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Chapter 4

Laboratory Operations

Designing Molecular Laboratories to Decrease Contamination

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Introduction

- Foundation for the molecular analysis of specimens in many laboratories starts with the polymerase chain reaction (PCR)
- **PCR Advantage:** Ability to amplify very small amounts of DNA
- **PCR Disadvantage:** Process is vulnerable to contamination
- Laboratory design paramount to decrease possibility of specimen contamination

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Real-Time PCR: Solution to Contamination?

- Provides direct measurement of amplicon product as reaction takes place
- An alternative to traditional post-PCR analysis methods; eliminates need to handle the sample
- Self contained automated system
  - No need to open PCR containers – no amplicon escape
  - Eliminates the need for separate processing rooms
- Although advantageous, contents of individual tubes made during PCR may need to be analyzed and containers may have to be opened
  - *Will still need separate room for analysis

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Contamination Issues

- Important sources of PCR contamination:
  - Development of PCR amplicon aerosols during post-analysis
    - Solutions:
      - Physical separation of the laboratory
      - Use of aerosol-proof pipettes
  - Target template
    - RNA templates less stable than DNA templates; makes RNA highly susceptible to DNA contamination

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Sources of Contamination

- Previous amplification and purification of plasmid clones
- Repeated isolation of template nucleic acids
- Previously amplified molecules (amplicons)

*The control and removal of PCR amplicons is the basis for a contamination control program.

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PCR Activities

- Sample preparation
- PCR reaction assembly
- PCR execution
- Post PCR analysis

* These activities can be classified in two major categories: **Pre-PCR and Post-PCR**

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PCR Activities (Cont.)

- Pre-PCR Activities
  - Isolation of nucleic acid
  - Assembly of reaction to amplify samples
    - Must determine minimum needed equipment for PCR sample preparation, reagent preparation, and assay set-up
  - Risk: creating aerosols in preparing RNA and DNA templates
    - Perform under hood
    - UV light in hood area prior to sample preparation
    - Bench-top sized cabinets equipped with UV light
- Post-PCR
  - PCR execution and analysis

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Contamination Control Program

- Space and time separation of pre and post PCR activities
- Use of physical separation aids
- Use of ultraviolet (UV) light
- Use of aliquoted PCR reagents
- Incorporation of numerous positive and negative control blank PCR's (water substituted for nucleic acid template)
- Use of one or more various contamination control methods

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Contamination Control

- Two broad methods of contamination control:
  - Physical methods to prevent dispersion of PCR amplicons
  - Chemical methods that inactivate the amplicon's ability to be templates in a new cycle of PCR
  - *Most successful PCR labs use a spectrum of these methods to effectively control contamination

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Contamination Control (cont)

- Physical Methods
  - Positive displacement
  - Barrier pipette tips to prevent aerosols
    - Both prevent the re-introduction of small amounts of a contaminating aerosolized sample into the next sample that is pipetted
    - Pipette tips recommended in pre-PCR areas of lab where samples are being processed and template nucleic acids are being isolated and purified
      - Tips are necessary and cost-effective in pre-PCR lab because there is already a large amount of amplicon present
      - Adjunct to tips is the use of laminar flow hood or biological safety cabinet to prepare PCR samples and reagents
        - Reduces chance of an external source of PCR amplicon contaminating the samples and reagents being manipulated for the subsequent PCR activities

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Contamination Control (cont)

- Chemical Methods
- UV Photolinking
  - Used in both pre-and post-PCR setting
  - Fast reaction; effective also for bigger amplicons greater than 700 base pairs
  - Most often used in a pre-PCR setting in which equipment is installed in a small table-top cabinet and then illuminated before the PCR's are assembled
    - Concerns:
      - UV light exposure
      - Photo reaction favors thymidine over cytidine 10:1; amplicons A:T rich sequences more effienciently disabled than A:T poor sequences
      - Decreasing length of amplicon usually gives a lower rate of protection; shorter amplicons not well-controlled

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Contamination Control (cont)

- Chemical Methods (cont)
  - Uracil-DNA-Glycolase
    - Enzyme also known as UGD
    - Highly effective in destroying PCR amplicons when used for sample preparation

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Space and Time Separation

- Potential contamination reduction by separating the source of amplicons
  - Post PCR and Pre PCR rooms
  - If impossible, designate areas for sample preparation and PCR set-up that are separate from post-PCR analysis area
- Establish daily schedule for performing PCR
  - Sample preparation and pre-PCR activities should occur in the morning
  - Post-PCR analysis performed in afternoon

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Space and Time Separation (cont.)

- If all activities must be performed in a single room:
  - Sample preparation area should take place in a laminar flow hood with UV light
    - Walls of hood wiped with freshly made solution of 10% bleach before processing or prepping samples

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Space and Time Separation (cont.)

- Laboratory Space Arrangement
  - De-ionized water should be present in pre-and post-PCR areas
  - There should be separate centrifuges, storage freezers/fridges, and storage of supplies for both areas
  - Phones, computers, and electronic equipment should not be shared between the pre- and post PCR areas
  - *It's important to note that rarely is laboratory space allocated strictly for PCR; it's usually shared with other lab procedures
    - PCR protocols may be integrated into lab operations maximize sharing of facility and bench space

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Space and Time Separation (cont.)

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Space and Time Separation (cont.)

