BEST PRACTICES, FUHRMAN LAB

Little details often matter a lot (for safety, for good results, and to prevent wasting resources and time)!

CHEMICALS: [to avoid contamination of chemicals that are used by everyone now and into the future] Never dip a spatula into any chemical for weighing, but rather tap out what you need into a clean weigh boat or weigh paper and return the excess (exceptions - "chunky" chemicals that don't pour, OK to use a carefully cleaned plastic spatula or clean plastic pipette to break up chunks). Similarly, it is much preferable to pour liquids from an original chemical stock (e.g. acid) into a cylinder for measuring rather than dipping a pipette. If you MUST use a pipette, be sure it is clean and be careful not to touch it to the inside of the bottle at the bottleneck. Do not use plastic pipettes in solvents unless you are completely sure of full compatibility first (do not test in the reagent bottle!)

CONTAINERS/PLASTICS: Plastics all have their own chemical compatibilities and incompatibilities. Be cognizant of the material of any plastic items you use (check the embossed labels!) and what is OK to put in them. It is not obvious! There are tables on the Nalgene Website (and should be up on the lab corkboard). For example, you cannot store bases in polycarbonate, nor organic solvents in many plastics. Centrifuging requires particular care (in the type, max g force, chemical compatibility, and the exact correct rotor or adapter to prevent tube/bottle failure – match the exact shape of the tube bottom). Sometimes a chemical is compatible at room temperature but incompatible when heated. Carefully considering this will not only help your experiments and prevent wasted time, but also save the lab considerable money in replacing damaged plasticware.

Compatibilities are not just about containers, but also includes components and instruments. For example, the mesh filters and nets we use to separate plankton are made of NYLON, which is incompatible with acids (dissolves in acid). So do not wash the Nytex 80 um filter mesh in the acid bath! Also, most zip ties are made of nylon, and they will also dissolve in acid.

Do not mix up the caps of different types of screw-capped bottles. Even if they screw in they may not seal properly (i.e. the seals may not match up). Also some caps have been used for very toxic materials and may contain toxic traces (even after cleaning) that can ruin subsequent materials they contact. Such caps should be clearly labeled TOXIC and kept with the associated container they match.

For any container that will hold live marine bacteria (intended to stay alive and active, especially for activity measurements and cultures), pre-rinse the receiving container 3 times with enough seawater to splash on all interior surfaces, shake it out fully between rinses. If using typical reusable labware it should be acid washed (e.g. 5% HCl, i.e. 5 parts concentrated HCl + 95 parts deionized water) and rinsed with deionized water first. Use HCl because it is volatile and does not leave a residue when it dries.

Cubitainers or other carboys to be used for live seawater should be acid washed and stored closed tightly with a small amount of acid (can be e.g. 25 ml) inside to keep them clean and avoid microbial growth on the walls. Be sure they do not leak (e.g. pinholes in Cubitainers are common). If a cubitainer is found to leak, cut it in way so that it will not be mistakenly used in the future and throw it in the trash. Before performing experiments where you will be submerging cubitainers in an incubator, consider the

first step of your experiment to be testing the cubitainers for leaks in the lab. Fill them up completely because sometimes leaks are not obvious until the cubitainers are full.

Keep containers and tubs containing the wash acid (5% HCl) air-tight sealed to avoid fumes that are not healthy and also condense inside the air conditioning system and slowly destroy our ventilation (would fill the lab with little aluminum chloride flakes), and rust any steel nearby. Use them in the fume hood whenever possible.

DO NOT MIX UP labware that has held very toxic material like formalin, toxic metals (Hg, Cu, etc.), TCA, etc. with labware to be used for live organisms! It is very hard to remove traces of the toxins and the labware will remain toxic indefinitely and ruin other people's experiments if mixed up. Native marine microbes are remarkably sensitive to tiny traces of chemicals that "lab rat" microbes tolerate. All labware to be used for such material should be clearly labeled TOXIC (REMEMBER TO LABEL THE CAPS ALSO!) and should be cleaned carefully after use in a way that any remaining toxin (after proper disposal of waste) does not spread to clean labware (do not wash at the same time as clean containers or share cleaning tubs or acid baths), also rinse the sink and cleaning area are very carefully after cleaning these.

Dilute DNA or RNA solutions (ng/ul) should be kept in low-bind tubes (made for nucleic acids), or dilute proteins in low protein binding tubes – otherwise the macromolecule will stick to the walls and be lost. Avoid refreezing very low concentration nucleic acid solutions (low pg/ul), which also causes their loss – instead make initial aliquots containing the amount you need at one thaw event. Often the nucleic acid completely "disappears" upon the second freeze-thaw (not sure why - maybe binding irreversibly to the tube). Store DNA in TE, not water. Store RNA in citrate buffer made for this purpose, not water (NOTE: most kits and protocols call for RNA to be kept in pure water – which may be OK for lab cultures and large quantities that can be replaced. But with natural extracts RNA will easily be lost on storage in water, even when mostly kept at -80 C (it needs to be warmed up to use it, and degrades during that time).

