as a template upon which to record the particulars of the experiment and any changes you make to the procedure.

Immunoprecipitation of the 68K Protein—^{32}P-Labeled 68K protein was immunoprecipitated from cell lysates containing equivalent amounts of protein using an antiserum directed against the bovine brain 87K protein as previously described (4). In some cases, an antiserum directed against purified mouse brain 68K protein was used. The two antisera gave identical results. Immunoprecipitated 68K protein was separated by electrophoresis on 8% SDS-PAGE gels according to Laemmli (9), and ^{32}P-labeled 68K protein was visualized by autoradiography using Kodak X-Omat x-ray film and intensifier screens. Where indicated, autoradiograms were scanned on an LKB Ultroscan densitometer.

IMMUNOPRECIPITATION PROTOCOL

Start with cell lysates in approximately 100 µl lysis buffer on ice (protein concentration 1.5 µg/µl). Keep samples at 4 degrees for all subsequent steps.

Preclear with 50 µl Protein A Sepharose beads (Sigma #P-3391) (50% slurry in PD). Add beads and nutate 15 minutes at 4C.

Spin tubes at 7000 rpm, 2 minutes, 4C, in TOMY with swing out Rotor.

Carefully transfer supernatants to new eppendorf tubes.

To supe, add 5µl antibody/150 µg protein. Nutate 1 hour at 4C.

Add 50 µl Protein A Sepharose beads and nutate 15minutes 4C.

Spin as before. Save the supernatant for gel to confirm depletion by antibody.

Wash beads two times with Wash Solution A

Once with Wash Solution B

Once with Wash Solution C

ie, add 1 ml buffer, vortex, spin as before, discard supernatant.

Resuspend beads in SDS sample buffer, boil 5 minutes and run on SDS Page gel.

Preparation of Protein A Sepharose Beads

To 1.5g Protein A Sepharose Beads add 30ml PD

Nutate 15min. at 4C

Centrifugre in Kneewell 1500 rpm, 10min, 4C

Aspirate Supe

Repeat this Wash procedure 2 times

Add 6ml fresh PD to washed beads so that final volume is 12ml.

Add 2.4mg Sodium Azide (final concentration is .2mg/ml)

PROTOCOL 2.

These immunoprecipitation protocols are from the same lab. The first is from a published manuscript (Rosen et al., *J. Biol. Chem. 264: 9118–9121*), the other from the actual protocol used in the laboratory. (Protocol courtesy of Alan Aderem, University of Washington, Seattle.)

TO TAKE BEAUTIFUL FLUORESCENCE PICTURES WITH THE ZEISS

Turn on microscope (knob on right, back)

Load camera (on right):
- Align red line and red dot and pull out cannister
- Slide out of holder
- Use crank to be sure there is no film in cannister (! button on front, right should have a steady light is there is a film in, and a flashing light if there is no film)
- Take off cannister
- Push silver button to open film holder
- Slide film in place, pull across, and thread
- Close film holder
- Slide film holder into holder and cannister
- Slide cannister into microscope with red dot and red line aligned
- Rotate cannister up
- Push B (front button) 3 times to advance film
- Set ASA (front of microscope)

Turn on fluorescent bulb (box on right)

Cut off visible light source (black knob on left)

Set fluorescence filters (black knob on right, 2nd from top)
- 1st stop, 4 lines = green for rhodamine
- 2nd stop, 3 lines = blue for fluorescein
- DO NOT PULL FILTER ALL THE WAY OUT WHEN FLUORESCENCE BULB IS ON - YOU CAN HURT YOUR EYES

Focus

Cut off light to ocular (black knob, on right, top). You will see crosshairs, and use it to be sure focus is okay, but light will be very dim.

Press A (button on front, right) to take picture. Exposure meter on right tells you what proportion of the exposure is completed, exposure is complete at 1. 3200 ASA will take approximately 15-30 seconds, and 160 ASA will take more than 3 minutes.

Slide filter (behind objective) to block fluorescence. Use this, with visible light button (left, back) to take visible light picture.

Unload camera:
- Slide out cannister
- Use crank to wind film completely
- Remove film from cannister and holder and replace cannister

To turn off:
- Fluorescence bulb off
- Power off
- Wipe objectives gently and completely with lens paper only

PROTOCOL 3.

A protocol can be made for a procedure as well as for an experiment, and may be posted near the particular piece of equipment whose use it describes.

INTERPRETING RESULTS

You must examine your data with the same attention to detail that you give to analyzing the experiments of a competitor. Lay out the data and ask yourself:

1. **Did the experiment work?** Check your *controls.* Look at your procedural controls first to ensure that your equipment worked. Did the cells eat? Did the molecular weight markers run as you expected? Examine your positive control. If this worked, your experiment was probably properly executed. Now look at the negative control. If there is an effect where you didn't expect one, you must decide whether this is a real effect or background. If your negative control appears to be negative, all is probably well. If it is positive, either the experiment was not planned correctly or another variable asserted itself during the experiment.
2. **What are the results?** Compared with the controls, and minus the background, *did you get an effect?* How much of an effect? Two fold? Fifty fold? Do all the computation and graphing, so you are comparing data rather than subjective effects. Are two effects synergistic or additive? Did the effect vary over time?
3. **What does the experiment mean?** Does the result make sense? Is the result what you expected? Do you have any explanations for spurious results? Would additional controls help toward an understanding?
4. **Do other investigators understand the experiment?** Talk to other lab members. Discuss the results with the person from whom you obtained the protocol, or from someone versed in the technique. Go back and read background papers again. Don't get too excited yet, until the results have been repeated.
5. **Is the result repeatable?** Do the experiment again. Include controls that would strengthen the result and solve any questions you have about the result.