- Environmental Concerns
  - Air Handling
    - Air handlers need to be separate and air pressure individually adjusted in each lab
      - Pre-PCR area: slightly positive air pressure compared to air in connecting hallway
      - Post-PCR area: slightly reduced pressure to pull air in from the outside and prevent escape of amplicons
    - Air handlers should be connected to separate air ducts
      - Each must lead to a separate location for exhaust

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Protective Clothing/Equipment

- Each technologist should have lab coats designated only for:
  - General molecular lab
  - Pre-PCR
  - Post-PCR
  - In extreme cases, disposable gowns and booties may be used
- Adhesive paper or “stick mat” at lab entrances
  - Prevents trace amounts of dust and debris from entering lab

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Reagent Sterilization

- Because PCR labs perform some methods that require sterile reagents, some may need to be autoclaved
  - Most critical reagent: water (H2O)
  - Sterile USP water can be converted to PCR water by filtering it through two 0.45 micron nitrocellulose filters
    - Filters have high binding capacity for nucleic acids and proteins
    - If lab is involved in amplifying very small quantities of bacterial DNA, USP water should be autoclaved separately from all other reagents before filtration
  - Reagents and solid items destined for pre-PCR lab should be autoclaved separately from other supplies

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Sample PCR Laboratory Design

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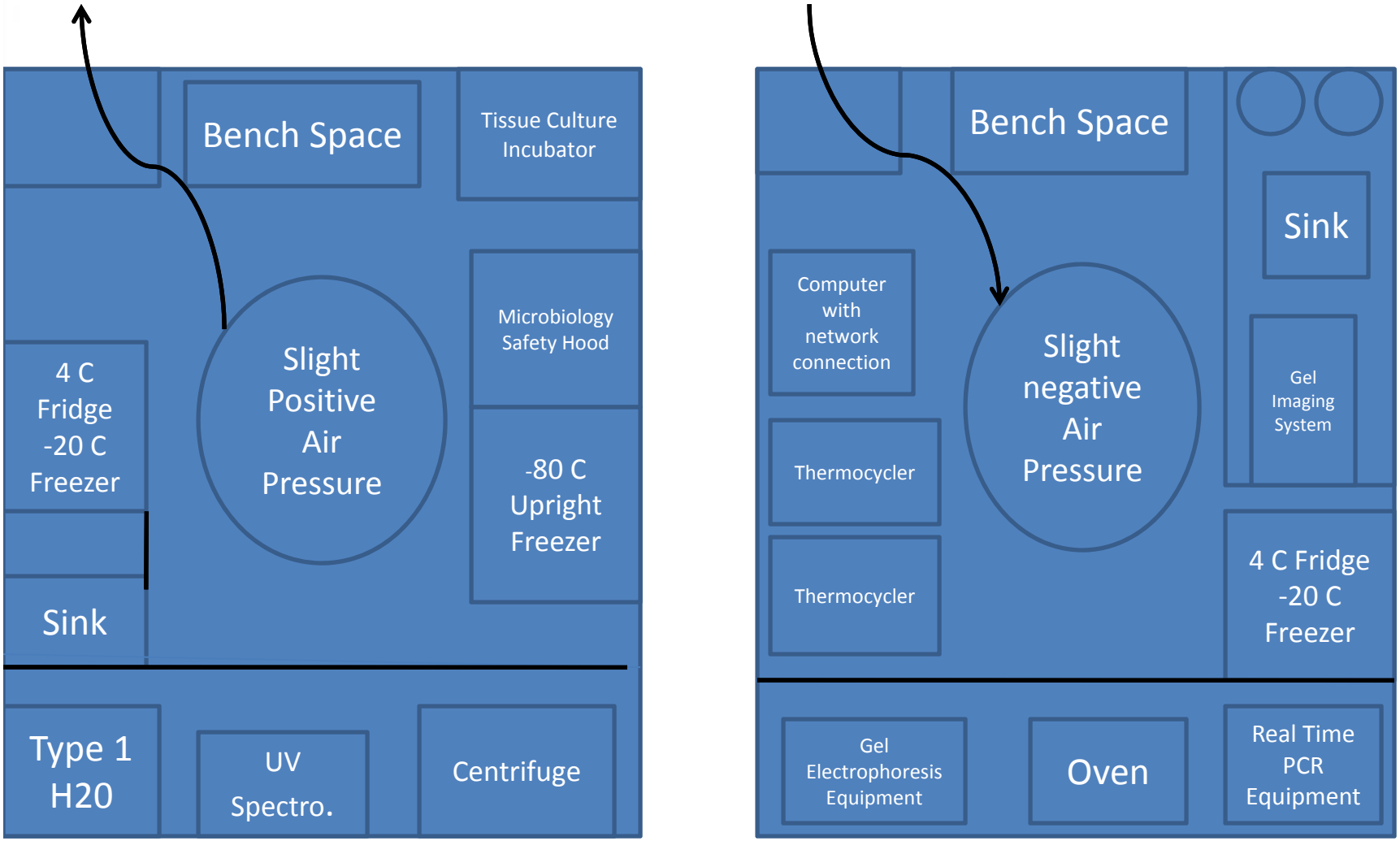

Pre-PCR Lab → **Post-PCR Lab**

Flow of samples for PCR analysis

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Sources

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