GLOVES: We usually use gloves to protect our samples from hand contamination, not just to protect us from caustics. Latex (tan) gloves and latex tubing are deadly toxic to marine plankton. DO not use them with live materials. Nitrile (usually blue or purple) are better. Preferably wash your 'hands' (in the gloves) after you put on the gloves and periodically while using them. It is often preferable (environment-wise) to wash your gloved hands regularly with soap and water, rinsing off carefully, rather than arbitrarily changing gloves. Consider washing your gloves with 'DNA away' before setting up PCR reactions and don't allow your gloves to give you a false sense of security! While they are probably cleaner than your hands, they immediately start accumulating contamination without due care, even old PCR products. Remember everything you touch with gloved hands (like doorknobs, pipettes, phone, face, etc.) can contaminate the gloves.

WATER: Deionized water – currently purified in our lab by a Barnstead High Capacity then High Purity cartridge, with a 0.2 um capsule prefilter before DI and at the dispenser end. Feed water is the building's DI water (usually quite good itself). Check color indicators of the cartridges regularly to know they are not exhausted. Change filters every 6 months to one year or if clogged.

FILTRATION: Filter types: We use filtration very much in our work. Different filters have very different properties. Don't mix them up. They vary in size distribution they capture, charge effects (good or bad, depending on the application), porosity and flow rate, chemical compatibility, optical properties for microscopy. One type that says "0.45 um" MAY capture more bacteria than others rated the same or even "0.22 um." Use the HA 0.45 um Millipore (mixed ester) for thymidine and leucine incorporation, 0.2 um Durapore (or Supor if the protocol calls for it) for DNA or RNA collection, 0.2 um black polycarbonate for acridine orange, etc. Do NOT use 0.45 um Sterivex (Durapores inside) to collect bacteria, because about half of the bacteria pass through. No glass filters retain all marine bacteria, but the 0.3 um GF75 come closest. Remember free marine bacteria are typically 0.2-1 um, viruses mostly 0.02 -0.2um, protists 0.6 –hundreds of um…

Filter holders: Use the right ones for your application! Most inline filter holders are meant for flow only in one direction (or else the filter cracks or punctures). Some need to have air gaps vented out or else liquid will not pass. Most filter holders have O-rings or gaskets and will leak terribly, or not work at all, if they are missing or the incorrect size or folded or dirty. Polycarbonate filters have special inline holders that work best for them (sandwich, two gaskets).

Vacuum/pressure pumps: ALWAYS have the 'pump protector' filters in place on the vacuum inlet side right next to the pump, even if you have a vacuum trap up the inlet line (also have a protector on the outlet) – these protectors allow gases but not liquids to pass. Seawater destroys the pumps from the inside, and we have lost several of them to corrosion. Make sure the protector is oriented right and not clogged (if it is clogged or waterlogged, try blowing it out backwards on the pressure side of the pump, then rinse with DI water and dry it). Clean off any spilled seawater from the pumps when you are done. Many of our pumps have been ruined or are not adjustable because someone spilled/splashed seawater onto the metal parts and failed to rinse them off. Remember seawater is one of the most corrosive materials we work with. The pumps cost about $500 each and it is generally a waste to need to discard them due to carelessness. Don't "wait until tomorrow" to clean seawater off pumps or other lab equipment, even if it is painted or looks like stainless steel.

SAMPLE/REAGENT PROTECTION: When you take materials from the freezer or refrigerator to use out in the lab, keep them on ice or in a special chilled tube rack. This applies especially to DNA or RNA samples (not just SPOT) and enzymes. Do not think it's OK to leave them out and use them quickly because "I only have them out for a little while." Especially with materials that should last years (e.g. SPOT DNA) even short intervals out of refrigeration add up. And it is easy to be distracted or forget, then leave them out for a long time. Several irreplaceable samples have degraded completely already, sometimes found out on the bench a day or more after being taken out.

FILTERED SEAWATER: We often prepare filtered seawater to remove organisms, 0.2 um (Durapore GV, Polycarbonate, cellulose nitrate/acetate like Millipore GS) to remove bacteria and larger cells, or 0.02 (Anodisc) or ultrafiltration (e.g. 50 kD) to also remove viruses. Usually some cells get through 0.2 filtration (especially Polycarbonate), so bacteria and their viruses will likely grow back up to a million per ml in a few days (room temp) or weeks (refrigerator). Don't assume that if you filtered the water it stays cell free! This may be prevented by truly sterile filtration conditions (autoclaved filter holder with filter

in place, sterile filter air vent) and sterile filters like a 0.22 Millipore GS, then using truly sterile technique to handle it later on. Alternatively you may heat the filtered water to ~50 C to inactivate native marine bacterial cells and viruses (some spores or heat tolerant cells can survive this) – but if you do not use sterile technique it will eventually get contaminated. If it is OK for the water to be toxic (e.g. for dilution in microscopy prep) you can add a preservative like a very small amount, e.g. 0.1%, formalin; in this case the container and cap should be labeled TOXIC.

