SUPPLEMENTARY NOTE 1, Yuan DS *et al.* (2005)

Suggested Procedures for Avoiding Contamination in TAG PCR

INTRODUCTION

The labeled extracts used in yeast UPTAG and DNTAG microarray applications consist of PCR products at molar concentrations much higher than those typically encountered in cloning applications. This high molarity is a consequence of both the high primer concentrations (5 μM) and the small product size (56 bases). Experience has shown that PCR contamination is difficult to avoid even with good conventional laboratory practices.

The definitive test of freedom from contamination is the lack of a 56-bp product in a standard reaction (using asymmetric PCR) when no template is added, coupled with the presence of this product when TAG template is added at 1 fM final concentration to a reaction run in parallel. It goes without saying that this test is meaningless unless valid negative controls can be demonstrated.

This note outlines measures to try when negative PCR controls fail. Some of these measures may seem extreme, and none is in fact "proven", but most are simply elaborations on the theme of avoiding propagation of contamination via aerosols, dusts, or direct contact. Many are borrowed from procedures used for handling infectious agents or radioactivity.

The key idea is to dedicate "clean" and "dirty" rooms for handling different levels of TAGs. While all areas are regarded as contaminated, the clean room is maintained with scrupulous care while the dirty room is reserved for handling hazardous levels of TAGs. All negative controls are assembled first in the clean room before taking them to the benchtop for adding any reagent that contains tags. PCR analysis, hybridization, and washing should be performed exclusively in the dirty room.

CLEAN ROOM PROCEDURES

Clean room usage policy -- No tag reagents are *ever* allowed in the clean room. Conversely, anything needed for negative controls for PCR -- water, buffer, stock primers, enzymes, aliquots, mixtures, tubes, pipette tips, marker, tube racks -- is prepared nowhere else but the clean room. A laminar flow hood is desirable.

Handwashing -- The clean room must still be regarded as contaminated, because it takes only one colleague to disregard an isolation rule and unwittingly contaminate the entire laboratory. In this light, hands must be washed and dried after touching *anything* that someone else may have touched, including fixtures such as faucets, door handles, and light switches. Remember that the only guaranteed clean materials are supplies in their original unopened containers, running tap water, and paper towels in a clean dispenser (the touchless "EnMotion" dispenser from Georgia Pacific is useful). A surgical scrub (elbows to hands, soap, avoid touching the faucet and avoid dripping down to hands) is a good idea. Arms should also be washed before using a laminar flow hood (to avoid contaminating the hood). Gloves may be more of a hindrance than a help because of the frequent washing required (10-40x if no one is available to open containers etc), and also because of the possibility of latex or starch dusts on the gloves.

Use of hood -- Turn the blower on and UV light off. Drape the work surface with paper towels or a sheet of Whatman 3MM filter paper; this defines the usable work area. Take notes on a paper towel with a specially clean pen, or ask a colleague to take dictation.

Tubes -- Aliquot tubes into disposable plastic food boxes that are newly opened. Shake tubes out of their original bags into the food boxes without touching them. Do not use any form of adhesive label or tape; label tubes directly. Tube racks are difficult to wash and should be purchased brand-new and transferred to the clean room for dedicated use. (One obstinate contamination problem was traced to a plastic 96-well rack, even though it had been thoroughly scrubbed beforehand with laboratory soap and exhaustively rinsed.)

Buffers and instruments -- Consider autoclaving these supplies as an adjunct to preventive measures.

Reagents -- Aliquot reagents once it is well-established that tubes are free of contamination. Give each aliquot its own number and keep detailed notes to identify which aliquot is in current use; debugging is impossible otherwise.

Thawing reagents -- Tubes of enzymes and primers must be thawed and centrifuged before opening. Because heater blocks and centrifuges may be contaminated and are difficult to clean, the exterior of these tubes should be also regarded as contaminated, i.e., isolated on their own clean tube rack and handled last. Alternatively, thaw tubes in a floating rack in a clean tub of water.

Storage -- Leave clean materials in a washable covered plastic box that stays in the clean room. Ensure that covered boxes for externally stored reagents are clean for handling.

