Setting Up a PCR Laboratory

The PCR laboratory should consist of three distinct work areas (Fig.3). In order to avoid the contamination problems, each area should be dedicated to a single procedure. Specimen preparation occurs in the first area, reagent preparation and PCR set-up in the second area, and amplification and detection in the third area.

The entire procedure can be performed in a single room if proper precautions are taken. The following practices will diminish the potential for contamination.

- Each area should have dedicated supplies and reagents.
- Color coding of reagents and supplies identifies those that belong to a particular area.
- Reagents, supplies and equipment should never be taken from one area to another; three sets of pipettors are therefore essential.
- The workflow must be unidirectional from " clean" (pre-PCR) to " dirty" (post-PCR).
- Dedicated labcoats and gloves should be worn at each worksite; when moving to a new area, workers should put on new gloves and labcoats.

Specimen Carryover: The Major Pitfall of PCR:

Careful attention to technique is essential to performing PCR. Because of the high sensitivity of the procedure, the introduction of

even a minute amount of a positive template into a negative specimen can lead to the generation of a false-positive result. An amplification consisting of 25 cycles yields up to 10^3 copies/mL of the amplicon. A 0.1 μL aliquot of this mixture contains 10° amplicons. By comparison, there are only 1.4x 10 copies of a single-copy gene in a microgram of human DNA. As a result, such contamination results in a false-positive reaction. It is not surprising that the biggest problem associated with the successful use of PCR is this potential for specimen carryover.

This carryover can be controlled through a number of different techniques, as follows:

Biochemical sterilization of previously amplified material:

Commercial kits are now available (Carryover Prevention kit, Cetus Corp., Norwalk, CT) that cause the enzymatic degradation of previously amplified material. The methodology incorporates deoxyuridine triphosphate (dUTP) into the amplicon instead of deoxythymidine triphosphate (dTTP) into the amplicon instead of deoxythymidine triphosphate (UNG) is added to reaction material. UNG specifically digests any DNA sequence that contains uracil. A template that contains thymidine will not be affected. Use of this method eliminates any product generated during a prior amplification.

Sterilization of DNA by exposure to ultraviolet light:

The exposure of double-stranded DNA to ultraviolet (UV) light results in the formation of dimmers between adjacent thymidine residues. These altered bases are incapable of extension by the enzyme. Exposure to UV light does not affect the action of either enzymes or single-stranded primers. It is therefore possible to treat all reagent mixtures with UV light prior to the addition of template DNA in order to eliminate any contaminating DNA. It is also preferable to treat the work area with UV light.

Strict adherence to proper laboratory technique:

It is essential to prevent the introduction of previously amplified material into future reaction mixtures. To this end, the following suggestions have been made.

- **Physically isolate PCR preparations and products:** A separate clean room and biological safety cabinet are required for set-up of the reaction. Processing of amplified material must be performed in a totally different work area.
- **Autoclave solution:** Autoclaving degrades DNA into fragments of low molecular weight.
- **Aliquot reagents:** Do not repeatedly open containers of buffers, enzymes, nucleotides, or primer stocks. Instead, prepare single-use aliquots and discard any remaining material after set-up of the reaction.
- **Use disposable gloves and change gloves often during set-up:** DNA may splash onto gloves when the tops of

microcentrifuge tubes are opened. Frequent changing of gloves reduces the possibility of transferring DNA between specimens. The powder that is used in latex gloves also interferes with the action of Taq DNA polymerase, It is essential to wash any excess powder off the outside of gloves prior to setting up a reaction.

- **Avoid splashes:** Perform a quick spin in a microcentrifuge before opening it, and pay close attention to careful pipetting techniques.
- **Use positive-displacement pipettes or aerosol resistant tips on air-displacement pipettes:** There are essential to prevent carryover. DNA contaminates the barrel of conventional air-displacement pipettes. The contaminating DNA is easily transferred to subsequent reactions.
- **" Premix" reagents:** In order to avoid excessive pipetting while setting up a reaction, prepare master mixes that contain all components except for the sample DNA.
- **Add DNA last:** adding the DNA as the last step decreases the chance of carryover.
- **Choose positive and negative controls carefully:** Positive controls that contain large amounts of template DNA should be avoided, because their use would increase the likelihood of cross-contamination. Instead, use the lowest dilution's that generate a positive result.