MICROFUGE TUBES: If you open these with your hands, expect some cross contamination is possible or even likely unless you use care each time (and don't pop it open with your thumb! instead pull it open from the top). This can ruin experiments, cause all sorts of blank problems for you and others, etc. A cap opener is preferable

Some microfuge tubes are suitable for boiling and some are not. If performing DNA extraction in microfuge tubes, check that they will not pop open or break upon boiling. Freeze the tube first if your tubes with samples will be coming directly from the freezer. Not checking your tubes for caps that will pop open with boiling can result in complete loss of samples.

PIPETTES: Most of our work depends on using these just right, and it is not as simple as it looks. Remember not to dip the tip more than a few mm into the liquid you are pipetting, for example. New lab users should check their technique and accuracy by weighing distilled water at the top, middle, and bottom end of each pipette range. It is easy to be quite off, especially with the 1 ml pipette (even for experienced users). Take your time when working near the top of each pipette's range. Watch out for stray droplets inside and outside the tip.

When pipetting viscous material like glycerol, it is preferable to use the special red positive displacement pipet (each tip has a plunger inside).

TUBING AND STOPPERS: Some material used commonly in labs are deathly toxic to many native marine bacteria. These include new latex tubing (tan, rubbery – also as GLOVES), some black rubber tubing, and heavy black rubber stoppers. Avoid using them. Black neoprene is usually OK.

PROTOCOLS (most are available as separate files):

DNA Extraction, Thymidine*, Leucine*, SYBR counts (25 and 13 mm), Acridine Orange counts , agarose gels and gel documentation "system", DNA concentration measurement, ARISA whole procedure from DNA to binned data.

*replace stocks after 1 year. Keep thymidine in 70% ethanol to reduce decay, and buy only tritiated thymidine labeled on the methyl group!

PCR product handling to minimize spreading it everywhere and ruining future experiments. Careful separation of PCR preparation areas and pipettes/tips from PCR analysis areas and pipettes/tips.

SYBR GREEN best practices

While SYBR green microscropy slides theoretically store for years at -20, save years of anguish by counting slides fresh or at least counting a few fields from each filter before storage!

Aliquot reagents into very small aliquots so that they only need to be thawed a few times. SYBR green method can be hit-and-miss with previously thawed reagents. We find best success with 'fresh' reagents.

NOTEBOOKS and EXPERIMENT PLANNING: Develop good habits early. As stated above "the devil is in the details," so they must be planned minimize variations with an experiment, and all recorded carefully.

It is super important that you record in your notebook (not random paper in the lab, paper towels, benchcovers, etc.) pretty much everything that can matter in interpretation of data and reproducing experiments, including finding out if a step or reagent may be a problem. So when you use a kit, record which exact kit (full name and manufacturer), lot number and date received/expiration, time of day you did each of the different steps (or start and finish of procedures that need to be done rapidly in succession), step descriptions (abbreviated description usually fine) and exact volumes even if just following instructions, exactly which buffers if not from the kit, quantitation of DNA and RNA with the underlying data and which dye kit (Pico Green, SYBR Green, Qubit, etc. date we got it, what standards, computer file name is using one, etc.). You probably won't remember it all later no matter how good your memory.

Also when processing many samples from time series, experiments, ocean transects, etc., **record very carefully** how samples are arrayed in strips or plates- **and double check that**! It is very easy to make mistakes with those, and hard (or impossible and expensive) to recover. Have a 'foolproof' system to keep track of exactly where "you are" when repetitively pipetting many things one after the other, which might include speaking aloud and voice- or video-recording the label IDs with your phone as you pipet. That way if you lose track (which anyone can do!) and are not sure what you just did, you can replay to check. It is often helpful to use a multipipette that dispenses 8 at a time. Remember it may take days to prepare a set of samples, but weeks or many months to analyze the data, which will affect your results and ability to interpet them - so take extra precautions to avoid transpositions or pipetting mistakes, even if they take a little extra time.

Keep a copy of ALL kit instructions (we actually should have a 3-hole notebook with sleeves to hold these, in an office) as they change over time and someone may throw out instructions with an old kit.

One important factor is to keep well-labeled extra ds cDNA in the -80 freezer (like all field DNA and RNA also) - with labels someone else could understand. Tubes are too small for all relevant stuff, unless we use a barcode system (maybe a good if very late idea). It is wise to print/write details and your name (and dates) on a paper in the box in the freezer, as I think you probably do.