CLEAN REAGENTS AND SUPPLIES

- Deionized water
- PCR buffer and enzyme
- PCR primers
- Microfuge tubes, PCR tubes; plastic boxes for aliquots of tubes; tube racks (washed); Saran wrap
- Filtered pipette tips; new 0.5-10 µl and 20-200 µl pipetters
- Covered plastic box for storing the above (except enzyme and primers)

BENCHTOP PROCEDURES

The aims here are to avoid contaminating PCRs that would otherwise be clean, and to avoid further contamination of the laboratory environment.

Workspace -- Again, drape the work area with clean paper towels or sheet of Whatman 3MM filter paper. Maintain a covered sharps container or 500 ml flask for dropping pipette tips and used tubes. Cover bottles with Saran wrap to avoid dust; remove existing dust by washing and drying, not by wiping.

Tube precautions -- Centrifuge all liquids before opening, and also after vortexing or if dropped. This includes 50 ml tubes, microfuge tubes, and PCR tubes. Ideally the centrifuges would be isolated also. Never touch the inside of the cap or the mouth of the tube; e.g., always use two hands to open a tube.

PCR precautions -- Keep tubes covered unless they are being handled. Individual tubes should be capped; 8-strip tubes can lay on their sides on a paper towel (the liquid will stay in the tubes); 96-well plates should be covered by a box without touching the wells. Tubes must already contain PCR cocktails and negative controls; after adding templates, mix by stirring. When sealing tubes, take great care to avoid touching the inner surfaces of the tubes or caps.

Pipette tips -- Use filtered tips only; avoid boxes that other people have opened and left uncovered. Ideally, a dedicated set of pipetters would be maintained by each person. Autoclaving is not necessary, but it is important to keep boxes covered.

Pipetting technique -- Be aware of the pipetter shaft at all times and keep it away from the walls of receptacle tubes. Avoid splashing or bubble formation. Do not transfer tag solutions with the liquid at the end of the pipette tip. Instead, use a reverse pipetting technique (i.e., evacuate the pipetter all the way, draw up the desired volume by judging the position of the liquid in the pipette tip, then carefully draw the liquid up into the tip). Avoid pipetting to the cap region of a tube or to the very bottom. Do not blow out the last bit of liquid. Mix PCR samples by pipetting up and down and stirring at the same time. Dispose of each tip by unscrewing it and dropping it into a tall covered receptable, rather than ejecting it.

DIRTY ROOM PROCEDURES

Dirty room usage policy -- Anything that contains or has been exposed to high-grade tag solutions (see list above) may be opened *only* in this room. Contamination in this room is inevitable. Never leave the room without thorough handwashing.

Waste disposal -- All trash must stay in the dirty room. Never fish around in the trash; the likelihood of contaminating one's clothes is virtually certain. Store all used hybridization supplies in the dirty room. Store all other materials (e.g., used gels, genomic DNA preps, tag stock solutions) in washable plastic containers or racks wrapped in plastic wrap if they need to be stored elsewhere.

DIRTY REAGENTS AND SUPPLIES

These can be classified as either low-grade (fM-pM) or high-grade (nM-μM) hazards. These are listed below in order of use:

- Genomic DNAs (low grade)
- PCR controls and spikein mixtures (low grade)
- Tag stock solutions and dilution series (high grade)
- PCR products (high grade)
- Electrophoresis chambers, used buffers, used gels (high grade)
- Hybridization and washing chambers, trays, tools, tubes, used buffers
- (high grade)
- Used microarray slides (low grade)

Refridgerators and freezers should also be segregated by hazard level and treated accordingly.

DECONTAMINATION

Surfaces -- To the extent practical, wipe down or soak surfaces with a 1:10 dilution of fresh bleach. Dry with a paper towel, then repeat twice more. This applies to all instruments, centrifuges, containers, and benchtops, especially when contamination persists. Decontaminate laminar flow hoods with UV lamps (if so equipped).

Pipetters -- Dissemble, soak shafts and ejectors in the bleach, and thoroughly rinse all non-metal and non-rubber surfaces. Blow dry with compressed air in a separate room; beware of aerosols.

Buffer spills -- Buffer spills from gel boxes in a dirty room, e.g., from being jarred or decanted, are a major hazard because of their commonplace occurrence and the ease of dissemination. Hybridization buffers are probably just as bad. Likewise, the sink and benchtop may be heavily contaminated. It may help to think of the buffer as full of ink to appreciate how a small spill can contaminate the entire laboratory. Clean up as for any surface, but with multiple dilutions due to the extremely high concentrations that must be removed.