> The only way to learn to interpret experiments is to do a lot of them. You will develop a feel for what an experiment means, or for what it needs. Experience with different kinds of experiments will enable you to "know" instantly when an experiment has or hasn't worked. DO a lot of experiments.

When Experiments Don't Work

You did the experiment, and got a completely unexpected result.

1. If it is a procedural problem, *check your equipment.* Make sure plugs were attached, that you used the right buffer. Go carefully through your notes to see if you omitted anything.
2. *Redo the experiment.* This often takes care of the problem, because many mistakes with positive and negative controls are manipulation mistakes.
3. *If the problem recurs, redo only that part of the experiment* that is in question. An experiment with only positive and negative controls is usually what is needed.

> For example, if a negative control gave a strong signal in your assay, check only the negative control and a negative control obtained from another source in a very small repeat experiment, against a positive control. If only the original negative control "behaved" incorrectly, there is a problem only with the negative control, and not the rest of the experiment. If both negative controls give a positive result, you must begin to check buffers and other components.

4. When you have identified a probable source of the problem, *do a small experiment* to see whether the problem is fixed. Do not be tempted to jump in and repeat the original experiment yet—wait until the result has been explained.
5. When you cannot find the source of the trouble, when you have asked advice and done everything you can, *repeat the experiment.* And repeat. And repeat.

> The difficulty is in distinguishing between an experiment that has yet to work, and one that never will. It takes practice and experience, and it will get easier—but it will always be a part of life at the bench.

Switching projects

Most projects start out with hope and excitement. As they are nurtured and explored, as so many hours are poured into experiments, the investigator can lose perspective. Emotion and ego are involved in the success of a project. It sometimes seems impossible to give a project up.

It is imperative that, as a scientist, you learn when to stop a project. And since, with such emotional investment, you won't always be able to make such a decision, you should solicit and accept advice.

Knowing when to halt a project is not always clear. In fact, it is very seldom clear. Some indications are:

> *An idea often precedes the available technology. No matter how spectacular and important the idea is, if you can't reliably prove it, you should not work on it.*

- *The data are not reproducible.* If you cannot replicate your results, even if you heartily believe in them, you cannot further the project. It may be that there are problems with a particular assay or piece of equipment, and this should be investigated before the project is dropped. It may also be that such variations are inherent in the system, or that the effect is not important, or that the effect you are studying is too small to be explored with the technology available.
- *The project has no support from the P.I.* Laboratories vary greatly in the amount of independence each lab worker has. In some places, you will choose your own project and how to go about it, and will receive very little actual instruction from the P.I. But you will probably get advice or opinions, and you should listen. Even if you have been given complete free rein, it makes no sense to continue with a project that the P.I. does not believe in: It is very difficult to work well on a project the lab head does not like. Of course, try to convince him or her with your data. If that doesn't work, consider a change of project.

There are P.I.s who care little for personnel and assign projects with little chance of success. This is usually done because he or she has an idea that he is interested in, but has no real chance of working. If you feel you are being sacrificed, do something immediately. Talk to the P.I., and talk to others in the department: It is a character assessment as well as scientific acumen you must judge. If he or she refuses to remove you from a project that is obviously doomed, you should consider leaving the lab.

- *The direction of the project has changed.* Unexpected results may send the project down a path that neither the lab nor the investigator wants to follow. An example would be a student who started working in a fly lab on a protein thought to be critical in *Drosophila* physiology. After cloning and sequencing the gene, he discovers that the protein is involved in mammalian neurogenesis, and that the role in *Drosophila* is of minor importance. The project has changed, and the student must decide whether to study neurogenesis in a lab equipped for fly genetics, join or collaborate with another lab, or drop the project.
- *The project is too difficult.* The difficulty of a project has to be judged against the time you have to work on it. If your visa will expire in 2 years, you don't want to work on a project that will take 4 more years to continue. One option is to have someone ready to take over the project, and share in the kudos. It is not a failure to switch projects! Most failure-to-thrive problems come from inexperienced researchers clinging to a nonviable project.

"Waiting for the bus in the rain" or "I've invested too much time in this project to quit now."

You arrive at the bus stop. You wait for the bus. It is late. You look at your watch, you look at the rainy streets, you look at your watch. This bus is never late—usually. But it is today. There is another bus you could take, if you hurry around the corner. But yet...you have invested so much time in this bus, what if it comes just after you have left?

Well, what if it does? What have you really lost if you get on the other bus? Research is like waiting for the bus in the rain. There will be no neon sign telling you when to change projects, but you must make an informed decision. You can't worry about time you think you have wasted if you switch projects. Think about the time you will waste if you don't switch when you should.

RESOURCES

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